In studies of salmonellosis (Sporn et al., 1950) and *Diplococcus pneumoniae* infection (Robinson & Siegel, 1944) in rats, riboflavin deficiency produced no observable differences in resistance. Riboflavin-deficient rats infected with the rickettsia of murine typhus fever succumbed rapidly; there were no deaths among controls receiving a complete diet (Pinkerton & Bessey, 1939). Rasmussen and associates (1944b) were unable to find any effect of riboflavin deficiency on Theiler mouse encephalomyelitis. A slightly increased resistance to type 2 poliovirus was evident, as judged by less frequent paralysis.

The effect of riboflavin deficiency on *Trypanosoma cruzi* infections in albino rats maintained for 13 weeks on deficient diets was studied extensively by Yaeger & Miller (1960b). The peak of parasitemia was reached earlier, and the severity of cardiac damage was slightly increased. Pair-fed controls indicated that the effect was attributable more to decreased food intake than to lack of riboflavin.

After initial infection with heavy doses of *Nippostrongylus muris*, two of ten rats on riboflavin-free diets died within 12 days; there were no deaths among controls (Watt, 1944). After animals with relative immunity from previous infection were fed a single dose of larvae, an average of 60% of worms was recovered from six deficient animals, compared with less than 6% from controls sacrificed after 12 days. Furthermore, the plasma from hyperimmunized deficient animals was not protective, but that from hyperimmune controls was.

In contrast to thiamine deficiency, riboflavin deficiency has been reported to be associated with antagonism in only one study, but the possibility has been little explored. The general conclusion is that riboflavin deficiency is likely to aggravate many infectious diseases.

**Niacin**

Table 11 lists the few known studies on niacin deficiency and resistance to infection.

Niacin deficiency sufficient to induce black tongue increased the susceptibility of dogs to *Fusobacterium fusiformis* (Smith et al., 1937). Sporn and co-workers (1950) failed to observe any effect on deaths among rats fed infective doses of *Shigella dysenteriae*. Larsh (1952), however, reported a greater incidence of amebiasis among dogs receiving stock diets than among animals given a black-tongue-producing ration.

Niacin deficiency increased the intensity of parasitemia in chickens infected with *Plasmodium lophurae*, but with no prolonged course of the disease (Roos et al., 1946). Similar studies of ducks by the same investigators showed no effect, a finding attributed to the extreme virulence of *Plasmodium lophurae* for this species.

To summarize, little work has been done on interactions between niacin deficiency and infection. The evidence that does exist suggests that niacin
deficiency has less influence on infection than any of the nutrients thus far discussed.

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusobacterium fusiformis</em></td>
<td>Dog</td>
<td>Synergistic</td>
<td>Smith et al. (1937)</td>
<td>Infection spontaneously present in deficient animals with &quot;black tongue&quot;</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Rat</td>
<td>No effect</td>
<td>Sporn et al. (1950)</td>
<td>No increased fatality in deficient animals following oral administration</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plasmodium lophurae</em></td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Roos et al. (1946)</td>
<td>Parasitemia greatly exaggerated in deficient animals but duration of infection not affected</td>
</tr>
<tr>
<td><em>P. lophurae</em></td>
<td>Duck</td>
<td>No effect</td>
<td>Roos et al. (1946)</td>
<td>Infection was too virulent for effect to be manifest</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Dog</td>
<td>Synergistic</td>
<td>Larsh (1952)</td>
<td>When fed cysts, twice as many dogs on a black-tongue-producing diet showed positive rectal swabs at 17 weeks</td>
</tr>
</tbody>
</table>

**Pyridoxine**

The numerous studies on pyridoxine deficiency and infection are listed in Table 12. In investigations of several bacterial infections of rats—including *Diplococcus pneumoniae* (Robinson & Siegel, 1944), *Shigella dysenteriae* (Sporn et al., 1950), and *Corynebacterium kutscheri* (Serdonde et al., 1956)—pyridoxine deficiency had no effect on the resulting severity of the disease. Among pyridoxine-deficient guinea-pigs infected with *Mycobacterium tuberculosis*, gross lesions were less severe, even though the skin reaction to tuberculin was depressed (Axelrod et al., 1963).

For viral and systemic protozoal infections, synergism and antagonism are almost equally frequent. Growth of influenza and mumps viruses in the chick embryo was inhibited by administration of deoxypyridoxine, which produced a deficiency by blocking the action of pyridoxine (Cushing & Morgan, 1952). Leftwich & Mirick (1949), in studies of viral pneumonia of mice, found that an acute deficiency of pyridoxine led to less severe lesions and a lower fatality in laboratory animals, whereas chronic deficiency (Mirick & Leftwich, 1949) gave a progressively increased susceptibility.

Bodian (1948) encountered what appeared to be a naturally acquired poliomyelitis infection in a pyridoxine-deficient monkey. The reaction was measurably more severe than that in monkeys in good nutritional state.
### TABLE 12. EFFECTS OF PYRIDOXINE DEFICIENCY ON INFECTIOUS DISEASE

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Rat</td>
<td>No effect</td>
<td>Suronie et al. (1956)</td>
<td>Slight loss of natural resistance to this normally occurring organism</td>
</tr>
<tr>
<td><em>Diplococcus pneumoniae</em></td>
<td>Rat</td>
<td>No effect</td>
<td>Robinson &amp; Siegel (1944)</td>
<td>No effect on resistance after intratracheal inoculation</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Rat</td>
<td>No effect</td>
<td>Sporn et al. (1950)</td>
<td>No increase in deaths of deficient animals following oral administration</td>
</tr>
<tr>
<td><em>Myxobacterium tuberculosis</em></td>
<td>Guinea-pig</td>
<td>Antagonistic</td>
<td>Axelrod et al. (1963)</td>
<td>Gross tubercular lesions less in deficient animals</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural respiratory infections</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Yeager &amp; Miller (1960)</td>
<td>More frequent in deficient animals than in <em>ad libitum</em> or pair-fed controls</td>
</tr>
<tr>
<td><em>Mouse pneumonia virus (PVM)</em></td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Lefkovich &amp; Mairick (1949)</td>
<td>Acute deficiency after inoculation consistently decreased severity of lesions and number of deaths</td>
</tr>
<tr>
<td><em>Mouse pneumonia virus (PVM)</em></td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Mirick &amp; Lefkovich (1949)</td>
<td>Increase in susceptibility roughly proportional to amount of time animals were fed deficient diet before inoculation</td>
</tr>
<tr>
<td><em>Mouse encephalomyelitis (Thiel)</em></td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichstein et al. (1945)</td>
<td>No consistent difference in susceptibility to intracerebral inoculation between deficient and well-fed animals</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td>Rat</td>
<td>Synergistic</td>
<td>Yeager &amp; Miller (1960)</td>
<td>With pyridoxine-free diet, animals more susceptible to infection; higher, more persistent parasitemia and greater degree of myocarditis than pair-fed control rats. Deficiency counteracted normal increase in resistance with age</td>
</tr>
<tr>
<td><em>Eimeria nieschulzi</em></td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Becker &amp; Dilworth (1941)</td>
<td>Pyridoxine administration to deficient animals significantly increased oocyst yield</td>
</tr>
<tr>
<td><em>Plasmodium berghei</em></td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Ramakrishnan (1954)</td>
<td>Multiplication of parasites markedly less in deficient animals</td>
</tr>
<tr>
<td><em>Leishmania donovani</em></td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Actor (1960)</td>
<td>Pyridoxine deficiency induced by diet or antagonists or both produced marked increase in</td>
</tr>
</tbody>
</table>
inoculated intracerebrally with the virus. Experimental infection was obtained in 2 out of 11 monkeys. Lichstein and co-workers (1945) were unable to demonstrate any effect of pyridoxine deficiency on the resistance of mice to Thieier encephalomyelitis virus.

Rats on a pyridoxine-free diet were more susceptible to *Trypanosoma cruzi* than were pair-fed controls (Yaeger & Miller, 1960d). Parasitemia persisted longer, myocarditis was more severe, and the expected increase in resistance with age was inhibited. Parasitemia of *Plasmodium berghei* infection was decreased in pyridoxine-deficient rats (Ramakrishnan, 1954). There were higher oocyst counts in deficient animals infected with *Eimeria nieschulzi* than in a control group given the same diet supplemented with pyridoxine (Becker & Dilworth, 1941). Deficiency did not affect *Giardia muris* infection in the mouse (Scholtens, 1962).

With *Leishmania donovani* infection in mice, a strong synergistic effect was evident at 48 days (Actor, 1960). This occurred even when the deficiency, induced by limiting dietary intake and administering deoxyribo-

In summary, no consistent pattern has been observed in the interaction of pyridoxine deficiency and infectious disease. No effect was observed in two studies involving bacterial diseases, and antagonism was observed in a third. Synergism and antagonism are almost equally frequent in systemic viral and protozoal diseases.

**Pantothenic acid**

Despite uncertainty about its natural occurrence in man, pantothenate deficiency and its effect on resistance to infection have been studied extensively in animals, as evidenced by the investigations summarized in Table 13. A significant synergistic effect was noted in most instances.

Pantothenic acid deficiency was produced in two strains of rats. Both groups were infected with a corynebacterium not normally an infectious agent. A fatal infection followed in one instance and serious disease in the

---

**Table 12 (continued)**

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania donovani</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia muris</em></td>
<td>Mouse</td>
<td>No effect</td>
<td>Scholtens</td>
<td>Same number of trophozoites in deficient animals compared with controls</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1962)</td>
<td>given same diet plus pyridoxine or laboratory chow</td>
</tr>
</tbody>
</table>

number of leishmania at 48 days even if deficiency started at 24 days; both *ad libitum* and pair-fed controls
### Table 13. Effects of Pantothenate Deficiency on Infectious Disease

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>Rat</td>
<td>Synergistic</td>
<td>Seronde (1954); Seronde et al. (1955, 1956); Zucker et al. (1955)</td>
<td>Fatal infection in one strain of deficient animals; less effect in another strain; no serious infection in controls. Susceptibility increases in both strains as animals remain longer on deficient diet. Young rats died sooner. Survival time extended to that of adults by very small supplement of pantothenate.</td>
</tr>
<tr>
<td><em>Diplococcus pneumoniae</em></td>
<td>Mouse</td>
<td>No effect</td>
<td>Day &amp; McClung (1945)</td>
<td>No significant increase in fatality in animals injected intraperitoneally 7-38 days after restriction to experimental diet.</td>
</tr>
<tr>
<td><em>D, pneumoniae</em></td>
<td>Rat</td>
<td>Antagonistic</td>
<td>West et al. (1943-44)</td>
<td>Pantothenic-acid-deficient animals more resistant to nebulization of agent.</td>
</tr>
<tr>
<td><em>D, pneumoniae</em></td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Robinson &amp; Siegel (1944)</td>
<td>No effect on resistance to intratracheally introduced pneumococci.</td>
</tr>
<tr>
<td>Natural respiratory disease</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Yaeger &amp; Millers (1960c)</td>
<td>Increased susceptibility to spontaneous respiratory disease in deficient animals as noted at necropsy.</td>
</tr>
<tr>
<td><strong>Rickettsia</strong></td>
<td>Man</td>
<td>Synergistic</td>
<td>Bean et al. (1954)</td>
<td>Increased frequency during 35-day period of experimental deficiency in 4 subjects; eliminated by return to a good diet.</td>
</tr>
<tr>
<td><em>Rickettsia prowazekii</em></td>
<td>Rat</td>
<td>Synergistic</td>
<td>Fitzpatrick (1948)</td>
<td>50% fatality with diet very low in pantothenic acid; no fatality in controls.</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Lichstein et al. (1944)</td>
<td>Less paralysis in deficient animals.</td>
</tr>
<tr>
<td>Poliovirus, type 2</td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichstein et al. (1944)</td>
<td>Same amount of paralysis in deficient and well-nourished animals.</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plasmodium gallinaeum</em></td>
<td>Chicken</td>
<td>Antagonistic</td>
<td>Brackett et al. (1946)</td>
<td>Deficiency suppressed blood-induced infections, but not those induced by sporozoites.</td>
</tr>
<tr>
<td><em>P. gallinaeum</em></td>
<td>Aedea aegypti</td>
<td>Synergistic</td>
<td>Tenzer et al. (1953)</td>
<td>Calcium pantothenate added to sugar decreased number of oocysts.</td>
</tr>
<tr>
<td><em>P. gallinaeum</em></td>
<td>Chicken</td>
<td>Antagonistic</td>
<td>Huff et al. (1958)</td>
<td>Average number of merozoites per schizont less in deficient birds.</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>Turkey</td>
<td>Antagonistic</td>
<td>Huff et al. (1958)</td>
<td>Average number of merozoites per schizont less in deficient birds.</td>
</tr>
<tr>
<td><em>P. lophiurae</em></td>
<td>Chicken</td>
<td>Antagonistic</td>
<td>Trager (1943a,b)</td>
<td>Extent of parasitemia of severely deficient animals was 50% less than in controls.</td>
</tr>
</tbody>
</table>
other (Soronde, 1954; Soronde et al., 1955, 1956). Two studies (Day & McClung, 1945; Robinson & Siegel, 1944) of a diet producing symptoms of deficiency did not appear to affect resistance of rats to pneumococcal infection. On the other hand, Yaeger & Miller (1960c) found at necropsy more spontaneous respiratory and pulmonary infections of undetermined origin in pantothenate-deficient rats than in well-fed controls. Pantothenic-acid-deficient rats proved resistant to nasal insufflation of *Diplococcus pneumoniae*, whereas three of seven animals on a control diet succumbed (West et al., 1943-44). The authors suggested that sulfapyridine may be therapeutically effective in pneumonia by blocking pantothenate, a nutrient needed by the pneumococcus.

Bean & Hodges (1954) studied experimental pantothenic acid deficiency in man, induced by a deficient diet and additional feeding of the metabolic antagonist, omega-methyl pantothenic acid. Frequent spontaneous upper respiratory infections occurred, especially pharyngitis. These infections ended promptly when subjects were returned to a normal diet.

In extensive investigations of B vitamins, Lichstein and co-workers (1944) encountered less paralysis in pantothenate-deficient mice infected with mouse Thiefer encephalomyelitis virus than in controls, but noted no differences with poliovirus type 2.
The deficiency suppressed blood-induced *Plasmodium gallinaceum* infections in chickens, a result not observed when infection was sporozoite-induced (Brackett et al., 1946). Initially, deficiencies were produced by inadequate diets. Similar findings were obtained when the chickens were fed any one of several pantothenic acid analogues, as antagonists to the vitamin. One analogue was four times as effective as quinine in controlling the disease. In the opinion of the authors, either the exo-erythrocytic stages of the infection require less pantothenate or, under experimental conditions, more pantothenate is available in the tissues than in the bloodstream.

Previously, Trager (1943a, b, 1947a) had reported that *Plasmodium lophurae* infection in chickens or *Plasmodium cathemerium* in ducks produced less than half as much parasitemia in pantothenate-deficient animals as in controls. Huff and associates (1958) described similar findings with *Plasmodium gallinaceum* in chickens and *Plasmodium fallex* in turkeys, with reduction in numbers of merozoites per schizont. On the other hand, addition of pantothenic acid to a glucose diet decreased the oocyst count in *Aedes aegypti* infected with *Plasmodium gallinaceum* (Terzian et al., 1955). Dependence of malaria parasites on pantothenic acid was shown even more clearly by in vitro studies. McKee (1951) found that a deficiency of pantothenic acid in culture media depressed multiplication of *P. knowlesi*. Trager (1954) has reported that, although pantothenic acid aids survival of *Plasmodium lophurae* in cells, it does not permit extracellular survival in vitro, where the more complex coenzyme A is needed for growth.

Pantothenate deficiency in rats inhibited elimination of *Eimeria nieschulzi* oocysts, but resulted in a significantly greater density of parasites in the blood (Becker & Smith, 1941-42). Since heat was used to destroy dietary pantothenic acid, other B-complex vitamins may also have been reduced. However, adding pantothenate to the diet was alone sufficient to restore normal resistance, and resulted in sparse parasite counts in the blood. Thiamine and pyridoxine supplementation had no effect. With pantothenate in the diet, oocyst elimination in the stools increased.

By contrast, greater parasitemia and longer duration of infection were noted in pantothenate-deficient rats infected with *T. leucici* (Becker et al., 1946-47) and *T. cruzi* (Yaeger & Miller, 1960c)—a synergistic effect. The cardiac lesions caused by *T. cruzi* were also more extensive.

An interesting shift from antagonism to synergism was observed in *Leishmania donovani* infection in mice (Actor, 1960). By the fifteenth day on a pantothenic-acid-deficient diet, parasitemia had declined to between one-third and one-half that found in pair-fed controls. By the twenty-sixth day, the effect was reversed. In the only study of an intestinal protozoan, *Giardia muris*, fewer trophozoites were found in deficient rats than in those receiving either the experimental diet with supplemental pantothenic acid or ordinary laboratory chow (Scholtens, 1962).
In summary, pantothenic acid deficiency in laboratory animals can be said to be frequently antagonistic to systemic viral and protozoal infections and synergistic with most others.

**Folic acid and vitamin B₁₂**

No reports on bacterial infections and folic acid or vitamin B₁₂ deficiencies in man have been found. However, Table 14 lists numerous studies of experimental viral, protozoal, and nematode infections.

Folic acid deficiency, produced by a deficient diet or the feeding of a folic acid antagonist, greatly prolonged survival of mice inoculated with the virus of lymphocytic choriomeningitis (Haas et al., 1957b). In chickens it prevented tumor growth after infection with the Rous sarcoma virus (Little et al., 1948). Acute folic acid deficiency had no apparent effect on poliomyelitis in monkeys, but chronic deficiency was definitely antagonistic (Lichstein et al., 1946a). Deficiency was defined as acute when animals first showed loss of weight, and as chronic when the weight dropped to such an extent that small folic acid supplements had to be given to assure survival.

In tissue-culture studies, folic acid was found to be not essential to growth of the agent of psittacosis. On the contrary, the bedsonia itself synthesized folic acid in amounts sufficient to meet the needs of host cells (Bader & Morgan, 1961).

The parasitemia of malaria was increased in folic-acid-deficient chickens (Seeler & Ott, 1945a, 1946). McKee (1951) showed that folic acid deficiency accelerated the development of *P. knowlesi* infections in monkeys. This was confirmed when Ramakrishnan (1954) also observed higher levels of parasitemia in folic-acid-deficient animals than in controls. These findings suggest that folic acid deficiency had reduced host resistance to the parasites.

That malarial parasites require this vitamin has been demonstrated by Trager's observation (1958) that folic acid enhanced multiplication of *P. lophurae* grown *in vitro* with duck erythrocytes. In extracellular *in vitro* preparations, however, the more complex coenzyme form of folic acid was required to promote parasitic growth. *In vitro* growth of *P. falciparum* in human erythrocytes was also favored by adequate folic acid. When *Aedes aegypti* or *Anopheles quadrimaculatus* mosquitoes were fed a refined sugar diet and exposed to *Plasmodium gallinaceum* by allowing them to feed on an infected chicken, addition of folic acid to the diet of the chickens decreased resistance of the mosquitoes, as determined by gastric oocyst density (Terzian et al., 1953).

Sadun and co-workers (1949, 1950) studied effects of a purified diet, deficient in folic acid and containing minimal vitamin B₁₂, on *Ascaridia galli* in the chicken. Chicks were placed on one of three experimental diets at 2 days of age, fed 500 embryonated eggs at 9 days, and sacrificed after 32 days. Worms were considerably more numerous in animals on folic-
acid-deficient diets. Worms in animals on a diet containing minimal vitamin B<sub>12</sub> measured only 2 to 4 mm, whereas those in animals on a commercial starter feed measured more than 20 mm. Worms decreased in number,

| Table 14. Effects of Folic Acid and Vitamin B<sub>12</sub> Deficiency on Infectious Disease |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Agent or disease                                | Host            | Response        | Reference       | Remarks                      |
| Folic Acid                                      |                 |                 |                 |                              |
| Bacteria                                        |                 |                 |                 |                              |
| *Lactobacillus casei*                           | Man (infants)   | Synergistic     | Matoh et al. (1964) | 80% of infants with diarrhea had folic acid activity below 40 µg/ml. Infants with infection showed mean value of 29.6 µg/ml. Severe folic acid deficiency often precipitated by infection in and outside alimentary tract. |
| Virus                                           |                 |                 |                 |                              |
| Lymphocytic choriomeningitis                    | Mouse           | Antagonistic    | Haas et al. (1957) | Survival of intraperitoneally or subcutaneously inoculated animals was greatly prolonged by either folic-acid-deficient diet or feeding of folic acid antagonist. |
| Rous sarcoma virus                              | Chicken         | Antagonistic    | Little et al. (1946) | Tumor growth prevented entirely by synthetic diet free of folic acid or by feeding folic acid antagonists from time of inoculation. |
| Poliomyelitis                                   | Monkey          | Antagonistic    | Lichstein et al. (1946) | Following intranasal and intracerebral inoculation, increased resistance with chronic but not with acute folic acid deficiency. |
| Protozoa                                        |                 |                 |                 |                              |
| *Plasmodium falciparum*                         | Chicken         | Synergistic     | Seele & Ott (1945a, 1946) | Parasitemia increased in folic-acid-deficient animals. |
| *P. gallinaceum*                                | *Aedes aegypti* | Antagonistic    | Terzian et al. (1952) | Adding folic acid to sugar diet increased oocyst density in stomach. |
| *P. knowlesi*                                   | *Anopheles quadrimaculatus* | Monkey       | Synergistic     | McKee (1951) | Parasitemia increased in deficient animals. |
| *P. knowlesi*                                   | *Anopheles quadrimaculatus* | Monkey       | Synergistic     | Ramakrishnan (1954) | Parasitemia increased in deficient animals. |
| Helminths                                       |                 |                 |                 |                              |
| Nematodes                                       |                 |                 |                 |                              |
| *Ascaridia galli*                               | Chicken         | Synergistic     | Sadun et al. (1949, 1950) | More and larger worms with folic-acid-deficient diet. |
| *Ascaridia galli*                               | Chicken         | Antagonistic    | Sadun et al. (1949, 1950) | Fewer and smaller worms when minimal addition of vitamin B<sub>12</sub> made to diet. |
with no increase in size, when 15 or 20 µg of folic acid were added to each 100 g of a deficient basal diet. In a subsequent experiment, chicks were infected with 600 parasite eggs at 6 days of age and sacrificed at 26 days. Liver extract, added to the purified rations as a source of vitamin B₁₂, had the same effect as folic acid.

To summarize, although synergism does occur, antagonism is the more usual finding in published reports on folic acid deficiency and was also observed in the one study involving vitamin B₁₂ deficiency.

**Biotin, inositol and choline**

All studies of biotin deficiency have recognized some degree of synergism, as shown in Table 15. One inconclusive report on inositol deficiency and two involving choline are also listed in this table.

In rodents, biotin deficiency has been found to be strongly synergistic with experimental avian malaria and also with *Trypanosoma lewisi* infections. Whether deficiency was induced by diet alone or by the addition of raw egg white to block available biotin, the result was a consistently higher parasitemia in deficient animals (Trager, 1943b, 1947a; Caldwell & György, 1943, 1947; Seeler et al., 1944; Ramakrishnan, 1954; Yaeger & Miller, 1965).

Deficiency induced by a diet of raw egg white also resulted in more deaths among mice infected orally with *Salmonella typhimurium*, as compared with deaths among *ad libitum* and pair-fed controls (Kligler et al., 1946). On the other hand, Sporn and co-workers (1950) saw no effect of deficiency on the killing capacity of *Shigella dysenteriae* in rats. Lichstein and associates (1945) included biotin and inositol in an extensive study of the interaction of specific B-complex vitamin deficiency with Theiler encephalomyelitis and with poliomyelitis type 2 infections of mice, but no effects were observed. Ruebner and co-workers (1958) maintained mice on diets with and without adequate choline and observed equal fatality in both groups following intraperitoneal injection of mouse hepatitis virus.

Choline deficiency produced responses in *Plasmodium lophurae* infections of chickens and ducks so slight as to have no practical importance. In four experiments with chickens, parasitemia increased slightly in deficient animals, in two of them so little as to have no statistical significance. Response in deficient ducks was mildly antagonistic, since parasitemia declined slightly six days after inoculation (Roos et al., 1946).

To summarize, synergism between biotin deficiency and infection was observed in several studies in laboratory animals. Few studies have been designed to investigate specifically the effects of inositol and choline deficiencies, and the reported results are inconclusive. Some of these deficiencies occur spontaneously in man.
### Table 15: Effects of Biotin, Inositol and Choline Deficiencies on Infectious Disease

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biotin</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Kligler et al. (1946)</td>
<td>Biotin deficiency induced by diet of raw egg-white increased fatality rate after oral infection compared with ad libitum or pair-fed controls</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>Rat</td>
<td>No effect</td>
<td>Sporn et al. (1950)</td>
<td>No increase in fatality rate following infection by mouth</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poliovirus, type 2 Mouse encephalomyelitis (Thielier)</td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichtstein et al. (1945)</td>
<td>No consistent difference between susceptibility of deficient and well-fed animals to intracerebral inoculation</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. berghei</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Ramabrahman (1954)</td>
<td>Rats died earlier but showed smaller parasite densities when biotin deficient</td>
</tr>
<tr>
<td>P. lactofermentans (P. lophurae)</td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Soeler et al. (1944)</td>
<td>Chicks receiving commercial ration with 15% raw egg-white developed biotin deficiency and higher parasitemia compared to controls fed cooked egg white or crystalline egg white</td>
</tr>
<tr>
<td>P. catenatum</td>
<td>Duck</td>
<td>Synergistic</td>
<td>Trager (1943b, 1947)</td>
<td>60% to 100% increase in parasitemia in biotin-deficient chicks compared with controls. More rapid in P. lophurae. Slower initial effect in P. catenatum</td>
</tr>
<tr>
<td>Trypanosoma lewisi</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Caldwell &amp; Gyorgy (1943, 1947)</td>
<td>Mild, moderate, and severe deficiency all significantly increased parasitemia and mortality compared with controls</td>
</tr>
<tr>
<td>T. cruzi</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Yager &amp; Miller (1962)</td>
<td>Significantly higher parasitemia when clinical evidence of egg white induced biotin deficiency</td>
</tr>
<tr>
<td><strong>Inositol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse encephalomyelitis (Thielier) Poliovirus, type 2</td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichtstein et al. (1945)</td>
<td>No consistent difference between susceptibility of deficient and well-fed animals to intracerebral inoculation</td>
</tr>
<tr>
<td><strong>Choline</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mouse hepatitis</td>
<td>Mouse</td>
<td>No effect</td>
<td>Reubner et al. (1958)</td>
<td>No difference in fatality rate in 8 animals inoculated intraperitoneally, after 11 days on deficient diet, and in 8 infected controls</td>
</tr>
</tbody>
</table>
Para-aminobenzoic acid

Table 16 was compiled on the assumption that the reported effects of milk diets on malarial and trypanosomal infections in a variety of hosts are due to the low para-aminobenzoic acid (PABA) content of milk. The assumption may not hold for all observations cited, although the addition of PABA counteracted the antagonistic effect of a milk diet in all cases tested.

The many investigations of milk or other PABA-deficient diets and malaria, following the initial observations in monkeys (Geiman & McKee, 1948) and rats (Maegraith et al., 1952), were undertaken in the hope that the findings might have some application to the control of malaria in man. From 1952 to 1955, 14 reports appeared; significantly, no more studies are found in the literature after this brief interest. Nevertheless, the results are worth summarizing.

When PABA was rigidly excluded from maternal diets, suckling rats and monkeys were more resistant to malaria (Hawking, 1954), an effect that was abolished by adding PABA to the maternal diet. In both rats and mice, a milk or casein diet had an antagonistic effect on resistance (Maegraith et al., 1952; Refaat & Bray, 1953; Ramakrishnan et al., 1953). The general action of PABA in partially abolishing the antagonistic effect of milk diets was confirmed (Refaat & Bray, 1953).

Seasonal fluctuation in the action of cow’s milk in protecting rats against P. berghei (Maegraith, 1953) was consistent with the known seasonal variations in vitamin content of milk due to differences in feed. The effect of a milk diet in increasing resistance of mice to P. berghei was not sufficient to overcome the decreased immunity following splenectomy (Kretschmar, 1963).

Ramakrishnan reported milder Plasmodium berghei infections in rats on milk diets, but found the opposite effect in chickens infected with Plasmodium gallinaceum (Ramakrishnan et al., 1953). In addition to a more pronounced parasitemia after sporozoite-induced infection in birds on the milk diet, he
<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmodium gallinaceum</td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Remakrishnan et al. (1953)</td>
<td>Greater parasitemia followed sporozoite-induced infections in birds receiving milk diet</td>
</tr>
<tr>
<td>P. berghei</td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Hawking (1954)</td>
<td>With PABA stringently excluded from maternal diet, suckling rats were more resistant to inoculation; adding PABA to maternal diet abolished effect of milk diet</td>
</tr>
<tr>
<td>P. berghei</td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Maegraith et al. (1952)</td>
<td>Human or cow's milk diets almost completely suppressed infections fatal to controls (effect varied with season)</td>
</tr>
<tr>
<td>P. berghei</td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Ramakrishnan (1954)</td>
<td>Milder infections occurred with milk than with vegetarian diet; most severe with mast diet</td>
</tr>
<tr>
<td>P. knowlesi</td>
<td>Monkey</td>
<td>Antagonistic</td>
<td>Bray &amp; Garnham (1953)</td>
<td>Milk diet inhibited immature blood schizonts, but not pre-erythrocyte schizonts nor free merozoites; thereafter, exacerbation upon return to normal diet</td>
</tr>
<tr>
<td>P. cynomolgi</td>
<td>Monkey</td>
<td>Antagonistic</td>
<td>Gaiman &amp; McKe (1948)</td>
<td>Addition of PABA to starvation diet increased parasitemia</td>
</tr>
<tr>
<td>P. knowlesi</td>
<td>Monkey</td>
<td>Antagonistic</td>
<td>Hawking (1954)</td>
<td>Stringent exclusion of PABA from maternal diet made suckling monkey resistant to inoculation; adding PABA to maternal diet abolished effect</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>Man</td>
<td>No effect</td>
<td>Miller (1954)</td>
<td>Whole milk diet for 21 days as compared with controls with added PABA 8 to 50 days, in 9 African children, did not affect parasitemia</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>Man</td>
<td>No effect</td>
<td>Chaudhuri &amp; Dutta (1955)</td>
<td>Ten subjects were fed milk and sugar alone for 3 to 11 days with no effect on parasitemia. Unable to confirm previous report of antagonism by Chaudhuri &amp; Chakraverty (1953)</td>
</tr>
<tr>
<td>P. gallinaceum</td>
<td>Aedes aegypti</td>
<td>Synergistic</td>
<td>Tizard (1950), Tizard et al. (1952)</td>
<td>Deficiency induced by sulfonamide decreased resistance, and para-aminobenzoic acid restored it</td>
</tr>
<tr>
<td>P. berghei</td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Refaat &amp; Bray (1953)</td>
<td>Infection inhibited by pure milk diet, or by 2% casein diet; effect partially reversed by PABA supplement, more complete reversal with vitamin B-complex supplement</td>
</tr>
<tr>
<td>Trypanosoma rhodesiense</td>
<td>Rat</td>
<td>No effect</td>
<td>Refaat &amp; Bray (1953)</td>
<td>Infection so virulent as to overwhelm any possible nutritional effect</td>
</tr>
</tbody>
</table>
also observed a higher fatality ratio. This synergistic response, which is in contrast to the antagonism usually observed, remains unexplained. From work with monkeys, Bray & Garnham (1953) concluded that the adverse effect of a milk diet was mainly on immature blood schizonts, since the pre-erythrocyte schizonts and free merozoites remained in latent form until the animals returned to a normal diet.

A failure to obtain consistent results in studies on man is the chief reason for the rapid loss of interest in milk diets. In reporting preliminary results, Chaudhuri & Chakravarty (1953) claimed that adults were protected from *Plasmodium vivax* by diets of milk alone, but Chaudhuri & Dutta (1955) were unable to confirm this. Similarly, Miller (1954) compared *P. falciparum* and *P. malariae* in children fed whole milk diets for 21 days, with and without PABA, and failed to find a difference.

PABA deficiency may affect the parasite in the mosquito as well as in the animal host. In a complicated series of experiments, Terzian (1950) and Terzian and co-workers (1952) showed that sulfonamide-induced PABA deficiency increased oocyst density in the stomach of *Aedes aegypti* and *Anopheles quadrinaculatus* infected with *Plasmodium gallinaceum*. Administration of PABA nullified this effect, although excessive amounts of PABA again raised oocyst density. In these trials, mosquitos were fed refined sugar alone or sugar plus the test substance.

As would be expected, the effect of milk diets has also been tested on trypanosomes and other parasites. Keppie (1953) found a dramatic reduction in the severity of *T. congoense* infection in mice on diets containing 20% to 30% of casein as the sole source of protein. On normal diets or on a bread, milk, and oat diet, the infection followed its normal fatal course. Presence of milk in the latter diet indicated that dietary antagonism was not
due to a direct anti-parasitic agent in milk, but probably to a deficiency. Similar trials with *T. rhodesiense* produced a fulminating infection that overwhelmed any nutritional effect, whereas *T. congolense* in mice produced a chronic infection. Efforts to use milk diets to control *Babesia canis* infections in puppies also failed (Maegraith, 1953).

Certain bacteria are dependent on specific nutrients, such as PABA, which are not required by man. Bacon and co-workers (1951) observed that virulent strains of *S. typhi* were independent of PABA and certain purines for growth. Avirulent strains, however, failed to grow *in vivo* when these nutrients were lacking. They became virulent when the nutrients were combined with the infectious agent.

Egg production by *Hymenolepis nana* and *Syphacia obvelata* was stopped in rats by feeding them a milk diet (Becker, 1933). Abundant eggs of both species were produced before and after the experimental period, when the rats were consuming a grain diet.

To summarize, despite the great interest arising from initial reports that milk diets, presumably deficient in para-aminobenzoic acid, successfully inhibited systemic protozoal infections in laboratory animals, such diets appear to have no therapeutic value for man.

**Mineral Deficiencies**

Table 17 summarizes studies of the effect of selected specific mineral deficiencies on infection.

**Iron**

Experiments with helminthic infections (Porter, 1935) showed that introduction of 300 larvae of the hookworm *Nippostrongylus muris* into rats resulted in recovery of much larger numbers of parasites from animals on a powdered whole milk diet, deficient in iron, than from animals on a stock diet. The longer the period of iron deficiency, the more marked was the difference. Addition of ferric citrate and copper sulfate to the milk greatly reduced the number of worms recovered, although not to the levels in control animals. In extensive studies, Foster (1936) produced the same effect in dogs and cats, either by a whole milk diet or by repeated bleeding; infection was induced by feeding *Ankylostoma caninum* to the animals biweekly.

It is surprising that no reports were found of clinical studies designed to measure the results, in terms of numbers of hookworms in the intestine, that follow correction of iron deficiency anemia. Cort & Otto (1940) and Otto (1965) reviewed in detail the evidence from animal studies, from which they concluded that nutritional deficiency must also be a determinant of the disease in man. Most well-nourished persons presumably have enough
### TABLE 17. EFFECTS OF MINERAL DEFICIENCIES ON INFECTIOUS DISEASE

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron</strong></td>
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</tr>
<tr>
<td><em>Nippostrongylus muir</em></td>
<td>Rat</td>
<td>Synergistic</td>
<td>Porter (1939)</td>
<td>Many more worms with diet of whole milk; addition of ferric citrate and copper sulfate resulted in fewer worms, although still more than in control animals</td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em></td>
<td>Dog</td>
<td>Synergistic</td>
<td>Foster (1936)</td>
<td>Resistant dogs made more susceptible to infection by biweekly feeding of larvae after repeated breeding of whole milk diet</td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em></td>
<td>Cat</td>
<td>Synergistic</td>
<td>Foster (1936)</td>
<td>Effect in cats fed same diet similar to that in dogs</td>
</tr>
<tr>
<td><strong>Phosphorus - Calcium</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Heterakis gallinae</em></td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Ciglham (1934a)</td>
<td>Diets low in calcium and phosphorus stopped growth and mineralization of bones and markedly decreased numbers of worms determined 24 days after 200 infective eggs fed</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Mouse encephalomyelitis (Theiler)</em></td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichstein et al. (1946b)</td>
<td>Resistance of animals not affected by calcium deficiency</td>
</tr>
<tr>
<td><em>Poliovirus, type 2</em></td>
<td>Mouse</td>
<td>No effect</td>
<td>Foster et al. (1949)</td>
<td>No clear effect of low calcium when mice fed five dietary combinations of phosphorus and calcium with and without vitamin D</td>
</tr>
<tr>
<td><strong>Helminths</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Ascaridia gelli</em></td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Gasfar &amp; Ackert (1953)</td>
<td>When fed 200 eggs at 15 days of age, many more and larger worms in group receiving low calcium diet as compared with controls</td>
</tr>
<tr>
<td><strong>Phosphorus</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Mouse encephalomyelitis (Theiler)</em></td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Lichstein et al. (1946b)</td>
<td>Fewer mice paralysed &amp; to 15 days after inoculation when receiving 5%, 15%, or 30% of optimum phosphorus, compared with controls</td>
</tr>
<tr>
<td><em>Poliovirus, type 2</em></td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Foster et al. (1949)</td>
<td>Five combinations of phosphorus and calcium levels, each with and without vitamin D; low phosphorus sharply increased mortality</td>
</tr>
<tr>
<td><strong>Helminths</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Ascaridia gelli</em></td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Gasfar &amp; Ackert (1953)</td>
<td>When fed 200 eggs at 15 days of age, many more and larger worms in group receiving low phosphorus</td>
</tr>
<tr>
<td>Agent or disease</td>
<td>Host</td>
<td>Response</td>
<td>Reference</td>
<td>Remarks</td>
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</tr>
<tr>
<td><strong>Potassium</strong></td>
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</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Rat</td>
<td>Synergistic</td>
<td>Woods et al. (1961)</td>
<td>After 2 weeks of potassium deficiency, rats injected with <em>E. coli</em> had a much higher frequency of kidney abscesses and pyelonephritis than controls similarly treated</td>
</tr>
<tr>
<td>Virus</td>
<td></td>
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</tr>
<tr>
<td>Poliovirus, type 2</td>
<td>Mouse</td>
<td>No effect</td>
<td>Jones et al. (1947)</td>
<td>No effect of deficiency on paralysis or fatality rate in inoculated animals</td>
</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Lichstein et al. (1946b)</td>
<td>Lower incidence of paralysis 8 to 12 days after inoculation in animals with decreased potassium intake</td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
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</tr>
<tr>
<td>Virus</td>
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<td></td>
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</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Lichstein et al. (1946b)</td>
<td>Fewer mice receiving sodium-deficient diet were paralyzed at 7 to 15 days after inoculation</td>
</tr>
<tr>
<td><strong>Chloride</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichstein et al. (1946b)</td>
<td>Chloride-deficient diet had no effect on percentage of animals paralyzed</td>
</tr>
<tr>
<td><strong>Magnesium</strong></td>
<td></td>
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<tr>
<td>Virus</td>
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<td></td>
</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>No effect</td>
<td>Lichstein et al. (1946b)</td>
<td>Deficient diet had no effect on percentage of animals paralyzed</td>
</tr>
<tr>
<td><strong>Manganese</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diplococcus pneumoniae</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Hitchings et al. (1949), Hitchings &amp; Falco (1946a,b)</td>
<td>With minimal infective doses, susceptibility decreased as manganese content of a highly purified diet was reduced</td>
</tr>
<tr>
<td>D. pneumoniae</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Dworscky (1949)</td>
<td>Infected animals survived longer on white bread than on whole wheat diet; effect subsequently attributed by Hitchings et al. (1949) to manganese deficiency in white bread</td>
</tr>
<tr>
<td>Helminths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris galli</td>
<td>Chicken</td>
<td>Antagonistic</td>
<td>Gaffar &amp; Acker (1953)</td>
<td>Birds fed 200 eggs when 15 days old had fewer and smaller worms if diet low in manganese</td>
</tr>
</tbody>
</table>
### TABLE 17 (continued)

<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Selenium</em></td>
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</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Mouse</td>
<td>Mixed</td>
<td>DeWitt (1927)</td>
<td>Fewer pathologic changes; worms smaller and did not mature sexually even though more of them</td>
</tr>
<tr>
<td><em>Cobalt</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemonchus contortus</em></td>
<td>Sheep</td>
<td>Synergistic</td>
<td>Todd &amp; Gracey (1958)</td>
<td>Multiple worms; burden less when cobalt given to lambs grazing in cobalt-deficient area</td>
</tr>
</tbody>
</table>

immunity to avoid the clinical symptoms that appear in undernourished persons. Other authors have also expressed this viewpoint, but without presenting evidence in its support (Payne & Payne, 1940; Andrews, 1942; Scott, 1946). The need for clinical studies is apparent.

In a study of 603 infants fed a proprietary baby formula containing vitamins and 12 mg of iron per quart, the incidence of respiratory infections was approximately half that for a control group of 445 fed an evaporated milk formula with supplemental vitamins but no additional iron (Andelman & Sered, 1966).

### Calcium and phosphorus

A deficiency of calcium and phosphorus sufficient to stop growth and limit mineralization of bones resulted in markedly decreased numbers of *Heterakis gallinarum* in chickens (Clapham, 1934a). Chickens fed viable eggs of *Ascaridia galli* had larger and more numerous worms when their diets were deficient in calcium or phosphorus than when they were on a stock diet (Gaafar & Ackert, 1953). Other studies of calcium deficiency and resistance to infection are reported by Lichstein and co-workers (1946b), who used the Theliger mouse encephalomyelitis virus, and by Foster and associates (1949), who used poliovirus type 2, mice being the laboratory animals in both instances. No significant effect was observed.

Zucker (1965) described a positive correlation between calcium concentration in the rations and fatality from cecal coccidiosis in either naturally infected or inoculated chicks. When the cause of the relationship was investigated further (Zucker et al., 1967), the effect was found to be limited to the phase of excystation. A diet extremely low in calcium (40 ppm) fed
a few hours before and after inoculation with the oocysts prevented the development of clinical symptoms of coccidiosis. The authors conclude that the pronounced effect of low dietary calcium on coccidiosis of chicks is solely due to the activating effect of calcium ions on intestinal trypsin activity.

Among experiments with various levels of dietary phosphorus and calcium, with and without added vitamin D, Foster and associates (1949) noted a sharply increased fatality in mice infected with poliovirus and subsisting on a low-phosphorus diet. However, Lichstein and co-workers (1946b) observed a progressively lower incidence of paralysis in mice with the Theiler encephalomyelitis virus as dietary levels of potassium, phosphorus, or sodium were lowered. Deficiencies of magnesium or chloride had no effect.

**Potassium**

Jones and associates (1947) detected no effect of potassium deficiency on resistance to infection with poliovirus type 2. Two weeks of deficiency were sufficient to induce a much higher frequency of pyelonephritis and kidney abscesses in rats receiving approximately 100 million *Escherichia coli* parenterally. The coexisting deficiencies of sodium, phosphorus, and chloride in the basic diet were not judged responsible for these results, but rather, a tubular obstruction or intestinal hydronephrosis (Woods et al., 1961).

**Manganese**

Hitchings & Falco (1946a,b) reported an undetermined factor that inhibited development of *Diplococcus pneumoniae* in mice on a wheat diet; it was subsequently identified as a manganese deficiency, incident to a highly purified diet (Hitchings et al., 1949). Dworetzky (1949) found that mice infected with *Diplococcus pneumoniae* survived longer when their diet contained white bread in place of whole wheat bread, although ultimately all animals succumbed to the infection. On the basis of further findings, Hitchings and associates (1949) concluded that an absence of manganese was probably responsible.

**Selenium**

In a previous review (Scrimshaw et al., 1959), DeWitt's studies (1957a,b) with *Schistosoma mansoni* infection in mice deficient in “Factor 3” were interpreted as expressing synergism because of the increased number of worms. The author, however, considered his results indicative of antagonism, since the worms did not reach normal size nor mature sexually to the
point where they could produce eggs; hence they were less pathogenic. "Factor 3" was subsequently identified as selenium (Schwarz & Foltz, 1957) inasmuch as addition of this mineral to the diet restored normal susceptibility (DeWitt 1957a,b).

Cobalt

Heavy multiple nematode infections, including *Haemonchus*, *Ostertagia*, *Necator battus*, *N. follicollis*, *Trichostrongylus*, and *Cooperia* were found characteristic of lambs grazing in a cobalt-deficient area in Northern Ireland. Addition of cobalt to their diet resulted in a significant decrease in the mixed worm burden (Todd & Gracey, 1958).

To summarize, a single general statement about the effect of mineral deficiencies is impossible. The few studies made have given variable results, sometimes showing synergism and sometimes, antagonism. Nevertheless, the response to infection elicited by mineral deficiencies cannot be dismissed as either rare or unimportant.

Unknown or Obscure Factors

Altered resistance to infection has many times been attributed to dietary changes, with no clear indication of the specific nutrient or nutrients involved (Table 18). Some studies are sufficiently described and controlled to permit their provisional assignment to one or more nutritional categories. Others, of necessity, are disregarded because of extreme weakness in design and presentation.

Valuable research ideas often emerge from recognition of unexplained effects. A recent example is the realization that the pathologic changes once considered characteristic of cholera cannot be differentiated from those of malnourished patients dying of non-specific acute diarrheal disease (Gangarosa et al., 1960). However, no atrophy of intestinal mucosa or other evidence of malnutrition or malabsorption was observed by Dammin and co-workers (1965) in five fatal cases of cholera recently studied in Dacca.

Nevertheless, there is increasing support for the view that the occurrence and severity of clinical cholera in epidemic form depend not only upon contact with *Vibrio cholerae*, but also on the nutritional state of the human host. Thus far, efforts of a United States Naval Research group to implicate specific vitamin deficiencies have been unsuccessful (Beisel, personal communication, 1963). Significantly, the commonest deficiency, a relative lack of protein, has yet to be investigated. Vitamin B₁₂ deficiency, known to occur in persons consuming strictly vegetarian diets and to result in achlorhydria, also warrants consideration.
### Table 18. Effects of Unknown or Obscure Dietary Factors on Infectious Disease

<table>
<thead>
<tr>
<th>Agent or Disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Schneider &amp; Webster (1945)</td>
<td>A partially characterized &quot;Salmonella resistance factor&quot; (SRF) found in some foodstuffs has the potential of converting virulent salmonella to phenotypic avirulence; acts independently of deficiencies of other nutrients</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Schneider (1946, 1956), Colburn et al. (1962)</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Guinea-pig</td>
<td>Synergistic</td>
<td>Neib &amp; O'Dell (1963)</td>
<td>A factor in both raw and cooked cabbage reduced fatality in inoculated guinea-pigs fed a diet of 30% casein, sucrose, and mineral mixture. Deaths further reduced by improvement of the mineral mixture</td>
</tr>
<tr>
<td>S. typhosa endotoxin</td>
<td>Guinea-pig</td>
<td>No effect</td>
<td>Neib &amp; O'Dell (1963)</td>
<td>Above cabbage supplement had no effect on antibody levels or fatality</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-and-mouth disease</td>
<td>Guinea-pig</td>
<td>Antagonistic</td>
<td>Oltisky et al. (1929)</td>
<td>Malnourished animals more resistant to inoculation with virus, as evidenced by delayed appearance of primary and secondary vesicles</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>Dog</td>
<td>Synergistic</td>
<td>Kegy &amp; Faust (1930-31), Faust &amp; Kegy (1934)</td>
<td>Salmon diet caused rapid progression of amebiasis; liver extract beneficial in suppressing diarrhea and healing colonic lesions (cf. next reference)</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>Dog</td>
<td>No effect</td>
<td>Artigas &amp; Beaver (1951)</td>
<td>Salmon diet caused chronic non-specific inflammation and diarrhea whether or not amebiasis present</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>Guinea-pig</td>
<td>Synergistic</td>
<td>Lynch (1957)</td>
<td>Synthetic diet containing gum arabic and essential minerals resulted in fulminating severe amebiasis</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>Guinea-pig</td>
<td>Synergistic</td>
<td>Taylor et al. (1952)</td>
<td>Growth less and infection more severe with modified guinea-pig diet (50% dried skim milk) than with rat-breeding diet (21% dried skim milk); both diets inadequate</td>
</tr>
<tr>
<td><strong>Helminths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichomonas muris</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Wagner &amp; Eskridge (1937)</td>
<td>Liver diet more favorable to Trichomonas than predominantly carbohydrate diet</td>
</tr>
<tr>
<td>Hymenolepis nana</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>DeWitt &amp; Weinan (1964)</td>
<td>Diets low in crude fiber reduced worm burden</td>
</tr>
</tbody>
</table>
The effects of certain types of diet on intestinal amebiasis have distinct interest. Thirty years ago, Faust and co-workers (1934-35) reported that induced amebiasis showed rapid progression in dogs fed canned salmon. The same effect was obtained with autoclaved liver. Fresh liver, however, led to rapid healing of cecal lesions (Kagy & Faust, 1930-31; Faust & Kagy, 1934). Two groups of investigators have since conducted similar experiments (Artigas & Beaver, 1961; Villarejos, 1962) and noted the same pathologic changes. Both reached the conclusion, however, that the mucoid diarrhea, dysentery, and mucosal changes represented a chronic non-specific inflammation related to the salmon diet, and that it occurred whether or not amebic infection was present.

Both guinea-pigs and rats were fed (1) a modified guinea-pig diet containing 50% of dried skim milk and (2) a rat breeder diet with 21% of dried whole milk. Amebae were injected intracecally after laparotomy (Taylor et al., 1952). Although both diets were nutritionally complete, growth of the animals was less and severity of the infection was greater with the modified guinea-pig diet. No explanation was given.

Lynch (1957) produced a fulminating experimental amebiasis in guinea-pigs by feeding them a synthetic diet of gum arabic and salts that, although nutritionally adequate, altered the intestinal flora. Even after massive doses of bacteria (designed to change the intestinal flora present in animals on the diet) were given, the result was the same. The diet alone resulted in thinning and vacuolation of the intestinal mucosa, which may have been a factor in the increased susceptibility. Animals given a standard commercial diet had only mild amebiasis.

Another unidentified dietary factor is suggested by the work of DeWitt & Weinstein (1964). Mice fed nutritionally complete, purified, low-residue or powdered skim milk diets eliminated the tapeworm Hymenolepis nana and the nematode worms Aspicularis tetraptera and Syphacia obvelata. Intestinal motility was greatly decreased by the purified diets. When motility was restored by the addition of 20% of cellulose to the diet, the moderating effect on the infection diminished. Similar but less marked results were observed with the round worm Nematospiroides dubius. Worms were not completely eliminated, but worm burden and size and rate of egg deposition by female worms were reduced appreciably in animals receiving the purified diet.

Lambs on either a pelleted semi-purified diet or a hay and concentrate mixture were fed 50 000 viable Haemonchus contortus larvae initially and an additional 70 000 five days later (Vetter et al., 1963). Fecal egg counts were much higher in animals on natural diets. Lambs fed the semi-purified diets had no signs of dietary deficiency except for poorer weight gain, although they had fewer worms and negligible production of eggs. Egg counts promptly increased when the animals were changed from a semi-purified to a natural diet. When they returned to the semi-purified diet, egg
counts again dropped. The factors responsible for these differences remain unidentified.

Why conditions in the cecum of rats become more favorable for *Trichomonas* when animals are fed a diet containing liver instead of a high-carbohydrate stock diet remains unclear (Hegner & Eskridge, 1937). Equally uncertain is why raw or cooked cabbage suffices to lower deaths significantly among guinea-pigs infected with *S. typhimurium* (Nabb & O'Dell, 1963).

A series of studies by Schneider and co-workers concerned a factor in whole wheat that is neither a protein nor a known vitamin. Rats receiving a synthetic diet containing all normally required nutrients were more resistant to *Salmonella enteritidis* when this factor was added to the ration (Schneider & Webster, 1945; Schneider, 1946b). It was subsequently named the Salmonella Resistance Factor (SRF), and found to be secreted by avirulent but not by virulent *Salmonella typhimurium* (Schneider & Colburn, 1960).

Apparently, this factor reduces pathogenicity by converting virulent organisms to phenotypic avirulence. The substance is also present in some foods in which Gram-negative saprophytes have grown. Abstracts reporting further work on isolation and chemical characterization of this factor indicate that it is an iron co-ordination compound of unknown composition (Colburn et al., 1962; Hill et al., 1962; Schneider & Colburn, 1963).

**Inanition**

Limitation of food intake may have an important influence on resistance to infection (Table 19). Most early experimental studies ignored a reduced food intake in animals consuming diets deficient in specific nutrients. Although enough experiments now include pair-fed controls to make certain that results are not wholly explained by inanition, the results of many studies nevertheless indicate an outcome greatly influenced by reduced food intake.

Both Klügler and associates (1945) and Guggenheim & Buechler (1946a; 1947) concluded that, in vitamin-A-deficient rats infected with *Salmonella typhimurium*, the apparent effect of the deficiency was actually due to reduced food intake. Similar results were observed in pair-fed controls. Lassen (1931) also had demonstrated earlier that restricted intake of a normal diet was sufficient to lower resistance of rats to *Salmonella*, a conclusion supported more recently by the results of Miller & Bohnhoff (1962). Seele & Ott (1944) demonstrated a similar synergism in studies on chickens fed a riboflav-in-deficient diet and infected with *Plasmodium lophurae*.

A synergistic effect may follow fasting in mice for periods as short as 24 to 48 hours either before or after infection with staphylococci or Kleb-
<table>
<thead>
<tr>
<th>Agent or disease</th>
<th>Host</th>
<th>Response</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Dubos (1955), Dubos et al. (1956)</td>
<td>Seventy of Klebsiella pneumonia increased by 24 to 48 hours of fasting before or immediately after inoculation</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>McGuire &amp; Floyd (1958)</td>
<td>Fasting lowered number of organisms required for parenteral LD50 dose and increased susceptibility to oral infection, despite higher oral LD50 — increase in number of fecal carriers, longer duration of intestinal infection, and greater incidence of Shigella bacteremia</td>
</tr>
<tr>
<td>Sh. flexneri</td>
<td>Guinea-pig</td>
<td>Synergistic</td>
<td>Formal et al. (1965)</td>
<td>After 4 days' starvation, inoculation with Sh. flexneri was fatal; probably effect of reduction in normal flora, which ordinarily provides protection</td>
</tr>
<tr>
<td>Anthrax</td>
<td>Pigeon</td>
<td>Synergistic</td>
<td>Corda (1923)</td>
<td>Starving birds susceptible; those receiving polished rice plus dried asparagus resistant</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Guinea-pig</td>
<td>No effect</td>
<td>Formal et al. (1958)</td>
<td>Resistance to Esch. coli well maintained after 4 days' starvation</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Rat, Mouse</td>
<td>Synergistic</td>
<td>Guggenheim &amp; Büchler (1947)</td>
<td>Caloric restriction to 60% and 80% of intake of controls greatly increased infection rate</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Kliger et al. (1945)</td>
<td>Markedly lowered resistance both in animals receiving adequate vitamin A and in pair-fed controls when both groups on low food intake</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Miller &amp; Bohr (1962)</td>
<td>Fatality rate increased by 24 hours' fasting before inoculation</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Rat</td>
<td>Synergistic</td>
<td>Lassen (1931)</td>
<td>Relative inanition from restricted intake of normal diet, up to 17 weeks, caused moderate, but significant, decrease in resistance</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>Guinea-pig, mouse</td>
<td>Synergistic</td>
<td>Burrows et al. (1947)</td>
<td>Fasting for 1-4 days plus opium to reduce peristalsis made normally resistant animals susceptible to Vibrio cholera</td>
</tr>
<tr>
<td>Staphylococcus (coagulase-positive)</td>
<td>Mouse</td>
<td>No effect</td>
<td>Smith &amp; Dubos (1956)</td>
<td>Partial food restriction sufficient to prevent weight gain did not alter resistance</td>
</tr>
<tr>
<td>Staphylococcus (coagulase-positive)</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Smith &amp; Dubos (1956)</td>
<td>Resistance lowered by acute fasting 36 to 48 hours before infection</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Boyer &amp; Lemensans (1961)</td>
<td>Resistance lowered by fasting</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>Pigeon</td>
<td>Synergistic</td>
<td>Corda (1923)</td>
<td>Unlike well-fed birds, those starved for 4 days died 2 days after infection</td>
</tr>
<tr>
<td>Agent or disease</td>
<td>Host</td>
<td>Response</td>
<td>Reference</td>
<td>Remarks</td>
</tr>
<tr>
<td>----------------------</td>
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</tr>
<tr>
<td>Rickettsia</td>
<td>Guinea-pig, rat</td>
<td>Synergistic</td>
<td>Pinkerton (1949)</td>
<td>More rickettsiae in peritoneal and auricular exudates of starved animals</td>
</tr>
<tr>
<td><em>Rickettsia prowazekii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus</td>
<td>Rabbit</td>
<td>Antagonistic</td>
<td>Sprunt (1942)</td>
<td>Three to 12 times as much virus required to infect fasted animals, and lesions were smaller (probably due to increased interstitial fluid)</td>
</tr>
<tr>
<td>Mouse encephalomyelitis (Theiler)</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Davies et al. (1949)</td>
<td>Diet low in calories or in total food intake reduced frank signs of paralysis</td>
</tr>
<tr>
<td>Poliovirus, type 2</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Foster et al. (1944a,b)</td>
<td>Paralysis and death less on restricted than unrestricted complete diet; maximum difference on 17th day</td>
</tr>
<tr>
<td>Poliovirus, type 2</td>
<td>Mouse</td>
<td>Antagonistic</td>
<td>Rasmussen et al. (1954)</td>
<td>Decreased food intake due to either thiamine deficiency or pair-feeding resulted in moderately increased resistance to infection</td>
</tr>
<tr>
<td>Poliovirus, type 2</td>
<td>Cotton rat</td>
<td>No effect</td>
<td>Weaver (1946, 1947)</td>
<td>Partial inanition did not alter susceptibility as judged by any of eight methods of infection</td>
</tr>
<tr>
<td>Canine distemper virus</td>
<td>Dog</td>
<td>Antagonistic</td>
<td>Miller et al. (1965)</td>
<td>Animals fed only 15 calories per kg per day more resistant than animals receiving 30 or 45 calories</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Rat</td>
<td>Antagonistic</td>
<td>Ramakrishnan (1954)</td>
<td>Five days' starvation reduced number of parasites; ten days' starvation almost eliminated parasitemia</td>
</tr>
<tr>
<td><em>Plasmodium berghei</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. jophurae</em></td>
<td>Chicken</td>
<td>Synergistic</td>
<td>Seeler &amp; Ott (1944)</td>
<td>Higher parasitemia at 0-13 days at half the food intake of ad libitum-fed controls</td>
</tr>
<tr>
<td><em>P. knowlesi</em></td>
<td>Monkey</td>
<td>Antagonistic</td>
<td>Gilmour &amp; Maskel (1948)</td>
<td>Starvation inhibited parasitemia</td>
</tr>
<tr>
<td><em>Plasmodium Sp.</em></td>
<td>Man</td>
<td>Antagonistic</td>
<td>Ramakrishnan (1954)</td>
<td>Parasitemia reported suppressed during Bengal famine of 1942, relapses when food supply improved</td>
</tr>
<tr>
<td><em>Trypanosoma duttoni</em></td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Shippe &amp; Adams (1957)</td>
<td>When infected with non-pathogenic trypanosomes, kept at low temperature, many partially starved mice died; no deaths among well-fed controls</td>
</tr>
<tr>
<td>Helminths</td>
<td>Mouse</td>
<td>Synergistic</td>
<td>Lash (1957b)</td>
<td>With reduced food intake, increase in cysticercoles corrected by multiple vitamins, even without increased food</td>
</tr>
</tbody>
</table>
siella pneumoniae (Dubos, 1955; Dubos et al., 1955; Smith & Dubos, 1956). Elimination of food for 36 to 48 hours before infection lowered the resistance of mice to coagulase-positive staphylococci; however, no further effect could be obtained from restriction beyond the point at which weight started to decline (Smith & Dubos, 1956). Boyer & Lamensans (1961) found that mice subjected to 30 hours of fasting before intravenous administration of Staphylococcus aureus were at least ten times more susceptible than were controls. Early reports (Corda, 1923) showed starving pigeons to be much more susceptible to anthrax than birds on a diet of polished rice and dried asparagus. Four days of starvation were sufficient to render guinea-pigs susceptible to fatal infection with Shigella flexneri, although resistance to Esch. coli was not altered (Formal et al., 1958). This result was mediated by alterations in the bacterial flora. Both starved guinea-pigs and starved rats became more susceptible to typhus fever, with increased concentrations of rickettsiae in the peritoneal and scrotal exudates (Pinkerton, 1949).

In studies of riboflavin-deficient rats infected with Trypanosoma cruzi, Yaeger & Miller (1960b) concluded that the slightly increased cardiac damage and parasitemia were due to reduced food intake rather than to riboflavin deficiency per se. Fasting increased the susceptibility of mice to Shigella flexneri (McGuire & Floyd, 1958) and Trypanosoma duttoni (Sheppe & Adams, 1957).

Larsh (1947b) observed that the increase in cysticeroids of Hymenolepis nana that accompanied reduced food intake in alcohol-debilitated mice was relieved by multiple vitamins even without increased food intake. McCance (1960) reported greater abdominal distention and a proportionately heavier mixed nematode burden in underfed swine than in control animals.

In contrast, 24 hours of starvation caused the disappearance of gravid proglottids of Raillietina cesticillus from chickens (Reid, 1942). Wong (1954) subjected chicks to fasting for 5, 10, and 15 hours after feeding them...
500 metacecarii, and then returned them to normal rations. Six days later, the starved animals averaged 62 to 108 flukes per chick, compared with 3.3 for chicks fed the basal oatmeal *ad libitum* diet and 0.5 in birds fed all-purpose chick feed. Birds fasting five hours before infection and then fed the oatmeal diet *ad libitum* averaged 30 worms at the end of six days. This study emphasizes the possible significance of even short periods of starvation at critical times in the course of an infection.

These examples of synergistic interaction indicate that reduced food intake should have consideration in any experimental or epidemiologic investigation in which malnutrition or starvation of the host may be involved.

Reduced food intake may be antagonistic to systemic diseases of viral or protozoal origin. Studies of vaccinia in the rabbit (Sprunt, 1942), Theiler encephalomyelitis (Davies et al., 1949), type 2 poliovirus (Foster et al., 1944b) and *Trypanosoma duttoni* in the mouse (Sheppe & Adams, 1957), distemper in the dog (Miller et al., 1965), and malaria in the monkey (Geiman & McKee, 1948) and rat (Ramakrishnan et al., 1953; Ramakrishnan, 1954) are examples of severe food restriction inhibiting an infection. Rasmussen and co-workers (1944b), in mice, and Weaver (1945, 1946), in cotton rats, noted no effect of inanition on susceptibility to poliovirus type 2. The animals were susceptible only to intracerebral inoculation; food restriction did not engender susceptibility as tested by eight other routes of infection.

Ramakrishnan (1954) reports the only instance in which a nutritional deficiency conceivably increased man’s resistance to infection. Parasitemia in malaria was stated to be suppressed in starving people during the Bengal famine of 1943. When the food supply improved, the frequency of relapse and death increased.

In summary, severe inanition is synergistic with most infections. It is antagonistic to some viral or intracellular protozoal diseases. There is a great need for experimental and field studies on the effect of restricted food intake on the resistance of man to infection.

**Carbohydrate and Fat**

In tropical regions, an excess incidence of microbial and parasitic disease is frequently coexistent with high-carbohydrate diets. There is little evidence of an interrelationship (Adolph, 1954). Only the study of Chandler and associates (1950) on *Hymenolepis diminuta* infection in rats directly concerned itself with the effect of dietary carbohydrate. When sucrose, corn starch, or glucose was the sole source of carbohydrate, worms were smallest in animals receiving sucrose and largest in those consuming corn starch.

The effect of dietary fat on mice injected with viable and non-viable BCG (Hedgecock, 1955, 1958) and chickens with avian tubercle bacilli (Solotorov-
sky et al., 1961) has also been investigated. Hedgecock (1958) found that the addition of fatty acid mixtures to a total of 5% of a synthetic diet regularly increased the survival times of mice, irrespective of varying amounts of the six individual fatty acids, so long as the total was about 5%. Solotorovsky and co-workers (1961) found that relatively severe challenging infections masked the dietary effect of fat in avian tuberculosis. With less severe infections, low dietary fat prolonged median survival time of chicks and led to a reduced number of tubercles per tissue section of spleen.

In contrast, diets high in fat were antagonistic to both Plasmodium berghei infection in rats (Ramakrishnan, 1954) and Entamoeba histolytica in monkeys (Gopalan, personal communication, 1961). It is also noteworthy that Plasmodium berghei and Trypanosoma congoense infections were suppressed in rats fed diets high in the unsaturated fatty acid fraction of cod liver oil. Since this effect was prevented by vitamin E, which has an antioxidant action, it was considered to be due to peroxides of the cod-liver-oil fraction acting on the sulfhydryl groups of protozoal enzyme systems (Godfrey, 1957). However, it is also possible that the vitamin E deficiency induced by the cod-liver-oil diet may have been responsible.

When monkeys maintained on diets rich in polyunsaturated fatty acids developed severe diarrhea, examination of the feces revealed both trophozoites and cysts of E. histolytica (India, Council of Medical Research, Nutrition Research Laboratories, 1965). It was concluded that the diet aggravated the effects of amebic infection in these monkeys.

A special atherogenic diet containing cholesterol, sodium cholate, and thiouracil definitely aggravated tuberculosis in rats after intravenous injection of a relatively avirulent human strain, H37RV. Severe fatty infiltration of the liver and lymphoid tissue (Costello et al., 1962) appeared after 60 days.

In view of the significant effects observed in all four experiments utilizing high-fat diets and excess carbohydrate, additional studies are obviously needed of the ways in which variations in the proportions and kinds of dietary energy sources affect the resistance of man to infection.

**Nutrient Excesses**

The many studies claiming enhancement of resistance from intake of vitamins far exceeding physiologic requirements need brief consideration. Such claims are the usual product of an unwarranted enthusiasm for some new method of therapy by persons unwilling or unable to conduct properly controlled studies by approved "double-blind" techniques. No attempt is made to assemble such reports nor to analyse the many opinions advanced. Generalizations will suffice, because in most instances they do not warrant individual consideration.

Such reports as "vitamin B-complex in combination with heavy doses of vitamin C seemed to abort colds in 24 to 48 hours" (Woolstone, 1954),
or "citrus flavonoids seem effective in treating upper respiratory infections in 23 patients, 7 to 70 years of age" (Biskind & Martin, 1954) require no serious regard. Other typical articles concern ascorbic acid, administered "sufficiently early" and "in large enough doses", and "found over years of private practice" to abort colds in 50% of cases (Markwell, 1947); or again, "one to two grams of ascorbic acid every two to four hours cleared up 60 cases of 'paralytic polio' in three to five days, eight cases of herpes zoster, six of viral encephalitis, five of influenza, four of chickenpox and three of herpes simplex" (Klenner, 1949). Neither introduction of controls nor statistical evaluation of the data complicated the experimental design of these investigations.

The amount of a specific nutrient required for growth cannot be assumed to be the same as that needed for maximum resistance to disease. Hill & Garren (1955) provided a clear-cut demonstration, in chicks fed Salmonella, that relatively high levels of all known required vitamins increased resistance. In order to be fully effective, some vitamins were required in amounts far exceeding those needed for maximum growth. The same authors also suggested that oversupplementation may decrease the effect. Theoretically, at least, too much vitamin A or D may increase susceptibility to infection, and will certainly provoke clinical signs and symptoms of an excess.

Excessive amounts of the water-soluble vitamins are well tolerated, although without benefit. For example, Squibb (1963), studying excessive vitamin supplementation in Newcastle disease of chicks, found that 30 times the required thiamine, riboflavin, pantothenic acid, niacin, pyridoxine, and biotin, and seven times the required balanced B-complex mixture were non-toxic and apparently did not affect weight gain of White Leghorn cockerels studied from birth to four weeks of age. With the exception of riboflavin, the vitamin additions were associated with more deaths among chicks infected with Newcastle disease, fatality being 3% to 13% higher than in non-supplemented birds with normal body reserves of these vitamins. In a replicated experiment employing two levels of virulence of Newcastle disease virus, riboflavin supplementation reduced the fatality by 10% and 17%. Antibody response, as determined by hemagglutinin inhibition titers, had no correlation with vitamin supplementation, growth of the chicks, or number of deaths.

Increased attention is currently given to imbalance of specific nutrients, such as amino acids, with particular emphasis on the extent to which excess consumption of certain nutrients markedly alters the requirement for others (Harper, 1957-58; Hsu, 1963). Excess methionine, through increasing the requirements for tryptophan to the point where a relative deficiency is created, is a particularly clear example. Gershoff and co-workers (1952) cited reduced susceptibility to infection after such an interaction, excess methionine proving antagonistic to type 2 poliovirus in mice. An antagonism between the virus and tryptophan deficiency had been demonstrated in an earlier
study. The methionine-induced tryptophan deficiency was further accen-
tuated by the analogue 6-methyl tryptophan.

Squibb (1964b) found that Newcastle disease in chicks responded
synergistically to both deficiency and surfeit of protein, the surfeit being
induced by diets containing 41% of casein. Two possible mechanisms were,
first, an amino acid imbalance caused by the metabolic use of protein to
meet energy needs, and, second, increased virus proliferation caused by the
extra protein. The first explanation was deemed the more reasonable.

Dogs made obese by being fed excess calories in amounts as high as 90 to
100 calories per kilogram of body-weight per day showed lowered resistance
to infection with distemper virus and poorer survival rates than dogs
maintained on 70 to 75 calories per kilogram or those fed a low-calorie diet
of 40 to 50 calories per kilogram of body-weight per day. Although the
mechanism of action is obscure, the results justify the conclusion that the
over-fed dogs had a greatly reduced resistance to infection with the distemper
virus (Newberne, 1966).

In summary, the preponderance of reports ascribing beneficial effects to
extreme doses of ascorbic acid, B-complex vitamins, and other specific
nutrients are, with few exceptions, the result of uncontrolled and uncritical
observations. In most instances, the cited claims are wholly invalid. There
is no convincing proof that an intake beyond the physiologic range favors
increased resistance to infection. Indeed, in some cases, excesses have the
opposite effect.

**Generalizations**

Certain patterns of behavior become apparent from the many studies here
reviewed and summarized. Table 20 provides a numerical summary of
responses so far as they may be determined within limits of available
knowledge. The cited interactions refer to individual reports in which a
specific host, agent, and nutritional deficiency were found to produce a
specific result. When several articles refer to the same interaction, a single
one has been selected for entry in the table. As in most fields, the reporting
of negative results has been casual, incomplete, and commonly incidental to
positive findings.

Synergism is the characteristic reaction with infectious agents such as
bacteria, rickettsiae, intestinal protozoa, and intestinal helminths. With
bacterial infections, over 150 instances of synergistic interactions are
recorded, and only 13 instances of antagonism. Five examples of antag-
onism among intestinal protozoa concern either *Giardia* or *Eimeria*. On
the other hand, antagonism is relatively common with viruses, which are
intracellular and highly dependent upon the metabolism of the host cell.
The systemic protozoa also frequently show antagonism. Although bed-
<table>
<thead>
<tr>
<th>Nutritional deficiency</th>
<th>Bacteria</th>
<th>Rickettsiae</th>
<th>Bedsonias</th>
<th>Viruses</th>
<th>Protozoa</th>
<th>Nematodes</th>
<th>Total</th>
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<td></td>
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<td>SAO</td>
<td>SAO</td>
<td>SAO</td>
<td>SAO</td>
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<td>7</td>
<td>10</td>
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<td>29</td>
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<td>—</td>
<td>3</td>
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<td>—</td>
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<td>1</td>
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<td>—</td>
<td>—</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Other B-complex</td>
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<td></td>
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<td>1</td>
<td>6</td>
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<tr>
<td>Inositol</td>
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<td>—</td>
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<tr>
<td>Choline</td>
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<td>Calcium</td>
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<td>—</td>
<td>2</td>
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<tr>
<td>Phosphorus</td>
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<td>—</td>
<td>—</td>
<td>2</td>
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<tr>
<td>Potassium</td>
<td>1</td>
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<td>—</td>
<td>1</td>
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<tr>
<td>Sodium</td>
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<td>—</td>
<td>—</td>
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<td>Chloride</td>
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<td>—</td>
<td>1</td>
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<td>Manganese</td>
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<td>—</td>
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<tr>
<td>Selenium</td>
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<td>—</td>
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<td>Cobalt</td>
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<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
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<td>Iron deficiency and starvation</td>
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<td>1</td>
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<td>Totals</td>
<td>158</td>
<td>13</td>
<td>23</td>
<td>11</td>
<td>1</td>
<td>29</td>
<td>28</td>
</tr>
</tbody>
</table>

* S = Synergy; A = antagonism; O = no effect.
soniae and rickettsiae are typically intracellular, only synergism has been reported between these organisms and the several nutritional deficiencies.

Patterns of interaction may also be defined in terms of type of nutritional deficiency. General inanition is synergistic with most infections, although antagonism has been found with some viruses and protozoa. Protein deficiency is usually synergistic but, again, with occasional instances of antagonism, especially with specific amino acid deficiencies. Vitamin A deficiency is regularly synergistic, whereas vitamin D deficiency frequently has no influence on infection. The consequences of vitamin deficiencies depend upon the particular host and agent, and collectively are responsible for a large proportion of known antagonistic responses. Thiamine, pyridoxine, pantothenate, and folic acid are particularly active in this regard. Lack of vitamin C, like lack of vitamin A, is almost always synergistic in its action. The effects of specific mineral deficiencies, as with those of the B-vitamins, depend upon agent, host, and experimental conditions; and antagonism is as common as synergism.

Severe deficiencies result in synergism— a consistent finding of major clinical and public health significance. Table 21 summarizes the studies of Tables 1 through 19 in which man is the host. An antagonism of clinical significance between nutritional deficiency and specific infection has not been demonstrated in man under either experimental or natural conditions. Furthermore, the harm caused by the more frequent bacterial infections resulting from synergistic effects would presumably exceed any possible benefit from antagonism.

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Number of studies</th>
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<tr>
<td></td>
<td>Bacteria and rickettsiae</td>
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<tr>
<td>Multiple</td>
<td>12</td>
</tr>
<tr>
<td>Protein</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>7</td>
</tr>
<tr>
<td>Vitamin D</td>
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<tr>
<td>Vitamin C</td>
<td>4</td>
</tr>
<tr>
<td>Thiamine</td>
<td>1</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>1</td>
</tr>
<tr>
<td>Folic acid and B₁₂</td>
<td>1</td>
</tr>
</tbody>
</table>

The addition of nutrients to an already adequate diet brings no benefit. In some instances an excess reduces resistance to infection or has other harmful effects.

The data support a general rule that moderate to severe nutritional deficiencies increase the seriousness of infectious disease in man.
CHAPTER 4

DETERMINANTS OF THE EFFECTS
OF NUTRITION ON INFECTION

Introduction

Nutritional factors can modify the characteristics of both host and infectious agent. An organism’s response to nutritional influences is conditioned by such innate host features as genetic make-up, age and physiologic state, and by the presence of a complicating illness.

The direct effect of malnutrition on host resistance is well documented. Nutritionally induced changes in antibody formation, phagocytic activity, tissue integrity, inflammatory response, intestinal flora, endocrine metabolism, and non-specific protective mechanisms have been studied intensively by investigators in a variety of fields. Their findings are reviewed below.

To understand better the antagonism sometimes existing between a specific nutrient deficiency and an infection, attention is also given to the metabolic requirements of infectious agents.

Innate Characteristics of Host and Agent

Host characteristics, including resistance to infection, are basically the result of genetic variation and environmental influence. The important consideration is that resistance is dependent on an interaction of the two, an interaction that may be recent or remote. In interpreting the nutritional influences on innate resistance, it is thus necessary to take into account the age, sex, and physiologic and pathologic states of a person.

The more that is learned of environmental effects, and especially diet, the more significant these influences appear in accounting for differences among people that were once considered wholly inborn. Resistance may be expected to be highest when a favorable inheritance is combined with good nutrition; and greatest susceptibility is anticipated in persons with poor heredity and severe malnutrition. Between these two extremes, dietary

— 143 —
factors often become highly important. Physiologic variables can influence nutritional status and hence resistance to infection. For example, owing to the increase in nutritional requirements during pregnancy and lactation, a diet that was previously satisfactory often becomes barely adequate, and hence susceptibility to infection is increased.

To tabulate the many pathologic states capable of diminishing resistance of the host would not serve any useful purpose. The subject has been extensively reviewed in *Natural Resistance and Clinical Medicine*, by Perla & Marmorston (1941). Typical examples are the debilitating effect of cancer (so extensive that the eventual cause of death is commonly a secondary infection), the infections directly associated with accidental or surgical trauma, and the respiratory infections so common in persons with pneumocociosis.

In summary, the innate resistance of animal hosts to infection is determined by many factors. The interaction of the genotype with a constantly changing environment leads to the result that the organism is progressively renewing itself yet constantly aging. As a consequence, nutritional influences on innate resistance must of necessity be interpreted in the light of a changing individual.

The microbic agents of naturally occurring infections are also affected by genetic variations, a basic biologic fact sometimes ignored in experimental studies. Laboratory animals, themselves often highly inbred, are frequently infected with a relatively homogeneous strain of micro-organism. Under such circumstances, the infectious agent may be either so virulent or so attenuated that the outcome cannot be influenced by dietary manipulation. Similarly, the host may be genetically so susceptible or so resistant that neither his nutritional state nor variation in virulence of the agent will affect the outcome. Conclusions derived from experiments conducted under these conditions, therefore, may have little or no direct application to what happens in heterogeneous general populations of animal or man.

That both innate resistance of the host and virulence of the infectious agent are governing factors in determining the effects of dietary deficiency on resistance is well illustrated by the classical experiment of Schneider (1950) (Fig. 3). He chose three host genotypes corresponding to selected resistant, selected susceptible, and unselected strains of mice, and exposed each of the three to three different cultures of salmonella—one composed entirely of a virulent genotype, another that was uniformly avirulent, and the third a mixed culture containing both virulent and avirulent genotypes. Taking survival or death as the criterion, the nine genetic combinations of host and agent, each tested with an adequate and an inadequate diet, gave rise to one of three results:

1. When natural resistance was high in relation to the virulence of the agent, disease did not result no matter how the diet was varied.
2. When natural or constitutional resistance of the host was low in relation to the virulence of the agent, severe infectious disease ensued regardless of nutritional factors.

3. When there was an equilibrium between natural or constitutional resistance and the virulence of the agent, nutritional factors did affect the outcome of the infection.

The fact that only one of the nine test groups presented in Fig. 3 showed this effect does not mean that this proportion reflects the usual situation in nature. From a public health point of view, the contrary is true. The results were correctly interpreted by the author, but they have been misused to derogate the importance of nutrition in resistance to infection. In only one of the test groups did the situation correspond to that characteristic of a general population; this was also the only one showing an effect of diet.

With a normal distribution of agent virulence and innate host resistance in a population, as usually results when there is an ecologic balance, the most frequent situation will be an intermediate agent virulence combined with an intermediate host resistance. If normal distributions are assumed both for virulence of the infectious agent and for host resistance, and if the
extremes of virulence, avirulence, resistance and susceptibility are arbitrarily defined as lying outside the range ± 1 standard deviation, it is possible to construct the distribution diagram shown in Fig. 4. From this it can be seen that diet should then influence the severity of infectious disease in nearly 50% of cases, whereas the four extreme combinations in Fig. 3 together account for only 10% of cases.

FIG. 4. SCHEMATIC REPRESENTATION OF PROBABLE DISTRIBUTION, IN NATURE, OF AGENT VIRULENCE AND HOST SUSCEPTIBILITY, AS SHOWN IN FIG. 3, INDICATING THEORETICAL PERCENTAGES OF POPULATION SHOWING DIET EFFECT

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Intermediate resistance of host and virulence of agent.</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5%</td>
<td>10.8%</td>
<td>2.5%</td>
</tr>
<tr>
<td>10.8%</td>
<td></td>
<td>Nutritional effects likely</td>
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<td>46.8%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2.5%</td>
<td>10.8%</td>
<td>2.5%</td>
<td></td>
</tr>
</tbody>
</table>

A shift in the distribution of either agent virulence or host resistance would result in greater or smaller numbers of cases in which no nutritional effect would be expected. If the range virulence—avirulence is arbitrarily taken as one and a half or even two standard deviations on either side of the mean, a more likely circumstance in nature, the proportion of a population affected by diet would be even greater than indicated in Fig. 4.

On theoretical grounds, the deduction clearly follows that the nutritional state of an organism is often the deciding factor in a particular infection. Although there will undoubtedly be some cases in nature in which nutri-
tional status will have no effect on the outcome of infection, the tendency in laboratory experiments is for such situations to occur with exaggerated frequency. Unfortunately, no other investigator of the effect of malnutrition on resistance has explored the reasons for observed results as thoroughly as Schneider (1950, 1956).

As already pointed out, the failure in many instances to demonstrate an effect of nutrition on infection was due to the fact that the virulence of the infectious agent or the innate susceptibility of the host was too extreme to permit any other result. This observation has important practical implications. Experiments on the interaction of nutrition and infection that give a negative result are usually much less relevant to practical problems in public health than are those that show a significant relationship. On the other hand, the data presented in the preceding chapter suggest that, even when conditions are sufficiently favorable to end in demonstration of an effect of diet on resistance, some nutrient deficiencies are more likely than others to exert that influence. Furthermore, a deficiency must always be relatively pronounced in order to produce a significant result.

At the beginning of this discussion, the statement was made that genetic variations in virulence of infectious agents are closely related to variations in their metabolic requirements. The particular characteristics of the agent often determine that a given deficiency has a greater effect on agent metabolism than on mechanisms of host resistance. Furthermore, the degree of antagonism between an infectious agent and a nutrient deficiency varies greatly among different genetic strains of a micro-organism, as suggested by the tables in Chapter 3.

Some infectious agents are today mainly restricted to the tropics and sub-tropics, the same regions in which malnutrition is commonly prevalent. This enhances the opportunity for interaction. It also makes difficult the separation of the synergistic effects of infection and malnutrition from influences such as coexisting ignorance, poverty, and poor environmental sanitation. For example, Entamoeba histolytica is widely distributed in man, but causes serious disease mainly in tropical areas.

In summary, the outcome of a potential interaction between malnutrition and infection depends in part on innate host factors, such as age and genotype, and in part on abnormal physiologic states, metabolic disorders, and acquired immunity. Interaction also depends on the genetic constitution of the infectious agent. If the agent is uniformly highly virulent or uniformly avirulent, the effect of diet may be minimal. In the same way, the innate resistance of the host may be so slight or so great that diet is of secondary importance. In the ecologically balanced populations characteristically present in nature, the situation is an intermediate level of both virulence of agent and resistance of host, with the result that diet often determines the outcome. Dietary factors thus have greater significance in nature and in public health practice than some laboratory experiments suggest.
Synergistic Action of Nutritional Deficiencies

Antibody formation

The antigen-antibody relationship and its associated specific immunity is the best known and most extensively studied mechanism of resistance to infection. Most infectious agents either contain or discharge one or more protein molecules capable of stimulating production of specific antibodies by a host. Rarely, the antigenic substance is a carbohydrate. Antibodies have the capacity to bind or neutralize antigen; the infectious agent is thereby rendered more susceptible to phagocytosis or other resistance mechanisms, or is directly damaged so that its ability to harm the host is reduced or neutralized. Since, historically, recognition of the importance of immunity, of essential nutrients, and of nutritional deficiency disease came at much the same time, the influence of nutritional deficiencies on antibody production attracted early attention.

Vitamin deficiencies

One of the first attempts to demonstrate an effect of B-complex deficiency on antibody production was through injection of killed typhoid bacilli into rats; it was unsuccessful for reasons still not clear (Zilva, 1919). In other early experiments, Werkman (1923a,b) and Werkman and associates (1924a,b) induced deficiencies of vitamin A, ascorbic acid, or B-complex in rats, rabbits, and pigeons and failed to show that the vitamin deficiency had any effect on subsequent antibody response. Erythrocytes, killed typhoid bacilli, anthrax bacilli, and pneumococci were among the antigens used. Agglutinins, precipitins, hemolysins, and bacteriolyisins were measured. In view of subsequent investigations, the failure to observe a nutritional effect on antibody production is difficult to understand; some of the deficiencies were sufficiently severe to have affected resistance. Vitamin C would not, of course, have been expected to have an effect, since none of the animals used has an obligatory requirement for ascorbic acid.

Scorbutic guinea-pigs were found to have markedly reduced skin reactions to Corynebacterium diphtheriae toxin introduced either intracutaneously or subcutaneously (Arkwright & Zilva, 1924). This was confirmed by Bieling (1925), who viewed it as a partial explanation of the previously observed greater susceptibility of scorbutic guinea-pigs to large doses of diphtheria toxin. Even in guinea-pigs with a minimal deficiency, hemorrhage and necrosis were more marked at the site of injection; and survival time was reduced by half (King & Menten, 1935).

When killed typhoid bacilli were injected into rats fed a diet deficient in vitamins A and D, both agglutinin and bacteriolyisin response were less than in controls (Blackberg, 1927-28). With living organisms, the difference
between deficiency and control groups was somewhat less marked. In 1933, Greene reported that vitamin-A-deficient rabbits responded with lower average antibody titers to injection with sheep or ox erythrocytes, but with no loss of agglutinin response to Salmonella typhi.

More than a decade passed before attention focused again on this relationship. Stoerk & Eisen (1946) and Stoerk and co-workers (1947) showed that pyridoxine deficiency in rats brought about a striking reduction in growth, and also in antibody response to sheep erythrocytes. Protein, thiamine, riboflavin, or pantothenic acid deficiency, which reduced growth, did not influence antibody production. Agnew & Cook (1949) conducted experiments with pyridoxine-deficient rats inoculated with either sheep erythrocytes or killed cultures of typhoid bacilli containing the “H” antigen. They found a significantly lower antibody response than in either ad libitum or pair-fed controls.

In comprehensive investigations of vitamin deficiency and antibody formation, Axelrod and co-workers found a strong reduction in hemagglutinin response to human erythrocytes in rats deficient in pyridoxine and pantothenic acid. Riboflavin-deficient animals exhibited titers that were variable but lower than usual (Axelrod et al., 1947). Pair-fed controls indicated an effect directly attributable to the specific deficiency and not to inanition from reduced consumption of food (Ludovici et al., 1949). The eventual conclusion was that deficiencies of pantothenic acid, pyridoxine, or pteroylglutamic acid severely reduced antibody production in rats, that riboflavin, thiamine, biotin, vitamin A, or niacin-tryptophan deficiencies had a moderate effect, and that deficiencies of vitamins D and B₁₂ had no demonstrable influence (Ludovici & Axelrod, 1951a,b; Axelrod, 1952, 1953).

The known biochemical role of several of these nutrients accounts for the decrease in protein synthesis and lowered antibody response. Doubt was raised as to whether the antibody effect would hold when the antigen was a virus. Studies with influenza virus in rats confirmed the previous general result. Both pantothenic acid and pyridoxine deficiencies caused strong and specific diminution of antibody production, but thiamine deficiency had no effect (Axelrod & Hopper, 1960). Diphtheria toxoid produced similar results when injected intraperitoneally (Axelrod & Pruzansky, 1955; Pruzansky & Axelrod, 1955). Impairment was also observed with biotin or vitamin D deficiency.

In an excellent summary of these studies, Axelrod (1958) emphasized that simple inanition failed to modify antibody production and that vitamin deficiencies interfered equally with primary and secondary responses. Subsequently, both young and mature guinea-pigs were rendered pyridoxine-deficient, either by administering the antagonist deoxypyridoxine or by feeding a highly purified pyridoxine-free diet. The result with diphtheria toxoid was decreased formation of circulating antibody and a less marked early Arthus-type skin hypersensitivity (Axelrod et al., 1961).
Several other laboratories studied these problems at about the same time, with various results. Rats fed one-tenth of presumed optimal amounts of thiamine, pantothenic acid, pyridoxine, niacin, and riboflavin exhibited no effect on the production of complement-fixing antibodies following large doses of the rickettsiae of murine typhus fever. However, small amounts of antigen resulted in a depressed antibody response in rats deficient in pantothenic acid and thiamine (Wertman & Sarandria, 1951a,b). Subsequent experiments showed impairment in rats deficient in riboflavin, as well as in vitamin B_{12} and folic acid, when compared with either pair-fed or ad libitum controls (Wertman & Sarandria, 1952; Wertman, Crisley & Sarandria, 1952) and also in niacin-tryptophan-deficient animals (Wertman, Smith & O'Leary, 1954). Despite inconclusive data, claims have been made that administration of pantothenic acid improved antibody response when typhoid-paratyphoid vaccine was administered to rabbits (Meyer et al., 1955b, 1956) and also in patients with natural paratyphoid infection (Meyer et al., 1955a).

Zucker & Zucker (1954) observed that as many as half of the rats consuming a diet deficient in pantothenic acid died spontaneously from Corynebacterium pneumoniae, an organism not pathogenic to well-nourished rats. Inanition as a contributing factor was ruled out by pair-feeding. The break in resistance occurred even before physical signs of deficiency were apparent (Seronde, 1954). Further study showed that young rats deprived of pantothenate gradually lost their species resistance to a strain of Corynebacterium kutscheri originally isolated from a spontaneous pseudotuberculous lesion in a deficient animal (Zucker et al., 1955; Seronde et al., 1955). Neither low calories nor low thiamine intakes altered the ability of young rats to form agglutinins following administration of a vaccine made from a killed culture of the organism (Zucker et al., 1956; Seronde et al., 1956). No detectable agglutinins, however, were formed in pyridoxine-deficient animals.

In pantothenic acid deficiency, the capacity to form agglutinating antibodies was lost by some deficient animals and impaired in others, with the net effect an intermediate result about the same as that observed with deficiencies of thiamine and pyridoxine. It was concluded, however, that the ability to produce agglutinins does not influence the degree of resistance of rats to the live organism (Zucker et al., 1956).

Chicks fed diets partially deficient in either vitamin A, pantothenic acid, or riboflavin had a significantly lower agglutinin response to Salmonella pullorum than did controls (Panda & Combs, 1963). The deficiencies had no effect on thymus, spleen, and adrenal weights, although the weight of the bursa of Fabricius averaged less than in controls. Average eight-week gains in weight were approximately 1500 g for controls and for vitamin-A-deficient birds, and 1377 g and 1115 g, respectively, for those fed diets deficient in pantothenic acid or riboflavin. The results suggest that the need
for these nutrients was greater for optimum antibody production than for good growth.

Research workers in the USSR have had similar results. In guinea-pigs given thiamine, precipitin antibody response to *Ascaris* larvae was greater than in animals on a stock diet (Dolin et al., 1958; Dolin, 1961). Leutskaja (1964a) immunized chickens with an antigen from *Ascaridia galli* and obtained antibody levels 25% to 50% lower in vitamin-A-deficient animals than in well-fed controls.

Feller and associates (1942) kept five adult male patients on diets deficient in vitamin A or C and measured neutralizing antibodies and complement-fixation titers *in vitro* against influenza virus from infected mouse lung. In these studies the vitamin deficiencies had no observed effect.

Severe ascorbic acid depletion in guinea-pigs (Klimentova & Frjazinova, 1965) failed to influence the production of complement-fixing antibodies against *Rickettsia prowazekii* var. *typhi*, or the production of diphtheria antitoxin as determined in regional lymph nodes or blood serum. However, the administration to rabbits of 10 μg of vitamin B₁₂ per kilogram of body weight every other day or 25 μg per kilogram every five days significantly increased the average tetanus antitoxin titers of animals immunized with toxoid, compared with the response in controls (Tashmukhamedov, 1965).

Pigs on a vitamin-A-deficient diet after early weaning had a reduced antibody response to *Salmonella pullorum* until the deficiency was corrected (Harmon et al., 1963a). Similar results were produced by diets deficient in pantothenic acid, pyridoxine, and riboflavin (Harmon et al., 1963b). A vitamin-A-deficient diet did not, however, influence antibody formation against swine influenza virus in mice, although supplementation of the deficient diet with vitamin A increased resistance to the disease (Underdahl & Young, 1956).

Hodges and co-workers have demonstrated conclusively that vitamin deficiency in man can interfere with antibody response. Adult young men consuming a diet deficient only in pantothenic acid exhibited impaired antibody production in response to tetanus antigen but not to typhoid antigen. The addition of the antagonist omega-methylpantothenic acid abolished rather than augmented the effect. This led to speculation that the antagonist may have acted as an active vitamin for this particular function (Hodges et al., 1962c).

Pyridoxine deficiency, however, with or without administration of the antagonist, slightly impaired antibody response to both tetanus and typhoid antigens (Hodges et al., 1962d). Five young men were given a diet deficient in both pantothenic acid and pyridoxine, plus the antagonists, until clinical signs of deficiency appeared. At this stage, blood serum did not agglutinate either tetanus or typhoid "O" antigen, and only an insignificant reaction occurred with typhoid "H" antigen. The antibody rise was normal after the deficient vitamins were included in the diet. By contrast, all five men,
when still deficient, responded strongly to immunization with poliomyelitis antigens (Hodges et al., 1962c). There was no impairment of antibody production in subjects in whom only minor combined pyridoxine and pantothenic acid deficiency was allowed to develop.

Few studies have measured antibody response in man under conditions of natural vitamin deficiencies. Morey & Spies (1942) compared agglutinating titers following administration of a suspension of several strains of Pasteurella tularensis in 17 patients with mild deficiency disease with those in 31 patients showing signs of severe pellagra or moderately severe multiple vitamin-B-complex deficiency. The response was slower and maximum titers were much lower in the markedly deficient patients. Jayalakshmi & Gopalan (1958) described several previously reported false-negative tuberculin reactions in malnourished persons and added 20 cases of their own.

The possibility of diminished response to protective immunization among poorly nourished people must be taken into account by public health authorities, but is not of itself sufficient reason to postpone needed immunization.

Protein and amino acid deficiency

The interference of protein and amino acid deficiencies with antibody production is well documented. An early observation of differences in disease incidence between well-nourished and poorly nourished Africans led Orr and co-workers (1931) to conduct a comprehensive study of agglutinin reactions in malnourished sheep. They tested 80 castrated animals, divided into 16 groups, for the hemolyzing power of blood serum on rabbit red blood cells, its bacteriolytic effect on Escherichia coli and Salmonella choleraesuis, and its agglutinating properties with Brucella abortus and S. schottmuelleri. Responses were inversely proportional to the physical and nutritional status of animals maintained on protein-deficient diets. An improvement was observed with protein supplementation or when the animals were shifted to green pasture.

Madden & Whipple (1940) systematically gathered experimental evidence on the consequences of lowering plasma proteins. Dogs depleted by plasmapheresis exhibited a reduced ability to form specific antibodies and were more susceptible to infection (Miller et al., 1940). This could be reversed by feeding more protein and discontinuing the plasmapheresis. A dynamic equilibrium was shown to exist between circulating plasma proteins, including gamma globulins, and the proteins of the tissue cells (Whipple & Madden, 1944; Yuile et al., 1951; Bent et al., 1952).

Cannon (1942, 1945a) was among the first to emphasize the need for dietary protein in the synthesis of the antibody-containing gamma globulin fraction of blood plasma. Both young and adult rabbits rendered hypoproteinemic (Cannon et al., 1943) had a decreased capacity to produce
agglutinins to Salmonella typhi and S. paratyphi. Attention was paid to the speed of secondary immunologic response as being a more significant index of resistance than the mere presence of circulating antibodies (Cannon, 1945b).

Wissler and associates (1946b) found protein-depleted rats less capable of rapid antibody formation. Restoration of dietary protein increased antibody formation. These observations were extended by Wissler (1947b) to include intradermal injection of pneumococcus type 1 into protein-depleted rabbits. Not only was agglutinin response poorer in the deficient animals, but local lesions spread more rapidly and caused death. Similar results were obtained with rats, although the differences were not as marked (Wissler, 1947a). Lowered hemolysin titers to sheep cells in protein-deficient rats could be restored by isocaloric substitution of carbohydrate by food protein, protein hydrolysates, or synthetic amino acid mixtures (Wissler et al., 1946a,b).

Using 100 weanling rats fed various diets and exposed to Pasteurella tularensis, Berry and co-workers (1945) concluded that the average agglutinin titer was reduced by 50% in females and 75% in males maintained on basal diets not supplemented with casein. The ability of rats to form antibodies against sheep red blood cells or Friedländer’s bacillus decreased progressively with length of time on a low-protein diet (Benditt et al., 1949). Genovoy & Koffler (1949) also reported diminished precipitin response to beef serum in protein-depleted rabbits. In summarizing these findings, Cannon (1949, 1950) emphasized the potential clinical and public health importance of reduced antibody response due to moderate to severe protein deficiency.

The only known study in which the antigen was other than bacterial is that of Klimentova & Frijzinova (1963), who measured complement-fixing antibodies for Rickettsia prowazeki var. typhi in protein-deficient rats. Antibody reduction was three-fold in the blood and four- to six-fold in the regional lymph nodes of protein-deficient animals.

Little information exists about the effect of inanition on antibody formation because it is rarely possible to separate inanition from deficiency of protein or other nutrients. Ruckman (1946) has reported the development of neutralizing antibodies against the virus of Western equine encephalomyelitis to be significantly, although not markedly, impaired either in starved or protein-deficient mice.

Antibodies were once assumed to originate from preformed serum protein components specifically modified by contact with antigens. Studies of the direct synthesis of antibodies from isotopically labeled amino acids (Green & Anker, 1954; Gros et al., 1952; Taliaferro & Talmage, 1955; Yuile et al., 1951) indicate that a delay in production may be due to the need for antibody-synthesizing enzymes rather than to the formation of precursors. In either case, specific amino-acid or over-all protein deficiency would be the factor interfering with antibody production.
Cannon (1942, 1945a, b, 1949) and Cannon and co-workers (1944) presented evidence that synthesis of antibodies occurred in phagocytes with the following five essential amino acids particularly involved: lysine, methionine, tryptophan, threonine, and leucine. He and his associates proposed that a template protein was synthesized and retained in particular macrophages, the result being a rapid response to a reinforcing dose of antigen.

In *Nippostrongylus muris* infection of rats, lysine deficiency restricted an increase in the gamma globulin of blood serum. When compared, lysine but not methionine deficiency was found to produce not only an altered gamma globulin synthesis, but also loss of resistance, as shown by egg counts, hemoglobin levels, and other evidence of infection (Barakat, 1950). An extension of the theory of antibody formation (Schweet & Owen, 1957) led to the concept that antigens, in penetrating the nuclei of certain phagocytes, produce a primarily genetic change by altering the deoxyribonucleic acid molecule. As a consequence, new ribonucleic acid templates are formed and carried by further multiplication into daughter cells. This is an elaboration of the template concept of Cannon (1942).

The sequence of cell types involved in the process was studied in rats (Cannon et al., 1944; Wissler et al., 1957), typhoid bacilli, sheep erythrocytes, and Klebsiella pneumoniae being used as antigens. The development in two to four days of specifically sensitized antibody-forming cells from macrophages could be blocked by radiation, by protein depletion, and by specific amino acid analogues. Many other workers have demonstrated synthesis of antibodies within the reticuloendothelial system (Livieratos et al., 1954; Stavitsky, 1954) and shown that it could be blocked by amino-acid deficiencies (Cannon, 1945a, b; Stavitsky, 1957). The latter investigators used deficiencies produced by the amino acid analogues parafluorophenylalanine and gamma-ethylamidoglutamic acid.

Almost no work has been done on the extent to which human populations actually suffer through failure of antibody formation attributable to protein deficiency. The earliest clinical reference we have found was that of Krebs (1946), who described a single malnourished person with serum proteins of 3.1 g/100 ml and a serum albumin level of 2.0 g/100 ml. This person showed no antibody response to commercial typhoid vaccine. With a high protein diet, however, gamma globulin levels rose from 0.15 to 0.68 g/100 ml.

Gell (1948) had an opportunity to test the antibody response of 57 severely malnourished survivors of a German concentration camp at the close of the Second World War. Although he chose such unlikely antigens as tobacco mosaic virus and avian red cells, responses were much less among the malnourished than among 16 well-nourished British soldiers.

Wohl and associates (1949) followed the antibody response to typhoid immunization of 102 patients in a Philadelphia hospital; 88 had original serum albumin levels below 4.0 g/100 ml. The markedly slower response in patients with low albumin was improved when they were given a protein
supplement. Despite the many patients studied and the apparently clear-cut improvement in antibody production with protein supplementation, this report has largely gone unnoticed.

Balch (1950) reported no relation between amounts of serum protein, antitoxin response to diphtheria toxoid, and development of infection in 25 "grossly depleted" patients with terminal illnesses. This study has uncertain value because only 10 of the 25 subjects had total serum proteins below 6.0 g/100 ml and only seven had serum albumin values below 2.7 g/100 ml. Furthermore, his discussion leans heavily on undocumented statements that infection was not especially prevalent among malnourished civilian or prisoner-of-war populations in the Second World War.

Other seemingly negative studies should be mentioned. In infants on *ad libitum* diets, Dancis and co-workers (1953) found no difference in antibody response to a single injection of diphtheria toxoid whether the protein content of the diet was 10% or 20% of the caloric intake. Obviously, neither group of infants was protein deficient, since they consumed 3.0 g and 5.5 g, respectively, of protein per kilogram of body-weight per day.

A similar criticism applies to the finding that the ability to produce antitoxin after diphtheria toxoid was the same in 15 seriously wounded patients as in 11 healthy controls (Havens et al., 1954). Serum total protein levels were at the relatively high average of 6.8 g/100 ml in the experimental group and 7.4 g/100 ml in the controls. Only one patient had serum protein below 6.4 g/100 ml. Albumin levels averaged 3.3 g/100 ml in the wounded patients compared with 4.3 g/100 ml in controls.

Recent papers entitled "Antibody response in children with protein malnutrition", by Pretorius & De Villiers (1962), and "Serum antibody response of malnourished children as compared with well-nourished children", by N.A. Fernández (1960), report no differences in antibody response. The studies were carefully conducted to compare the agglutinin response to "H" and to "O" antigens of *Salmonella typhi* among children treated for kwashiorkor. They are particularly misleading, however, since they deal with patients receiving vigorous dietary therapy and hence no longer deficient in amino acids available for protein synthesis. Unpublished INCAP studies of antibody production in children under treatment for kwashiorkor gave similar results.

The ability of children with untreated kwashiorkor to form antibodies is clearly impaired or even completely inhibited. When Budiansky & Da Silva (1957) maintained children with severe protein malnutrition on a poor diet throughout an experimental period, antibody response to typhoid vaccine was inhibited. In another experiment, Olarte and associates (1956) obtained the same results when diphtheria toxoid was the antigen. Similarly, Reddy & Srikanthia (1964) showed that four of seven children with kwashiorkor given 30 g of protein per day and one of three receiving 50 g failed to develop antibodies to typhoid-paratyphoid vaccine. Of those showing an
antibody response, titers were higher with 50 g than with 30 g of dietary protein.

Brown & Katz (1965) compared the antigenic response of children with kwashiorkor admitted to a pediatric ward with that of children convalescing from tuberculosis and well nourished at the time of study. They found no impairment of antibody response to attenuated type 1 oral poliovirus vaccine or of clinical response to smallpox vaccination (Brown & Katz, 1966b). Details of diet are not given. They did note a complete absence of seroconversion at periods ranging from 5 to 14 days after administration of these vaccines in eight children with kwashiorkor compared with a positive response in four of six controls. In subsequent work, children whose only sign of malnutrition was retarded growth showed impaired conversion of Mantoux tests after being given BCG (Harland & Brown, 1965; Harland, 1965). Immunologic response to live measles vaccine administered with gamma globulin appeared normal (Brown, personal communication, 1965). Brown and Katz (1966a) have since described the results of administering the 17-D strain of yellow fever vaccine to eight children with kwashiorkor. None of the eight developed antibodies, while six well-nourished control children had a pronounced antibody response following the same procedure.

When visiting pediatricians in a number of developing areas, we have been told that children with kwashiorkor do not ordinarily react to tuberculin skin tests until after dietary treatment and partial recovery, even when tuberculous joints or pulmonary tuberculosis are found to be present. Until such impressions are confirmed by additional systematic and controlled studies, we suggest only that clinicians be aware of this possibility. The scientific relevance of the clinical impression arises from the importance attached to the skin test as an indicator of cellular immunity as distinguished from the more commonly measured antibody response.

The well-planned studies of Hodges and co-workers should be given far more weight than the negative results that have been reported. Three pairs of healthy men were fed diets containing approximately 0.1, 1.0, and 2.0 g of protein per kilogram of body-weight. One man from each pair received egg yolk, or egg yolk and skim milk solids, as sources of protein, the other, only skim milk solids. The men receiving the lowest protein intakes were in negative nitrogen balance. Antibody response to tetanus and typhoid "O" and "H" antigens was poor in the man receiving the low-protein diet of skim milk solids, but not in his colleague who ate yolk protein. However, as the quantity of egg yolk protein was further increased, the magnitude of antibody response declined (Hodges et al., 1962a). Large doses of gamma globulin administered intravenously to three other subjects approximately doubled the gamma globulin concentration in the serum, but resulted in a poorer antibody response to typhoid fever "H" and "O" antigens and to Asian influenza vaccine than in control subjects, although the response to tetanus toxoid was unaffected (Hodges et al., 1962b).
Golebiowska and associates (1965) reported that gamma₂ globulins had a tendency to decrease in undernourished Polish children, although this was not the case for gamma₁ globulin or for the production of iso-agglutinins.

In summary, protein deficiency, if sufficiently severe, inhibits normal antibody response. A widespread, but mistaken, impression among clinicians is that protein deficiency sufficient to cause this effect is not seen in human populations. On the contrary, not only is antibody production impaired in the large numbers of children who develop kwashiorkor in the developing countries, but there is also strong evidence that impaired antibody formation is frequent among persons with debilitating illnesses in all countries.

**Phagocytic activity**

Phagocytosis is generally viewed as the second major defense against infectious disease. Cells capable of phagocytic activity have a similar site of origin in the reticulo-endothelial system to those responsible for antibody production. Phagocytic cells are primarily the fixed macrophages located in the liver, spleen, and other reticulo-endothelial tissues, and, secondly, the wandering macrophages, among which the polymorphonuclear leukocytes are most prominent. Despite a more or less common origin, phagocytic activity and antibody formation are affected differently by nutritional state.

Under some circumstances, although production of antibodies is reduced resistance to infection is maintained (Zucker et al., 1956). In others, poor resistance is evident even when antibody formation seems unimpaired (Miles, 1951a; Kahn et al., 1957). Severe undernutrition and concomitant depletion of protein reserves will eventually lead to a marked atrophy of the liver, spleen, bone marrow, and lymphoid tissues, from which phagocytes originate (Cannon et al., 1944; Livieratos et al., 1954). In the severe protein malnutrition of kwashiorkor, concurrent infection sometimes results in little, or complete lack, of the anticipated leukocytosis (Trowell et al., 1954; Béhar et al., 1956).

Well-controlled animal experiments support the findings described in man. Asirvadham (1948) found that protein-deficient rats lost their ability to respond by leukocytosis to turpentine abscesses. Bone marrow smears showed atrophy of tissue with depletion of myeloid and lymphoid elements. After 18 days of re-feeding, leukocyte response was restored.

Prolonged protein deficiency in rabbits sufficient to cause hypoproteinemia resulted in poor antibody response to *Diplococcus pneumoniae* (Wissler, 1947a) and, also, in an inability of polymorphonuclear leukocytes to phagocytize the coccus. Similar, but less pronounced, effects were noted in protein-deficient rats (Wissler, 1947b).

When Steffee (1950) infected roosters with *Diplococcus pneumoniae* in numbers sufficient to cause death in half the control animals, 28 of the
29 protein-depleted ones died. Total food intake was equal in the two groups. The capacity of the depleted animals to clear the circulating blood of *Diplococcus pneumoniae* was greatly reduced, a result interpreted as due in part to ineffectiveness of the Kupffer cell macrophages of the liver.

Guggenheim & Buechler (1946b, 1948, 1949) stated that protein-deficient diets “invariably” impaired phagocytic regeneration in rats and that the effect was readily reversed by restoring dietary protein. Rats were fed different amounts of protein and inoculated intraperitoneally with 0.5 ml of a 24-hour broth culture of *Salmonella typhimurium*. Leukocyte counts and the phagocytic index were lower and bacterial counts higher in animals receiving a 3% casein diet than those fed 6%, 9% or 18% of casein. Maize protein at a level of 9% had about the same effect as the 3% casein diet; 9% of protein from egg or meat produced a result more nearly equivalent in bactericidal and phagocytic activity to that observed with 18% casein.

At lower levels of protein intake the effect of protein quality on phagocytic activity is readily measurable in laboratory animals. This demonstration lends urgency to clinical studies of the effect of protein malnutrition in young children in less developed areas. The need is for knowledge of the mechanisms actually operating in these human populations and responsible for the observed greater susceptibility to infection.

The situation in marasmus and inanition is not wholly comparable with that of specific protein malnutrition. In patients in the later stages of prolonged wasting diseases, no decrease was seen in numbers of circulating neutrophils nor in the phagocytic power of the cells against staphylococci (Balch & Spencer, 1954). In marasmus, amino acids mobilized from muscle cells are known to become available to the liver and presumably to the reticulo-endothelial system as well.

Decreased macrophage activity has been reported in deficiencies of vitamin A and ascorbic acid. This is significant in view of the frequent synergism of these deficiencies with a specific infectious disease. Ørskov & Molike (1928) found that vitamin-A-deficient mice had a reduced ability to remove and destroy *Salmonella* injected intraperitoneally. As a result, the infectious agent accumulated in liver, spleen, and peripheral lymph nodes and frequently gave rise to severe and often fatal septicemia. In his long series of experiments with *Salmonella* in vitamin-A-deficient animals, Lassen (1930, 1931) repeatedly observed the same behavior. He also demonstrated decreased macrophage activity, as measured intraperitoneally and in vitro. A combined deficiency of vitamins A and D in rats, sufficient to produce an effect on phagocytosis, also prevented growth of the host (Pottingham & Mills, 1943).

Previously, Findlay & Maclean (1925) observed that in rats fed a diet deficient in vitamins A and D to the point where the animals developed keratomalacia, the blood lost its capacity to kill staphylococci in vitro. The
blood leukocyte count was not significantly reduced and the blood serum was apparently not bactericidal for staphylococci; the results were attributed to decreased capacity of leukocytes to achieve phagocytosis.

Turner & Lowe (1930-31) reported no effect of vitamin A deficiency in rats on numbers or proportions of white blood cells in circulating blood, but this means little because they measured neither the response of leukocytes to infection nor phagocytic activity. Among 25 prisoners in Uganda jails receiving only the prison diet and an equal number who received 30 ml of cod liver oil daily for 14 days, Hennessey (1932-33) failed to demonstrate a significant difference in leukocyte counts after subcutaneous injection of Escherichia coli. Since both groups were “outwardly healthy” and dietary intake was not recorded, this can hardly be considered a study of the effect of vitamin A deficiency.

Indian workers (Hassan et al., 1947) have reported an inverse relationship between plasma vitamin A levels and mean neutrophilic leukocyte counts in 60 male medical students. They postulated a protective physiologic leukocytosis in vitamin A deficiency, but their data do not justify this conclusion. The average leukocyte counts in five groups, in order of decreasing plasma levels of vitamin A, were 7350, 7130, 7150, 7430, and 8633 per cubic millimeter. The marginally higher average values in students with the lowest plasma levels of vitamin A would seem more likely to have been due to greater frequency of infection in this group.

Scurvy in guinea-pigs has been associated with decreased numbers of granulocytes and a lessened phagocytic power. Irritating substances such as titanium dioxide failed to produce the inflammatory exudate, clouded by pus cells, so characteristic of normal animals (Lawrencowicz, 1931; Nungester, 1951). Leukocytes also showed a marked tendency to rupture and fragment (Nungester & Ames, 1948). The injection of supplemental ascorbic acid into normal mice enhanced phagocytic activity of leukocytes in peritoneal exudates, as indicated by tests with staphylococci (Marcus et al., 1953).

Vitamin B-complex deficiencies also reduce white blood cell activity. Monkeys on diets deficient in B vitamins developed a striking granulocytopenic leukopenia and a concomitant markedly lowered resistance to natural infections (Saslaw et al., 1943). Phagocytosis of Micrococcus candidus was reduced by 35% to 49% in rats fed a basal diet deficient in protein, minerals, and B-complex vitamins (Berry et al., 1945). Activity was not restored by adding casein or casein plus minerals to the basal diet, but only by addition of B-complex vitamins.

Wertman and co-workers (1953) described a leukopenia, due mainly to fewer lymphocytes, in thiamine-deficient, B-complex-deficient, and pair-fed rats suffering from inanition. The bone marrow showed a marked relative lymphocytopenia with a proportionate increase in granulocytes. Subsequently, Wertman & Groh (1959) reported no obvious effect on the cap-
acidity of leukocytes from thiamine-deficient or inanition control rats to phagocytize *Diplococcus pneumoniae*, despite a greater susceptibility of the deficient animals to this micro-organism.

Riboflavin deficiency also resulted in leukopenia in rats (Shukers & Day, 1943; Wertman et al., 1952, 1957; Wertman & Sypherd, 1960); but, again, the same result occurred in controls suffering from inanition. Comprehensive studies of blood cell response in rats with these deficiencies were initiated in an effort to explain the greater susceptibility to *Diplococcus pneumoniae* (Wertman & Groh, 1959; Wertman & Sypherd, 1960). Two important changes were believed to offer a sufficient explanation: first, a sharply reduced complement activity and, second, a reduced ability of phagocytes to migrate to the site of infection. Although no decrease in numbers of circulating leukocytes was observed, leukocytes were reduced in peritoneal exudates and in exudates from other inflamed areas.

Pyridoxine deficiency, which has such a markedly deleterious effect on antibody production, also reduced numbers of effective phagocytic cells. Mushett and associates (1947) fed chicks, puppies, and monkeys either a pyridoxine-deficient diet or the metabolic analogues deoxypyridoxine and methoxypyridoxine. Chicks developed hypoplasia of the lymphoid elements of the spleen, and dogs and monkeys, leukopenia and microcytic anemia. In accord with other authors, these investigators attributed the leukopenia to atrophy of the reticulo-endothelial system, especially lymph nodes and thymus. Autopsies gave supportive evidence, except that in dogs the ratio of spleen weight to body-weight was increased. Their experiments with pair-fed controls indicated that the leukopenia in rats fed a pyridoxine-deficient diet was due to the vitamin deficiency, not to the associated inanition. Thus, lack of pyridoxine appears more specific in its effect on leukocytes than lack of thiamine or riboflavin.

From experimental studies on monkeys and from clinical observations, Doan (1946) came to the conclusion that folic acid deficiency produces cellular inadequacy in mammalian bone marrow of sufficient extent to interfere with the production of leukocytes and largely to nullify the protective action of antibodies. Wertman and co-workers (1956) found that rats deficient in either folic acid or vitamin B₁₂ had leukopenia and that the migrating power of their leukocytes in response to an irritant was diminished. Other investigators have had difficulty (Lichstein et al., 1946) in maintaining folic acid deficiency in monkeys (*Macaca mulatta*) because of frequent leukopenia, severe dysentery, and high mortality. On deficient diets, the animals developed a striking granulocytopenia and had a high mortality from spontaneous infections and from experimental infection with group C hemolytic streptococci or influenza virus (Saslaw et al., 1943).

In these studies, reduced phagocytic activity was a more constant and important consequence of malnutrition than leukopenia. A clue to the mechanism is provided by the numerous intracellular enzymes identified in
phagocytes (Bazin, 1956; Cohn & Hirsch, 1960a,b; Hirsch & Cohn, 1964; Braunsteiner et al., 1964; Cohn & Wiener, 1963a,b; Saito & Suter, 1965). DeDuve (1959, 1964) has suggested that in many cell types lysosomes may play a defensive role. Histochemical observations have shown a transfer of basic protein from lysosomes to engulfed micro-organisms within the phagocytic vacuoles (Cohn & Hirsch, 1960a,b). The process by which acid phosphatase and other enzymes concentrate in lysosomes is still a problem. Tejada and associates (1964) found decreased alkaline phosphatase activity of leukocytes in kwashiorkor. A marked loss of acid phosphatase has also been described in the leukocytes of guinea-pigs treated with excess vitamin A (Janoff & McCluskey, 1962).

In summary, existing evidence indicates that a number of nutritional deficiencies, particularly of protein, vitamin A, and ascorbic acid, when sufficiently severe, can interfere with phagocytosis by leukocytes. This action would be expected because of the associated interference with the intracellular enzymes that digest micro-organisms and with the production of antibodies, which are an essential feature of the opsonocytophagic process. These deficiencies, and those of pyridoxine, folic acid, and vitamin B12, as well as primary inanition and inanition secondary to thiamine, riboflavin, or other nutrient deficiencies, may also interfere with the ability of the liver, spleen, and bone marrow to produce macrophages and microphages. Aschkenasy (1957), in experiments on rats, found that protein deficiency induced anemia and leukopenia because it deprived the hematopoietic organs of amino acids for new cell formation. The extent to which nutrient deficiencies, through their action on numbers and activity of phagocytes, have clinical and public health significance remains undetermined.

Non-specific protective substances

Blood serum and body fluids of normal animals have a capacity to kill or inhibit growth of many infectious agents independently of antibodies or phagocytosis. Several non-specific protective substances have been identified.

Properdin

Properdin is a euglobulin found in the blood serum of all normal animals thus far tested (Pillemer et al., 1954, 1955, 1956). It appears to be associated in some way with natural resistance to many diseases of bacterial, viral, and even protozoal origin (Hunter & Hill, 1958; Finkelstein et al., 1959; Hinz, 1956; Schubart et al., 1964; Blum, 1964). The presence of magnesium is essential to its action. Properdin seemingly functions by combining selectively with polysaccharides of high molecular weight (Wardlaw et al., 1955).
Following administration of bacterial lipopolysaccharides (Landy & Pillemier, 1956), properdin titers were lower in germ-free rats (Gustafsson & Laurell, 1960) and higher in germ-free mice. Removal of properdin eliminated the bactericidal activity of rat blood serum as tested against a variety of bacterial agents (Wardlaw & Pillemier, 1956). Properdin is not wholly independent of the antigen-antibody system since complement is necessary for its activity.

The properdin system is vulnerable to nutritional deficiency at several points — in the formation of the compound itself, in its need for appropriate complement, and in its demand for magnesium. To date, the only published confirmation of nutritional effect is the report of a marked reduction in properdin in rat serum in the presence of a deficiency of pantothenic acid sufficient to interfere with growth and with enzyme activity of the liver (Wiss et al., 1957). Thiamine deficiency sufficient to interfere with growth had no such effect, which would indicate that the results with pantothenic acid were not due to an accompanying inanition. The action of other nutritional deficiencies on the properdin system needs investigation.

Interferon

Interferon is a natural product of animal cells that protects them from attack by a number of viruses (Burke & Isaacs, 1960; Isaacs, 1961, 1963; Isaacs & Hitchcock, 1960; Friedman et al., 1962; Wagner, 1963; Neva & Weller, 1964; Finter, 1964a, b; Glasgow, 1965a, b; Merigan, 1967). It supplements other mechanisms of resistance to viral infection and presumably accounts for some of the resistance to a second viral infection when one virus is already present in cells. Its mechanism of action appears to be through uncoupling of oxidative phosphorylation. Glucose is still metabolized to lactic acid, but the process no longer produces the normal amount of adenosine triphosphate (ATP). Since viruses are unable to multiply within a cell unless plentifully supplied with ATP, the blocking mechanism theoretically interferes with viral replication without depriving the cell of sufficient ATP for its own needs.

The release of interferon appears to be a general reaction of cells to virus infection. Since it is a protein molecule, its formation may well be depressed in nutritional states in which protein synthesis is impaired, but as yet there is no information on this possibility.

Recently, interferon has been found in cells as an apparent response to Escherichia coli endotoxin and to infection with Serratia marcescens, Salmonella typhimurium, Brucella abortus, and Rickettsia tsutsugamushi (Youngner & Stinebring, 1964; Stinebring & Youngner, 1964). Even RNA and such products of RNA hydrolysis as adenosine monophosphate are effective in stimulating interferon production (Sigel, 1964).
Lysozymes

The existence in body fluids of enzymes that destroy pathogenic microorganisms, at least in vitro, is recognized, although these products are usually regarded as of minor significance. It is known, for example, that the circulating levels of a lysozyme-type of enzyme are significantly higher in guinea-pigs sensitized with heat-killed tubercle bacilli than in normal animals. This could not be related to either skin reactivity or leukocytic activity in response to purified protein derivative of the tubercle bacillus (PPD) (Janicki & Patnode, 1961). Lysozyme isolated from egg white has shown in vitro and in vivo activity in mice against pathogenic staphylococci (Ermol'eva et al., 1964). The authors state that this lysozyme preparation has been used successfully in the USSR for curing antibiotic-resistant staphylococcal carriers.

In addition to destroying bacteria, lysozymes are said to act on viruses in vivo and in vitro (Ferrari et al., 1959). An inhibitor effective against the viruses of poliomyelitis, herpes simplex, and Rous sarcoma has been demonstrated in the genital tract of women, but its nature has not been determined (Pannu & Sigel, 1963).

There is little doubt that enzymatic activity of this nature can be decreased or abolished as a result of nutritional deficiency. The greatly reduced lysozyme activity in the tears of two children with xerophthalmia “increased remarkably with five to seven days of cod-liver-oil therapy” (Anderson, 1933). Such findings may be of significance in view of the frequency of secondary ophthalmia, as well as systemic infections, in cases of xerophthalmia. Decreased secretion of lysozyme into the gastrointestinal lumen has been observed in vitamin A deficiency in man (Sullivan & Manville, 1937), although the bowel wall itself contained a higher concentration of lysozyme than normally observed.

The saliva of malnourished persons, in contrast to that of persons who were well nourished, had little or no bacteriolytic activity against a variety of bacterial agents, including Vibrio cholerae. Saliva from cholera patients showed a similarly reduced activity (Dawson & Blagg, 1950).

Cohn & Wiener (1963a,b), analysing selected hydrolases from rabbit peritoneal macrophages, found a two- to three-fold increase in lysozyme when such cells were stimulated by injection of killed BCG. They also noted a similar increase in acid phosphatase and lipase activities under these circumstances.

Inhibition of reproduction of trypanosomes during early stages of infection by a humoral substance has been described (Braude, 1963; Raffel, 1961; Pérez-Tamayo, 1961).

A normally occurring serum factor, β-lysin, is bactericidal for such Gram-positive bacteria as Bacillus anthracis and B. subtilis (Donaldson et al., 1964).
Another factor that reacts with the group-specific polysaccharide C-substances of pneumococci (C-reactive protein) is found in a number of other infections (Braude, 1963).

Other

Even intestinal helminths may be inhibited by non-specific protective substances secreted into the gut. Well-nourished horses were capable of elaborating a specific growth-inhibiting substance against Nippostrongylus muris (Schwartz et al., 1931), but production was largely suppressed by nutritional deficiencies. Similarly, an extract of mucus from adult dogs and hogs caused early death of the fowl nematode Ascaridia lineata when tested in vitro. Antigen-antibody mechanisms were ruled out by autoclaving the extracts before the test; no nutritional studies were conducted (Eisenbrandt & Ackert, 1941). The effectiveness of mucus as an inhibitor of Ascaris galli in chickens increased with age of the animals, but this was perhaps attributable to larger numbers of goblet cells per area of intestinal wall. The phenomenon was not studied in malnourished chickens (Frick & Ackert, 1941).

A well-worked-out, specific enzymic effect of an infectious agent comes from demonstration that protein deficiency reduces production of trypsin, which, in turn, is necessary for the excystation of oocysts of Eimeria in chicks (Britton et al., 1964). Severe protein deficiency or starvation markedly reduced trypsin production in 48 hours, with closely correlated amelioration of the infectious process. A direct relationship was then shown by adding trypsin to the Eimeria oocysts before feeding. Prompt restoration to full infectivity occurred as the sporozoites were released from the oocysts.

In summary, the significance of the various non-specific mechanisms of resistance to infection just cited is difficult to assess. The occurrence of interferon and of properdin in living cells is well established and potentially important clinically. Lysozymes, although known much longer, appear of marginal importance. Recorded investigations on these substances are few, and the subject has attracted little recent interest.

A non-specific substance inhibiting intestinal nematode infections has been described by several investigators. Other such substances may well exist.

Non-specific destruction of bacterial toxins

Neutralization of bacterial toxins in the course of resistance to infectious processes is ordinarily through combination with specific antitoxin generated by the animal host. There is some evidence of other mechanisms. Werkman and co-workers (1924b) found that rats suffering from deficiencies of B-complex vitamins or vitamin A were more susceptible than controls to
diphtheria toxin, although antitoxin production was unaffected and the rate of disappearance of injected toxin was normal.

Dubos and co-workers (1955) reported that, after 48 hours, fasting mice were susceptible to *Klebsiella pneumoniae* endotoxin when given 10% of the LD₅₀, whereas animals on an adequate ration were susceptible only to 50% of the LD₅₀. This sensitivity was reversed by 48 hours of good diet. The authors concluded that the effect was independent of ordinary immune mechanisms, since large differences were noted a few hours after injection and long before an immune response could have occurred.

Guinea-pigs starved or treated with carbon tetrachloride were more susceptible to intravenously injected *Klebsiella* endotoxin than normal animals. Since this was a first exposure and deaths in treated animals occurred within a few hours, these findings cannot have been due to a difference in antitoxic immunity. The authors provide no explanation of their results (Formal et al., 1960).

Fatty degeneration of the myocardium in guinea-pigs inoculated with diphtheria toxin is manifested biochemically by a depressed rate of oxidation of long-chain fatty acids in heart muscle, excessive accumulation of triglycerides, and a striking decrease in concentration of myocardial carnitine (DL-gamma-trimethylamino-beta-hydroxybutyrate), a compound known to stimulate long-chain fatty acid oxidation (Wittels & Bressler, 1964). Exogenous carnitine had a restorative effect in vitro on the depressed rate of palmitic acid oxidation by myocardial homogenates of the toxin-treated animals, suggesting a biochemical basis for resistance to this bacterial toxin.

**Tissue integrity**

Dietary inadequacies have long been assumed to diminish resistance to infection by reducing the integrity of various tissues. Nutrient deficiencies frequently result in gross epithelial lesions. Examples are the metaplastic hyperkeratosis due to vitamin A deficiency; the dermatitis, cheliosis, and angular stomatitis from riboflavinosis and pyridoxine deficiency; the characteristic dermatosis and mucosal atrophy of pellagra; the spongy gums and subcutaneous hemorrhages of scurvy; and the atrophy of skin and gastrointestinal mucosa of severe protein deficiency.

The mucosa of the gastro-intestinal tract of vitamin-A-deficient cotton-rats was more readily penetrated by poliovirus than the mucosa of control animals (Weaver, 1946). Similarly, Seidmon & Arnold (1931-32) found a significant increase in numbers of *S. typhimurium* in livers of rats deficient in vitamin A or B when cultures were made 30 to 60 minutes after inoculation. Stryker & Janota (1941), however, were unable to duplicate these results when *S. enteritidis* was administered to vitamin-A-deficient rats by stomach tube.
INTERACTIONS OF NUTRITION AND INFECTION

From work with guinea-pigs infected with *M. tuberculosis*, Grant (1926) suggested that an adequate balance of dietary calcium, vitamin C, and vitamin D was essential to normal resistance of the intestinal wall against entrance of bacteria into the blood-stream from the lumen.

Nutritional deficiency conceivably has an influence on resistance to infection through one or more of the following pathologic tissue changes (Horwitt, 1955):

1. alterations in intercellular substance;
2. reduction or absence of secretion of mucus;
3. increased permeability of intestinal and other mucosal surfaces;
4. accumulation of cellular debris and mucus, resulting in a favorable culture medium;
5. keratinization and metaplasia of epithelial surfaces;
6. loss of ciliated epithelium of the respiratory tract;
7. nutritional edema with increased fluid in tissues;
8. reduced fibroblastic response; and
9. interference with normal tissue replacement and repair.

The evidence now available does not permit judgment as to the relative significance of these several changes.

Lynch (1937) used a special synthetic diet containing gum arabic and various salts to produce fulminating amebiasis in guinea-pigs. The result was first attributed to alteration of the bacterial flora. Feeding massive doses of bacteria to simulate the change had no effect, however, on the severity of amebiasis in control animals. He concluded that the thinning and vacuolation of the intestinal mucosa in animals receiving the diet were a more likely explanation.

The "maturation resistance" to viruses described by Sabin (1941) was apparently due to a mechanism preventing the spread of virus from nasal mucous membrane to the brain. Four different viruses, infective by intracerebral inoculation, were also infective by intranasal inoculation at two to four weeks of age, but not thereafter. Deficiency of vitamin B-complex, thiamine, vitamin E, or total calories delayed the development of this "maturation resistance" to intranasal inoculation.

In summary, some of the tissue changes characteristic of nutritional deficiencies influence resistance to infection, but their relative importance is not well known and is sometimes over-emphasized.

Wound healing and collagen formation

Wound healing, fibroblastic response to local trauma, walling-off of abscesses, and collagen formation are all reactions closely related to nutri-
tional deficiencies. Too often evaluation of synergism and antagonism between nutrition and infection is restricted to the acute manifestations of infectious disease. The impact in terms of total resulting disability is frequently dependent on the rapidity with which the infection can be localized and contained without serious clinical manifestations.

The walls of induced sterile subcutaneous abscesses in protein-deficient rats are much thinner than in well-nourished animals and, when spontaneously or experimentally infected, show much less fibroblastic response (Taylor and Tejada, 1966). As a consequence, a fatal septicemia is frequent. Protein deficiency, and particularly lack of methionine, interferes not only with the conversion of procollagens, but also with the tensile strength of collagen fibers (Dunphy et al., 1956; Dunphy, 1957; Williamson et al., 1951). Fasting for seven days also reduces the procollagen content of guinea-pig skin (Gross, 1958). Skin collagen synthesis, as measured by the uptake of radioactively labeled glycine, was considerably decreased in rats fed diets deficient in amino acids (Nimni et al., 1962).

Early experimental observations by Menkin and co-workers (1934) showed the importance of ascorbic acid in the formation of collagen. Subsequent biochemical studies identified the dominant role of ascorbic acid in the synthesis of the amino acids from which collagen is formed (Gould 1960, 1961, 1963; Dunphy et al., 1956). Two amino acids are almost unique to collagen, hydroxyproline (Gould & Woessner, 1957) and hydroxyllysine (Sinek & Van Slyke, 1955). The skin of wounds of scorbutic guinea-pigs contained no hydroxyproline, which can be taken as indicating the absence of procollagen, but when ascorbic acid was restored to the diet, hydroxyproline was rapidly produced (Gould & Woessner, 1957).

Some confusion arose because, in spite of the biochemical evidence of collagen deficiency, sections of the scorbutic skin examined microscopically appeared to contain abundant collagenous material. This was due to accumulation of mucopolysaccharides, which resemble collagen histologically (Robertson & Hinds, 1956). Bavetta and associates (1961) designed experiments to measure the rate of collagen biosynthesis in rats fed different amounts of protein, with and without ascorbic acid supplementation. There was a highly significant increase in initial collagen formation in rats consuming the higher-protein diet and the added ascorbic acid. There is, as yet, little to connect nutritionally induced changes in collagen formation with resistance to infection, but the relationship should be investigated in such diseases as tuberculosis.

**Altered intestinal flora**

The micro-organisms ordinarily inhabiting the intestinal tract of man have in recent years been credited with increasing physiologic and pathologic significance. Since the initial observations of Metchnikoff (1908), many
studies in man and in laboratory animals eventually established that the
diet of a host can have a profound effect on the intestinal flora. Evidence
increases that changes in the so-called “normal” flora make the host more
susceptible to a number of pathogenic agents and that others that are usually
harmless become pathogenic in the presence of malnutrition.

Studies in swine have shown that the relative numbers of fecal Escherichia
coli, of other Gram-negative bacilli, of enterococci, and of clostridia are
influenced by the type of diet. Diets rich in animal protein and calcium
gave increased numbers of atypical Clostridium perfringens, from a normal
of about 200 per gram to about 500,000 per gram, as well as greater numbers
of Gram-negative bacilli (Mansson & Olsson, 1961a,b,c). Enterococci and
Clostridium perfringens were fewer after addition of citric acid to the diet
(Mansson & Olsson, 1962). The investigators reviewed the work of others
who also found a marked dietary influence on intestinal flora.

The in vitro growth of Streptococcus faecalis was halted by specific
deficiencies of certain amino-acids (Shockman et al., 1958). Lysine was
essential to culture media for growth of both cell wall and cell protein.
Deficiencies of valine, histidine, and threonine, however, interfered with
protoplasm synthesis; but cell wall production was stimulated to the extent
that the usual size of bacteria was doubled.

Lack of dietary protein is believed responsible for disturbances of the
microbial pattern and overgrowth of intestinal bacteria in kwashiorkor
(Smythe, 1958). The weight of stools from children with kwashiorkor may
be as much as four to five times normal figures (Hansen et al., 1962). The
bacteria of the lower intestinal tract also tend to migrate to higher levels in
patients with sprue (Frazer, 1949), and probably in severe malnutrition
associated with other conditions.

After sulfonamides and antibiotics came into common use, many studies
were reported of their effect on the flora of the intestinal tract. Although no
review is attempted here, it is to be noted that such therapy regularly induces
changes in the relative proportions of intestinal bacteria of various types,
and that total numbers of bacteria increase (Bridges et al., 1952, 1953).
Spatial distribution within the intestinal tract may also be altered (Anderson
et al., 1956).

For the most part, these therapeutic agents act by blocking either
nutrients or metabolic reactions essential to the bacteria. The selective
effect on the intestinal flora is of the same general order as that produced by
primary dietary deficiencies. Whether or not these changes in intestinal
micro-organisms influence susceptibility to infection is still an open question.

Some recognized pathogens probably find a more favorable environment
in the intestine of the malnourished host, with the result that micro-organisms
not normally pathogenic increase in numbers to such an extent that they may
cause diarrhea or other symptoms. Dubos and co-workers (1963c) have
recently summarized the evidence that “certain components of the indigenous
flora play a useful role in increasing resistance against virulent pathogens, and also against less virulent, but nevertheless deleterious microbial species which would otherwise become established in the intestinal tract". The microbial species responsible for this protective effect have not as yet been identified.

Dubos and co-workers (1963a,b) have pointed out that the intestinal bacteria of man range biologically from those so well adapted that they are never pathogenic to others that are always pathogenic. They consider the lactobacilli and the anaerobic bacilli included in the genus Bacteroides to be the truly normal flora of the intestinal tract of mice and of men, and their presence to be generally beneficial. Other common intestinal bacteria include enterococci, clostridia and Gram-negative enterobacilli (particularly Escherichia coli, the Proteus group, and Pseudomonas)—micro-organisms that ordinarily are not so numerous as the lactobacilli and Bacteroides. Dubos and co-workers (1963c) regard them as usually harmless invaders, which, under selected circumstances, may become pathogenic.

The animals of a mouse colony gained weight more rapidly and utilized protein more efficiently than did stock animals when they were kept essentially free of E. coli, the Proteus group of bacilli, and Pseudomonas aeruginosa, and when the greater proportion of the intestinal flora was restricted to organisms commonly classified as lactobacilli and Bacteroides (Dubos & Schaede, 1960, 1962a,b; Dubos et al., 1963a,b; Schaede & Dubos, 1962). When penicillin was administered, lactobacilli disappeared and enterococci and Gram-negative enterococci increased explosively. A striking observation was that E. coli, long thought to be a normal component of the microbiota, became abundant and was accorded a role in subsequently observed pathologic effects (Ashburner & Mushin, 1962). Clostridia (Lev & Forbes, 1959) and enterococci (Anderson et al., 1956) are among other bacteria presumably having a part in deleterious effects arising from the ordinary intestinal flora.

An inhibition of the protective action of the normal enteric flora was the explanation offered for the increased susceptibility of guinea-pigs to experimental shigellosis after either four days' starvation or antibiotic therapy with streptomycin, erythromycin, or nystatin (Formal et al., 1958). Control animals had a non-fatal infection; test animals had a prompt and high fatality. Starvation for a maximum of 36-48 hours resulted in chronic non-fatal disease.

The last chapter of this monograph emphasizes the high proportion of diarrheas among malnourished pre-school children in less developed countries where no known bacterial pathogen can be identified. It seems likely that some diarrheas are caused by infectious agents not normally pathogenic in the well-nourished child and not necessarily identified by present microbiologic methods. Gordon and associates (1957) found that feeding excessive numbers of even such normally desirable bacteria as Lactobacillus
*acidophilus* provoked diarrhea in children with severe protein malnutrition.

The dramatic increase in numbers of Gram-negative bacilli and *Clostridia* in the stools of babies fed a formula of cow's milk rather than breast milk may have significance even for the well-nourished child (Gyllenberg et al., 1957). It has been suggested that *Lactobacillus bifidus*, so regularly a feature of the intestinal flora of breast-fed infants, confers some degree of protection against the establishment of harmful strains of *Esch. coli* (Ross & Dawes, 1954) and other bacterial pathogens (Petuely, 1957; Gyorgy et al., 1962). Rose & Gyorgy (1955) have shown that, when children are fed breast milk, certain mutants of *L. bifidus* rapidly suppress other intestinal micro-organisms. At the University of Zagreb, in Yugoslavia, an experimental study is currently under way in which young children are fed large quantities of *L. bifidus* in order to determine whether or not the flora thus produced is beneficial in controlling enteric infections. The results are not yet available.

In the guinea-pig, six different diets each produced definite qualitative changes in the bacterial flora (Crecelius & Rettger, 1943). A number of intestinal micro-organisms benefit others by synthesizing the nutrients required, or, conversely, they may compete for the same limited supply of nutrients (Rosebury et al., 1954; Rosebury & Sonnenwirth, 1958). That some intestinal bacteria are truly symbiotic, to the benefit of the host, is suggested by the poorer growth commonly observed in germ-free animals when compared with that of normal animals on the same diet.

Newton & DeWitt (1961) found that germ-free guinea-pigs did not grow as well as animals conventionally reared, even though food consumption relative to body-weight was greater. A lack of bacterial flora was thought to hamper good nutrition. The germ-free animals, in addition to being smaller, had serum protein levels 25% less than those conventionally fed. Levenson & Tennant (1963) summarize the nutritional contribution of a normal flora as delaying the full effect of starvation, cirrhosis, and vitamin B deficiency in conventional as compared with germ-free animals. Scurvy is apparently augmented in conventionally reared guinea-pigs because their intestinal bacteria use the available vitamin C.

Experimental suppression of the enteric bacterial flora by antibiotics has resulted in decreased resistance to parenteral infection (Dineen, 1961). Observations on germ-free animals suggested that the normal flora somehow enhances the ability of the host to cope with infection. For example, germ-free guinea-pigs were susceptible to infection with *Sh. flexneri* alone; but, if first infected with *Esch. coli*, the animals survived (Forman et al., 1961).

Of possible relevance is the recent report that the normal flora has a significant impact upon ability of the host to mobilize and concentrate leukocytes in an area of injury (Abrams & Bishop, 1965). Quantitative comparison of aseptic, starch-induced peritonitis in germ-free mice and animals conventionally reared disclosed that extravascular migration of leukocytes, in response
to the sterile irritation, was significantly greater in animals harboring a living microflora.

An intestinal flora more favorable to growth of guinea-pigs resulted when part of the sucrose of the basal diet was replaced by 15% of gum arabic, 2.5% of potassium acetate, and 0.5% of magnesium oxide (Roine & Elvehjem, 1950). In general, most of the observed competitive microbial interactions seem to have occurred because of a pH unfavorable to growth of the specific host, or more commonly from action of an antibiotic substance (Florey, 1945, 1946; Rosebury, 1962). Some reports support the theory of such a competitive interaction as a possible factor in resistance, for example, to diphtheria (Mühlenbach, 1939) and to shigellosis (Friedman & Halbert, 1960).

The many different colicins are antibiotics that have attracted attention for at least forty years. They are derived from *Esch. coli* and other Gram-negative enteric bacilli (Fredericq, 1957). A pure culture of *S. enteritidis* from mouse feces prevented the growth *in vitro* of *S. typhi*, *Sh. dysenteriae*, and *Sh. flexneri* (Topley & Fielden, 1922). Later it became evident that *Esch. coli* can suppress *Vibrio cholerae* *in vitro* even in media favoring the vibrio. When the experiment was repeated *in vivo*, using isolated bowel loops in rabbits, no evidence of antagonism was obtained (Barua et al., 1963). Another example is the identification by Flippin & Mickelson (1960) of a non-pathogenic *Esch. coli* strain with an antagonistic action against a contaminating *Salmonella* introduced into egg-white medium. In a study in Egyptian villages, the probability of finding coliform strains capable of inhibiting *Shigella* was greatest when they were derived from the feces of patients with shigellosis. Next in order were those from family contacts, and last those from neighbors or persons having no direct contact with the disease (Robbins et al., 1958).

The use of non-pathogenic coliform bacteria as an adjunct to antibiotic therapy was tried by Stewart and co-workers (1964). They selected a strain of *Esch. coli* resistant to paromomycin or neomycin, the particular antibiotics to be used, and then introduced the organisms into the gut along with the antibiotic, treating two patients infected with *Sh. sonnet*, five with *S. typhi-murium*, and one with enteropathogenic *Esch. coli*. From this experience they concluded that the method might be useful in maintaining a coliform flora during prolonged antibiotic therapy, but that it did not contribute to elimination of the pathogens. Finegold and co-workers (1965) suggest studying these phenomena by selective inhibition of the various major elements of the intestinal flora with carefully selected drugs.

The increased gastro-intestinal motility and accompanying diarrhea associated with some deficiency states is another example of an alteration in the numbers and behavior of intestinal flora and of parasites. Loughlin & Mullin (1955) thought that ascarids and other helminths were less numerous in protein and vitamin A deficiencies because of "gastro-intestinal hurry",
that is, the more rapid passage of food through the digestive tract. On the other hand, it has been suggested that in chickens with vitamin A deficiency weakening of peristalsis may result in intestinal stasis and an accumulation of ascarids (Ackert & Nolf, 1931; Ackert et al., 1931).

The diets of herbivores are stated to lead, at times, to intestinal stasis, thereby favoring toxin production by Clostridium perfringens (Roberts, 1938; Parry, 1948).

Larsh (1945) presented evidence that the lowered resistance resulting from alcohol feeding or increasing age in Hymenolepis nana infections of mice was associated with decreased intestinal emptying time. He was able to produce the same effect with morphine (Larsh, 1947a).

Amebiasis became a fulminating disease in guinea-pigs fed a special synthetic diet that altered and increased the intestinal flora (Lynch, 1957). The result could not be duplicated by feeding well-nourished animals massive doses of the bacteria making up the greater part of the altered flora, from which the conclusion followed that the shift in flora was not the primary cause of the greater susceptibility. Hegner (1924) was impressed by the rarity of intestinal protozoa in many carnivores. Experimentally, he found that rats fed diets high in animal protein were less favorable hosts for Giardia muris, Trichomonas muris, and Hexamitus muris than animals subsisting mainly on vegetable proteins and carbohydrates.

The foregoing discussion demonstrates clearly that several forms of malnutrition alter types, numbers, and distributions of intestinal bacteria. Less is known of the effect this may have on resistance to pathogenic agents acting primarily on the intestine. Nevertheless, a number of convincing examples confirm a decreased resistance to intestinal infections brought about by nutritionally induced alterations of the gastro-intestinal flora. The severity of protozoal and helminthic infections of the intestine is frequently determined by dietary changes. Alterations in gastro-intestinal motility secondary to malnutrition may also play a part.

Endocrine imbalance

Endocrine activity is an integral part of the biologic mechanisms involved in resistance to infection; and malnutrition can produce endocrine abnormalities. The effect of protein deficiencies on endocrine function has been specifically reviewed by Leathem (1958). In laboratory animals, most endocrine responses are reduced once body proteins are so depleted that labile protein reserves are at a premium. In children with kwashiorkor, the urinary excretion of 17-hydroxy-steroids is lowered (Castellanos & Arroyave, 1961), and adrenal gland size is reduced (Stirling, 1959; Tejada, 1955). Kwashiorkor and less specific forms of severe protein malnutrition in adults also tend to induce panhypopituitarism and associated atrophy of other endocrine glands (Zubirán et al., 1955). Marasmus or starvation stimulates
the stress reaction, one manifestation being an increase in cortisone production.

Pyridoxine deficiency in rats reduces thyroid activity, as indicated by diminished $^{131}$I uptake (Hsu et al., 1959; Hsu, 1963). That iodine deficiency results in endemic goiter and possible hypothyroidism is well known.

The administration of thyroid hormone following infection of rats with pneumococci or streptococci (Sidorkina, 1950) apparently increased the number of survivors, and the time between inoculation and death was greater for the animals that died. Although a beneficial effect has been claimed for thyroid hormone in patients with scarlet fever, confirmation is lacking (Zimanyi, 1948). In mildly hyperthyroid chickens (Todd, 1949), *Ascaridia galli* attained a significantly greater length than in normal or mildly hypothyroid hosts. The opposite was true for *Heterakis gallinae*.

Several investigators have indicated that hypothyroidism has an adverse effect on the course of tuberculous infection (Joll, 1932; Fishberg, 1932; Webb, 1916; Delore, 1926; Lisser, 1934; Lurie & Ninos, 1956; Lurie et al., 1959). However, the reasons why an abnormal hormonal state alters the host’s resistance to tuberculosis are not known. Lurie and co-workers (1956, 1959) have demonstrated that hypothyroid rabbits have a lowered resistance to tuberculous infection, which suggests that this hormonal disorder may alter the inflammatory response of the host. The uptake and release of $^{131}$I has been shown to be reduced by pneumococcal sepsisemia in the rat (Shambaugh & Beisel, 1966). This was accompanied by a marked fall in the protein-bound iodine and circulating free thyroxine. The failure of serum thyroid-stimulating hormone levels to change appeared to be due to decreased pituitary response. Similar effects occurred with human subjects infected with *Pasteurella tularensis* or vaccinated with a living attenuated strain of Venezuelan equine encephalitis virus (Shambaugh & Beisel, 1967). Presumably, nutritionally induced endocrine changes could enhance these effects.

Chronic adrenal insufficiency in man, or Addison’s disease, and experimental adrenalectomy of animals markedly diminish resistance to infection and to stress in general (Kinsell, 1955). The mode of action is not clear, but the defect is correctable by adequate corticoid therapy (Selye 1946, 1949, 1950, 1951). Conversely, adrenocorticotropic-hormone (ACTH) or corticoid therapy favors extension of many infections by diminishing the protective inflammatory response (Kligman et al., 1951; Selye, 1951; Spink, 1957). The breakdown of tuberculous lesions, the spread of staphylococcal disease, the greater severity of varicella, and even the development of fatal vaccinia are all recognized hazards of such therapy. Top (1964) summarizes clinical experience in the statement that corticoids produce a reduction of symptoms but with danger of causing spread of infection in viral hepatitis, histoplasmosis, epidemic keratoconjunctivitis, measles, infectious mononucleosis, mumps, pneumococcal pneumonia, trichinosis, and typhoid fever.
There is also clinical and experimental evidence that some protozoan
diseases, such as trypanosomiasis (Wolf et al., 1951), malaria (Kass et al.,
1951), and amebiasis (Eiert et al., 1959), may be aggravated by steroid
therapy. Cruz and associates (1966) have reported five cases of fatal
strongyloidiasis developing during, or shortly after, a course of corticosteroid
therapy.

The increased susceptibility to Coxsackie virus of mice treated with
cortisone has been demonstrated by Bebehansi et al. (1962). Growth
hormone had the opposite effect. Administration of cortisone has been
reported to reduce the extent of acquired immunity of mice to typhoid
bacilli, as evidenced by a greater concentration of the infectious agent in
organs and tissues (Zhitova & Kudryashova, 1965). Where there is latent
corynebacterial infection, a natural occurrence in mice, a single injection of
10 mg of cortisone may precipitate the active disease, pseudotuberculosis
(Fauve et al., 1964.)

Germuth and associates (1951) reported that an inadequate amount of
ACTH or cortisone inhibited formation of antibodies and development of the
Arthus phenomenon in rabbits. Antibody suppression has been noted in
guinea-pigs (Kepinow, 1922), rats (Wyman, 1929; Ivanov, 1963), and
mice (Weiser et al., 1941) under similar treatment. Meyer and co-workers
(1964) were unable to demonstrate an effect of cortisone on the production of
precipitins and natural hemagglutinins in thymectomized and bursectomized
chicks.

In convincing studies, Hirsch & Church (1961) found that polymorpho-
nuclear leukocytes collected from rabbits given large doses of cortisone
exhibited a normal capacity to engulf and kill certain staphylococci and
enteric bacteria. The granulocytes of such animals also contained normal
amounts of the antimicrobial agents, lysozyme, phagocytin, and histone.
They concluded that the decreased resistance to infection occurring with
high doses of glucocorticoids was not associated with significant changes in
either the opsonic or the bactericidal activity of serum.

Adrenalecstony has been described as considerably decreasing properdin
levels in rats (Biró et al., 1964). The effect is increased by deoxycorticoster-
one, decreased by prednisolone, and abolished by aldosterone or cortis-
one.

Numerous claims of benefit from cortisone in the treatment of a variety
of acute infections have been made, but mainly in the early enthusiasm for
cortisone, when it was tried for almost every pathologic condition. Such
claims were usually based on poorly controlled and inconclusive studies or on
misinterpretation of a relief of inflammatory symptoms as control of infec-
tion.

Nevertheless, when cortisone was tested in mice for an effect on macro-
phage activity, as judged by splenic uptake of colloidal thorium dioxide, and
on the capacity of leukocytes of the peritoneal cavity to take up Staphylococ-
*Staphylococcus aureus*, both actions were significantly enhanced (Marcus et al., 1953). There is some evidence, moreover, that cortisone in mice is able to reduce the effect of a number of bacterial endotoxins (Boyer & Chedid, 1953). The protective action was observed only when cortisone was administered before the toxin (Geller et al., 1954; Berry & Smythe, 1963).

This action may be related to the protective influence of cortisone on endotoxin-induced disturbances of carbohydrate metabolism or to the ability of cortisone to mitigate tissue damage (Berry & Smythe, 1959). Another possibility is that cortisone slows the spread of infection by inducing tissue edema. Rabbits became more resistant to vaccinia virus by reason of increased tissue fluids produced either by hypertonic salt solution or by administration of estradiol. Spread of both virus and India ink particles from the site of infection was slowed down by edema (Taylor & Sprunt, 1943).

A possible relationship between resistance to avian malaria and gonadal hormones is indicated by studies of ducks infected with *Plasmodium lofhurae* (Trager, 1948). In egg-laying ducks with active ovaries the number of parasites introduced into the circulating blood was limited. In males, and in females with inactive ovaries, multiplication of trophozoites was observed. Sadun (1948) noted that both the gonadal hormones, testosterone and estradiol, increased the resistance of chickens to *Ascaridia galli* infection.

Addis (1946) showed that *Hymenolepis diminuta* in the male rat is ordinarily dependent on testosterone for normal growth, although progesterone can be substituted. The administration of hydrocortisone, testosterone, and, especially, progesterone to guinea-pigs inoculated with *Entamoeba histolytica* resulted in a greater frequency of hepatic abscesses than in untreated animals (Biagi et al., 1963).

Secondary infection is notably a common and severe complication of diabetes, although less now that long-acting insulin preparations have become available. High nitrogen losses were once a common result of the brief periods of ketosis so frequent when diabetics were dependent on regular insulin. Pollack (1955) suggests that maintenance of positive nitrogen balance with protamine-zinc insulin, despite some glucosuria, is the key to the greater resistance to infection characteristic of diabetics under modern management.

According to Cruickshank & Payne (1949), the bactericidal power of leukocytes is impaired in alloxan diabetic rabbits. In a later study, Cruickshank (1954) attributed the greater susceptibility to a peripheral circulatory failure, which inhibited migration of leukocytes to the infected tissues. Furthermore, the leukocytes of diabetic patients have a lower capacity to form lactic acid from glucose than do leukocytes of normal controls, a situation corrected by insulin (Martin et al., 1953). This is believed to be a significant consideration in view of the bactericidal action of lactic acid.
Kligman and co-workers (1951) mentioned the fungus *Trichophyton mentagrophytes* among cutaneous infections to which the cortisone-treated host is more susceptible. Fungal infections have not been mentioned previously in this monograph because of lack of published evidence that nutritional deficiencies affect them.

Pulmonary mucormycosis, an infection now recognized with increasing frequency, provides at least one exception. The disease is almost always associated with metabolic disorders, most frequently diabetes mellitus (Baker, 1956). Control of the diabetes appears to be the principal requirement for survival of patients with this mycosis (Harris, 1955). Rabbits with alloxan diabetes developed nasal, pulmonary, and cerebral lesions of mucormycosis after inoculation with the causative organism *Rhizopus oryzae*, even when inoculation of the fungus preceded the experimental diabetes by several days (Bauer et al., 1956). A less pronounced fungal susceptibility was also produced in rabbits by hyperglycemia induced by glucose infusion. The presence of degenerative changes in rabbit polymorphonuclear leukocytes in both types of hyperglycemia may be an important factor in pathogenesis of infection. It is noteworthy that thyroidectomy has been shown to increase the susceptibility of rats to this organism (Paplanus & Sheldon, 1965).

In summary, all mechanisms of resistance to infection are affected, in varying degrees, by the endocrine status of the host. Endocrine activity, in turn, is altered by many nutritional factors, including such common clinical deficiencies as those of protein or iodine. Starvation or marasmus, by producing a stress reaction, also affects endocrine balance. Part of the reduced resistance to infection characteristic of malnourished persons is almost certainly mediated through endocrine changes. It is probable that a better endocrine balance is sometimes partly responsible for the increase in resistance that follows an improvement in nutritional status. Moreover, specific endocrine disorders such as diabetes and Addison's disease must be controlled if the constant hazard of death from superimposed infection is to be avoided.

**Response to drug therapy**

Under special conditions, dietary imbalance markedly alters the therapeutic effectiveness of a number of drugs. Stibophen therapy of *Schistosoma mansoni* infection in mice was more effective after the mice were given supplemental vitamin K (Bueding et al., 1947). The observed effect was probably not attributable to direct nutritional mechanisms, because the level of vitamin K was one thousand times greater than the ordinary requirement for mice.

A similar non-specific nutritional effect on stibophen therapy of *S. mansoni* infection of mice was reported by Luttermoser & DeWitt (1961).
purified synthetic diet with a protein content of 8% to 30% was compared with standard animal chow. Up to 95% of parasites were killed by stibophen therapy in animals on the purified diet, compared with 12.5% in mice on the stock diet. The maximum fatality occurred when the protein content was low. The special diet had no effect on infections of untreated mice.

Subsequent studies in man showed that a high-protein diet had no direct effect on chronic S. mansoni infection. When stibophen treatment was started, however, the therapeutic response was more rapid among patients who had received a high-protein supplement for eight months (DeWitt et al., 1964).

*Plasmodium gallinaceum* infection of chicks responded 4 to 20 times more readily to treatment with sulfadiazine or metachloridine when the birds were on a purified casein diet rather than on a regular stock diet (Taylor & Greenberg, 1955). No effect was observed when infections were treated with quinine, chloroquine, atabrine, or other antimalarials. In subsequent studies, the accentuated therapeutic effect was eliminated by increased amounts of soy bean in the diet. An extract of tertiary amines, presumed responsible for the phenomenon, was eventually isolated (Greenberg et al., 1959). Pyridoxine supplements inhibited the therapeutic action of both quinine and atabrine on *P. lophurae* and *P. catheemerium* infections of birds (Seeler, 1945).

**Antagonistic Action of Nutritional Deficiencies**

Antagonism occurs when deficiency of a nutrient has a greater effect on the infectious agent than on the host. Sometimes the particular nutrient is required only by the agent, and not by the host. Under other circumstances, metabolic disturbances are induced that affect the agent more than the host, because the more complex host organism has alternative metabolic pathways.

Antagonism *in vivo* is best understood from such tissue culture studies as those in which thiamine-deficient mouse fibroblasts proved more susceptible to the filterable agent of psittacosis than cells from well-fed animals (Bader & Morgan, 1961). Oxythiamine-induced thiamine deficiency in minced chick embryo inhibited mumps virus and partially suppressed influenza virus (Cushing & Morgan, 1952). Deoxyribodoxine-induced pyridoxine deficiency in minced chicken embryo suppressed development of both mumps and influenza viruses, and methionine deficiency produced by ethionine blocked poliovirus replication in mouse fibroblasts (Brown & Ackermann, 1951; Brown 1952). Under the same experimental conditions, Mohajer & Gahams (1966) found that the methionine analogue 1-ethionine had no significant effect on the biosynthesis of two strains of poliovirus (Mahoney and Lansing) in HeLa cells, whereas in primary monkey kidney cells it markedly inhibited the biosynthesis of the Lansing strain of poliovirus.
Both ethionine and glycine methylester suppressed the replication of vaccinia virus in human Chang liver cells (Gabliks et al., 1967). The inhibitory effect of ethionine was partly reversed by an excess of methionine, but glycine addition did not prevent the effect of glycine methylester. Since an excess as well as a deficiency of either methionine or glycine inhibited vaccinia virus replication more than they inhibited cell growth, it appears that the balance of the amino acids in the intracellular pool can affect the magnitude of viral biosynthesis.

Many compounds currently used in chemotherapeutic and antibiotic management of infectious disease are closely related chemically to specific nutrients; they act by blocking normal participation of these nutrients in the metabolism of the agents. An example of current research of this type is the observation that a combination of 5-bromodeoxyuridine and N-methylisatinthiosemicarbazone is effective in eliminating vaccinia virus from cultured HeLa cells (Furusawa et al., 1965). To assure the necessary margin of safety, the metabolic requirements of the host for the blocked nutrients must be significantly lower than those of the agent.

Knowledge gained in the course of experimental studies on antagonism has been applied usefully in the search for more effective chemotherapeutic agents; for example, pyrimidines are required for the synthesis of nuclear material, and the systematic search for related compounds led to the discovery of paludrine (Curd et al., 1945).

Experimental production of antagonism in animals has not resulted in any direct contribution to control of infection in man. Deficiencies severe enough to produce antagonism almost always lower resistance to secondary infections, which then become a significant cause of death. Furthermore, antagonistic relationships usually require a highly specific and severe deficiency, obtained only under experimental conditions and rarely seen under natural conditions in either animal or human populations. Thus, the generally inadequate diets characteristic of less developed regions typically lead to synergism rather than antagonism.

From data summarized in the preceding chapter and viewed in relation to specific nutrients, certain generalizations about antagonism are possible:

1. Antagonism occurs more frequently with specific than with generalized nutritional deficiencies. The few instances of antagonism following acute fasting may have been due to a specific caloric effect, but none has been recognized as due to multiple deficiencies. This fits in with the concept that antagonism occurs because an infectious agent suffers from lack of a specific nutrient. In multiple deficiencies, any possible antagonistic effect is usually overwhelmed by synergism resulting from loss of host resistance, brought about by one of the mechanisms already discussed.

2. Among nutrients essential to man, certain ones are not essential to microbial metabolism, and their deficiency has not produced antagonism.
The lack of antagonism with vitamin A deficiency, for example, contrasts strongly with the frequent occurrence of synergism. In a comprehensive review of the literature by Guirard & Snell (1962) on nutritional requirements of micro-organisms, particularly the bacteria, vitamin A is conspicuous by its absence from the list of essential nutrients. The only report of antagonism that we have found was that *P. lophurae* parasitemia was less severe in vitamin-A-deficient chickens than in normally fed birds (Roos et al., 1946).

3. The nutrient deficiencies resulting in antagonism are those essential to microbial nutrition. In our tables, and in Guirard & Snell's (1962) review, these nutrients are: the vitamin B group, about half of which were originally discovered because they were essential for micro-organisms; a list of amino acids ranging from none to as many as 18, according to the particular organism; and a number of mineral ions, including K, PO₄, SO₄, Mg, Mn, Fe, Zn, Na, Ca, Mo, and Cl.

4. Micro-organisms need certain nutrients not known to be essential to the human host; as a consequence, they provide metabolic pathways susceptible to therapeutic attack. Examples are para-aminobenzoic acid, purines, peptides, pyrimidine bases, polyamines, and a long series of unidentified growth factors still under study (Guirard & Snell, 1962). Naturally, this circumstance is most frequent among infectious agents whose action is relatively independent of host metabolism. The prospect of finding nutrients specifically essential to a micro-organism diminishes with its increasing host dependence. Much of the success of antibiotic and synthetic antimicrobial agents depends on interfering with chemical processes unrelated to human metabolism (Davis & Feingold, 1962). One example of such an antagonistic effect is the dependence of malaria parasites on para-aminobenzoic acid.

**Host factors**

As already made clear, antagonism usually occurs because certain infectious agents are more dependent on specific metabolites than are their hosts. Animal hosts may ordinarily achieve biochemical homeostasis through multiple metabolic pathways. The simpler systems of microorganisms are more often dependent on single metabolic pathways. Microbial metabolism thus can be selectively blocked by inducing specific deficiencies. These can be produced most readily and effectively by use of antimetabolites.

The tables in the preceding chapter show that antagonism is most frequent among infectious agents highly dependent on host metabolism. From a practical standpoint, the diseases they cause are the ones most resistant to modern therapeutic measures. Infectious agents relatively independent of host metabolic processes can be successfully attacked by
means that do not directly affect the host's metabolic processes. Because information about such interactions aids understanding of the more complex metabolic interactions of host-dependent micro-organisms, a summary is now presented of pertinent views in this rapidly developing field of microbial metabolism.

Although reports are few, the possibility of nutritional interference with invasiveness and spread of infectious agents should also be considered. A good example is that fasting rabbits are more difficult to infect with vaccinia virus than are normal animals, a result attributed to accumulation of interstitial fluid (Sprunt, 1942).

Agent factors

Several reviews summarize important developments in bacterial metabolism. Davis & Feingold (1962) related metabolic research to prospects for developing new antimicrobial agents. Their main purpose was to find antimetabolites capable of blocking metabolic pathways required by the agent but not by the host. A variety of existing experimental evidence was introduced to illustrate the means by which substitute metabolites can be incorporated into complex chemicals to alter the function of infectious agents. From this came a hypothesis of "false feedback inhibition" to explain the resultant chemical blocking. The important point was made that most of the existing antibiotics do not act on well-known energy-yielding pathways but on previously unstudied metabolic pathways specific to micro-organisms, especially those concerned with the formation of cell wall and membrane.

Neidhart (1963) gave special attention to mechanisms of metabolic inhibition of the synthesis of the constituents of the cell wall membranes and the protoplast, and of RNA and DNA. In this connection, the report of Shockman and co-workers (1958) that growth of Streptococcus faecalis can be halted by specific amino acid deficiencies has particular interest. Lysine is essential for synthesis of both wall and cellular proteins. Deprivation leads to autolysis. On the other hand, valine, histidine, and threonine are needed for cellular protein but not for wall protein. In culture media lacking these amino acids, protoplast synthesis is halted while the cell wall doubles in size.

Another recent review (Panos & Aji, 1963) has related metabolic processes to pathogenicity. Virulent infectious agents are generally more active metabolically than are avirulent forms, although the relation may be reversed, as with the tubercle bacillus. Lost virulence in some strains of S. typhi, S. typhimurium, and K. pneumoniae is directly related to a developing metabolic dependence on purines and para-aminobenzoic acid, substances not normally available in animal hosts. A review by Seaman & Reifel (1963) considered metabolic pathways of protozoa as they relate to chemical structure and function.
The metabolic requirements of malaria parasites (McKee, 1951) are better known than most. Methionine deficiency retarded the development of *P. berghei* in mice and monkeys (Taylor, 1956) and of *P. knowlesi* in monkeys (Geiman & McKee, 1948). Ten days of starvation almost eliminated parasitemia with *P. berghei* in mice (Ramakrishnan et al., 1953; Ramakrishnan, 1954), and partial starvation inhibited *P. knowlesi* infections in monkeys (Geiman & McKee, 1948). A dramatic suppression of *P. knowlesi* infection was observed in vitamin-C-deficient monkeys; after ascorbic acid was administered, parasites increased overwhelmingly (McKee & Geiman, 1946).

The delicacy of balance between deleterious effects on host and on the parasite is indicated by the need for B vitamins in avian malaria. Moderate thiamine deficiency retarded *P. gallinaceum* parasitemia in chickens, but severe deficiency adversely affected the host and resulted synergistically in earlier death; high doses of thiamine accelerated parasitemia and hastened death, (Rama Rao & Sirsi, 1956). Pantothenate deficiency of chickens suppressed blood-induced *P. gallinaceum* infection, but not sporozoite-induced infections (Brackett et al., 1946).

More specific information on pantothenate requirements was obtained by *in vitro* culture of *P. lophurae* (Trager, 1954). Pantothenic acid was adequate for intracellular parasites, but extracellular forms required the more complex coenzyme A. Similarly, folic acid permitted *in vitro* growth of intracellular forms; infected erythrocytes actually contained more folic acid than normal cells. The more complex coenzyme form of folic acid was required, however, by extracellular cultures of the parasite (Trager, 1958).

Of particular interest because of possible therapeutic application was the demonstration that a milk diet inhibited plasmodial infection in rats and dogs (Maegraith et al., 1952; Maegraith, 1953). The milk diet produced para-aminobenzoic acid deficiency, which, in turn, depressed the parasitemia of rats and monkeys. A dietary supplement of para-aminobenzoic acid abolished the milk diet effect (Hawking, 1953, 1954). This confirmed the increased parasitemia in partially starved monkeys (Geiman & McKee, 1948). Results from feeding trials in humans were negative. Trypanosomal infections have a similarly reduced severity in the presence of certain dietary deficiencies.

Tissue-culture studies have given precise and detailed information on the nutritional requirements of viruses and bedsoniae. Poliovirus requires methionine, as shown by metabolic blocking with the analogue ethionine (Ackermann, 1951; Brown & Ackermann, 1951). Influenza and mumps viruses require pyridoxine and thiamine, as indicated by blocking with deoxyzyridoxine and oxythiamine (Cushing & Morgan, 1952). Thiamine is completely essential for the agent of psittacosis; and pantothenate, niacin, pyridoxine, and choline are needed for its maximal replication (Bader & Morgan, 1961).
In summary, antagonistic interactions between a nutritional deficiency and an infection are due in most instances to selective lack of one or more nutrients upon which the infectious agent is more dependent than the host. Only rarely does antagonism occur through such other mechanisms as physical interference with invasion or the readier spread of an infectious agent favored by altered tissue.
CHAPTER 5

PRINCIPLES OF FIELD STUDY
OF HUMAN POPULATIONS

Introduction

The evidence thus far introduced for an interaction of nutrition and infection has derived mainly from clinical investigation, from laboratory studies, and from animal experiments. This emphasis by investigators is a natural outgrowth of the development of experimental medicine during the past half century, the improved biochemical and biophysical methods of measuring life processes, and the fact that clinical research is now solidly based on scientific principles. The original concern with an interaction between nutrition and infection, however, arose out of observations in nature of the common association of war, famine, and pestilence. The community effect is still the dominant consideration if the knowledge gained, whatever its source, is to be applied to the benefit of the public health. The evaluation of community effect requires field investigation by epidemiologic methods.

Epidemiologic field study, too, has progressed in the course of years from a descriptive to an analytical discipline, and important advances have resulted from the application of modern methods of laboratory and clinical investigation to field observations. Formerly restricted to infectious diseases transmissible from person to person, epidemiologic investigation has now extended to practically all community diseases and injuries. In this transition, nutritional disorders have had a prominent part, especially through prevalence surveys of the nutritional state of representative groups of people. Nevertheless, several aspects of the epidemiologic field study of nutrition and its relationship to infectious and other diseases still remain largely untouched. Further progress depends heavily on good field studies.

As chronic disease processes came to be viewed from an epidemiologic standpoint, the technical methods of field study naturally developed along fresh lines. An outstanding feature was recognition that the procedures so well suited to sharply marked epidemics and to acute infectious diseases that run a rapid course did not wholly suffice for chronic diseases, of which mal-
nutrition is one (Anderson, 1965). The need was for long-term prospective study, for years instead of weeks or months, if incidence were to be accurately determined and causative factors identified. That method has become an essential part of chronic-disease epidemiology, and is being applied to a variety of morbid processes.

Field Study of Human Nutrition and Nutritional Disease

Field study has a long-established place in nutritional investigations. Lind’s observations (1757) on scurvy are an early example, although the “field” in this case was a ship on the high seas. Since then, changes in the nature of field studies are as many as in the other two fundamental approaches to knowledge of malnutrition: clinical investigation and laboratory experimentation.

Gordon & Le Riche (1950) distinguished three phases in the development of field work in nutrition and nutritional diseases. Initially, interest centered on food and its effect on the nutritional state of populations. The method was the dietary survey, although the procedure differed so much from present practice that it might better have been termed “food consumption survey”.

Identification of specific nutrients and an improved definition of nutritional disease entities led to a second stage, the study of the prevalence of individual dietary deficiencies and specific nutritional disorders. Laboratory tests were incorporated into field practice, and clinical methods were enlarged to give more precise measurements. Field study attained a new level of importance.

The current trend expands the nutritional survey to make it the nucleus of a general health survey. The emphasis is not only on nutritional disorders and the nutritional state as such, but also on the effect exerted by nutrition on the characteristics and behavior of other community disease (Gilles, 1964). Nutrition of the host influences resistance through a variety of mechanisms. Less appreciated is the fact that other mass diseases have a decided impact on the frequency and severity of nutritional disorders.

The range of interaction becomes increasingly apparent. In dealing with nutrition and infection, this monograph touches on only one of many such relationships. Others include such diverse interactions as that between calcium deficiency and fractures in the aged (Harrison et al., 1961) and the controversial connection between nutritional factors and chronic degenerative, metabolic or neoplastic disorders, such as coronary heart disease (Miller et al., 1956), diabetes (Wilkerson & Krall, 1947), and cancer (Jolliffe, 1962).

Nutrition has thus followed the course of other scientific disciplines concerned with health. Initially, there was emphasis on its own particular problems, but later these were considered as part of the general pattern of
clinical and epidemiologic effort toward community health and welfare. In the case of nutrition (Young & Trulson, 1960), this marked a return to the objectives of the original nutrition survey, but with better methods and greater achievement (Hankin et al., 1967; Keys, 1967).

As nutritional interests expanded from clinical management of patients to prevention and control of disease in general populations, epidemiologic techniques became an integral part of practice. At first, the methods were elementary and directed to case-finding—to identifying patients with nutritional deficiencies and assuring adequate treatment. Occasional outbreaks of nutritional disease brought into play the more complicated measures needed for epidemic control (Morley, 1963). Both endeavors are essentially operational, designed to remedy an existing situation, with minor concern for cause or effect.

The enlarged scope of the nutrition survey involved determinations of prevalence, accompanied by a need to know the characteristics of host and environment that determine the origin, course, and extent of nutritional disease in population groups. The result was a descriptive epidemiology.

As activities extended to the correlation of nutritional disorders with other diseases of a population, the straightforward methods of operational and descriptive epidemiology were insufficient. Changing incidence over time, multiple environmental factors, and cultural characteristics of the human host were important variables requiring a more sophisticated epidemiology (Williams, 1964; Ramos-Galván, 1965). Hypotheses to explain the complex pattern of causality had to be recognized and defined, then tested under representative conditions, with adequate controls, using quantitative measurements. At its best, this is field research in nutrition, and the method, that of analytical epidemiology.

In many countries, the effort expended on epidemiologic investigation of nutritional health and disease now ranks closely with that devoted to communicable diseases and injuries. Field studies have also extended to primitive places, ill-defined environmental conditions, and people whose customs and habits are little known (Jyothi et al., 1963; Marsden, 1964; Pharaon et al., 1965). Such investigations are informative, since the problems of nutrition are not only universal, but often clearest in less developed areas. Epidemiologic procedures find further application in food science and food technology—for example, in establishing the value of the iodization of salt (Marine et al., 1923). The incentive to develop Incarolina (Scrimshaw et al., 1961) as a cheap protein-rich food for young children and the demonstration of its desirable characteristics came largely from field investigations.

This does not imply that reliance has to be placed mainly on field study for furthering knowledge of nutritional disorders or any group of diseases. As Winslow (1948) stated, in the laboratory all factors can be held constant except the one studied, the influence of which can thus be determined in
definite and precise fashion. But the factors kept constant in the laboratory experiment may vary critically under natural conditions. Elimination of this variability may lead to conclusions that do not correspond to actual phenomena in nature. In both the laboratory and the field, serious errors are possible unless clinical identification of the condition studied is sound. All three approaches—laboratory, clinical and field—are essential and interdependent. Emphasis in a particular investigation frequently turns from one approach to another or combines all three. Clinical investigation requires laboratory support (Todd, 1960), and laboratory experiment without the test of applicability in clinic and field (Francis et al., 1955) lacks full proof. So, too, field procedure uses laboratory and clinical facilities in the measurements made, although modifications are often necessary to fit field conditions.

These interlocking interests are the main justification for presenting here the principles of the field practice of epidemiology. Many investigators competent in clinical or laboratory research are called upon to supplement their investigations by field trial. Too often this is done without appreciation that a well-ordered experiment in the field requires the same scientific exactness and care as a clinical or laboratory investigation. The essentials are adequate controls, appropriate selection of sample, and the use of statistically significant numbers. Careful definition of criteria for recognition of observed phenomena and established methods of measurement are indispensable. Sir George Pickering, in his Harveian Oration (1964) before the Royal College of Physicians of London, characterized science as an occupation not peculiar to laboratories: “The fact is that there are some questions that can be answered only by work in the field, which includes the clinic. Because a piece of work is done in the field does not make it less scientific. Because a piece of work is done in the laboratory and with elaborate apparatus, does not make it good science.”

Field studies are individually distinctive. Each has its motivation, incentives, procedures, and mode of attack. Each varies with time, place, and person. Some are associated with emergencies of short duration, the primary aim being practical control or amelioration of the situation. Others deal with persistent problems in which the need is to know origin and behavior as a means toward effective prevention. By its nature, epidemiology involves geographic pathology and is international in its interests. Socioologic and anthropologic considerations are also prominent, because so much of human disease is man-made. The three kinds of field study—operational, descriptive, and analytical—nevertheless share a common plan of action.

The Epidemiological Society of London was formed a hundred years ago, an event that conveniently marks recognition of epidemiology as a scientific discipline. The study of disease in nature is far older, for it reaches back more than two millennia to the *Airs, Waters, and Places* of Hippocrates (Adams, 1849). And yet, in 1942, the *Journal of the American Public*
Health Association asked editorially: "What and who is an epidemiologist?"

The answers differed so greatly (Winslow, 1948) that the question generally
remained unsettled. Indeed, much the same query was repeated by Terris
(1962), and the answers were equally diverse (Payne, 1962; Schweitzer, 1963;

An expanding field of interests within the past fifty years (Winslow et al.,
1952) accounts as much as anything for the varied interpretation of the scope
and content of epidemiology. The acute infectious diseases were once the
sole concern. Epidemiology now includes within its sphere of interests all
major community disease, notably chronic degenerative, metabolic, and
neoplastic processes, nutritional deficiencies, injuries, and mental and behav-
ioral disorders. Epidemiologic method, too, has progressed from simple
observation and description to analytic procedures designed to identify
through experiment (Hill, 1953) the causes of origin and behavior of
community disease. These developments have resulted in complicated
techniques drawing on many more scientific disciplines than just microbiol-
yogy. Epidemiology today is specialized in so many directions that at times
the appreciation of its fundamental characteristic comes close to being lost.

This primary attribute is the study of disease under natural conditions
in which a human population is the basis for observation. Within a general
population, the typical unit is a family or other group living together.
Epidemiology recognizes no boundaries as to the size or nature of the popu-
lations it studies, the "field" comprising such general populations as residents
of a village, town, or city; of an apartment house or a neighborhood; or of a
province, district, or nation, along with their associated environments.
Selected populations are persons within an institution, a factory or industry,
a hospital ward, or an army. The principle that epidemiology deals with
populations living under "field" or natural conditions and viewed collectively
rather than as an assembly of individuals is about the limit of common
agreement.

Scope and Content of Epidemiology

The epidemiologist approaches his field in a number of different ways
and with a variety of objectives. In his judgment of scope and content, he
usually evidences the wholly human propensity of favoring that with which
he is most familiar.

Any appreciable experience in epidemiology soon dissipates the rash
belief that agreement on "What and who is an epidemiologist?" will result
from deductive reasoning or any form of logic. But it may be worth while
to describe what happened and what has been seen in our collective experi-
ence, which has taken us from Hammerfest to Cape Town and circled the
tropics and the Arctic. Inevitably, a concept of principle in field work
evolves from interests as diverse as the acute communicable diseases, chronic non-communicable disorders, nutritional deficiencies, and the population problem. If illustrations used in this discussion are often from communicable diseases, it is because that is where most epidemiologic principles originated, despite the wider application today of field investigation.

The views expressed are far from wholly personal. They derive in large part from scores of former associates, more than any other from F.F. Russell. In his direction of investigations of yellow fever for the Rockefeller Foundation (Strode, 1951), he set an enduring pattern for study of disease — disease as exhibited broadly in clinic, field, and laboratory.

**Tracing reservoirs and sources of infection**

Early field work was largely operational epidemiology in communicable disease, an emphasis that still holds globally. The practical purpose was to identify sources of infection in the origin of epidemics (Goffman, 1965), to introduce indicated control measures, and to formulate preventive practices to ward off subsequent outbreaks. It soon acquired the highly descriptive name of “shoe-leather” epidemiology: the method was house-to-house enquiry, and the procedure was observational and descriptive. Where the source of the epidemic was traced to contaminated water, milk, or food, identification of the immediate source of infection was often enough for institution of proper control measures. Further recognition of reservoirs of infection, usually man—either as a patient or as a carrier—increased the chances of avoiding future epidemics.

An improving public health practice soon permitted the addition of individual case study, the epidemiologic case having as the unit of observation the household, which includes the patient and familial and other close contacts, along with the immediate environment (Gordon, 1965). Case study began with sporadic infectious diseases likely to evolve into epidemics. It came to include industrial poisonings, cancer, and other non-infectious processes. Eventually, as with tuberculosis, case study developed into case finding, with the purpose of discovering unrecognized illness. The gradual incorporation of laboratory procedures into field practice was an important result of these more complex activities.

Epidemics in the USA and many other countries are now less frequent, yet their investigation continues as the major obligation of epidemiology in official health agencies. With local differences, the prescribed diseases requiring case study also include nutritional deficiencies, poisonings, air pollution, and traumatic accidents. The tracing of sources of infection is not wholly routine; from time to time the epidemiologist faces the unknown as a fresh disease invades—St Louis encephalitis in Florida (Bond et al., 1963), hepatitis in New Delhi (India, Ministry of Health 1956) or cholera in Bangkok (Siddhichai & Grayston, 1960).
Administrative control of disease

In most health departments, the application of control measures, as determined by field analysis, is also the epidemiologist’s obligation (Rogers, 1963; Hilleboe & Larimore, 1965), especially emergency measures to remedy a deficiency in water supply, milk production, distribution of food, or the management of disease carriers. Other administrative duties (Anderson et al., 1962) include the planning and conduct of programs for specific immunization against diseases such as smallpox and diphtheria, based, in turn, on a preceding evaluation of existing levels of protection. Periodic surveys of disease occurrence are necessary to determine efficiency in disease reporting (Feemster, 1947). Recognition of changes in periodicity of endemic disease (Sartwell, 1965) and watchfulness for newly introduced infections (Comings et al., 1962) are continuing obligations.

All activities thus far noted fall within the scope of operational epidemiology. They are the means by which official health agencies seek to restrict disease. The larger goals of prevention require understanding of the broad biological behavior of disease. Thus, epidemiologic divisions of health departments increasingly assume investigative responsibilities, of greater or lesser complexity, commonly in collaboration with voluntary health organizations or academic centers concerned with medical research. This brings into play a descriptive epidemiology, still based on observations in nature, but emphasizing one of several other approaches.

Identification of agents of disease

Epidemiologists long ago added laboratory procedures in order to support the findings of traditional field study through identification of the inciting agent. Observations starting in the field are often carried to the laboratory, and those starting in the laboratory may end in the field. Both approaches are epidemiologic if they pertain to disease as it affects a human population.

There are differences in the relative emphasis placed on the two approaches, determined usually by the particular problem. When an outbreak of intestinal disease is clearly the result of food poisoning (Rubenstein & MacCready, 1953; Googins et al., 1961; Cockburn et al., 1962), the invaded population and the environment are the obvious objects of study in determining the source and instituting control. The laboratory identifies and confirms the suspected agent, although frequently not until the explosive episodes have ended. But, if the objective is to define the frequency and distribution of the reservoirs of infection, as in prevalence surveys of Shigella (Gordon et al., 1962c), or the significance of carriers in epidemic spread (Weiss, 1965), the laboratory dominates activities, with the field responsible mainly for proper selection and collection of specimens. Under other circumstances, as in community outbreaks of staphylococcal disease
with an origin in hospitals, both laboratory and field must make their full contribution (Nahmias & Eickhoff, 1961).

Several epidemiologic procedures of great merit have originated in the laboratory. The recognition of type differences within species of infectious agents permits sources of infection to be identified and aids in tracing lines of spread (Aycock & Foley, 1946). Through identification of antibodies in blood from representative fractions of a population (Paul, 1952), serologic methods demonstrate frequency of past infection and the limits of geographic areas involved (Tigerl et al., 1962). Differences in age distribution indicate when former epidemics occurred. Measurement of genetic markers, such as blood groups, certain enzymes, and abnormal proteins, helps in understanding hereditary influences (Blumberg, 1961). Comparison of successive samples of serum taken at short intervals from the same person establishes the occurrence of an active infection. Microscopic pathology serves similar purposes (Soper et al., 1934)—for example, suspicion of yellow fever or leishmaniasis may be confirmed by examination of punch biopsy specimens of liver tissue. Micro-analytical examination of the blood is used to determine nutrients in the field investigation of nutritional disorders (Arroyave, 1962).

Reference laboratories under the auspices of the World Health Organization are an application of laboratory epidemiology on a global scale. Located in various parts of the world are centers for influenza, salmonellosis, leptospirosis, and poliomyelitis. The purpose is to determine geographic distributions, the movement of communicable disease, and the nature of the prevailing infectious agent. Type determination and the demonstration of antibodies are made from specimens transmitted by regional laboratories.

The principle of associated laboratory and field investigation in epidemiology derives chiefly from the acute communicable diseases. Although it is not so widely employed in non-infectious diseases, its advantages have been demonstrated in chronic degenerative processes (Scrimshaw et al., 1957b), malnutrition (Béhar et al., 1960), lead poisoning (Ingalls et al., 1961), congenital anomalies (Ingalls, 1956), and injuries (Haddon et al., 1961). The laboratory contribution is no less important because the techniques are physiologic, biochemical, or enzymologic rather than microbiologic. Field investigation in these newer spheres of population pathology often fails to realize its full potential because of lack of laboratory support.

Geographic pathology

One of the oldest approaches to epidemiologic understanding is to compare the incidence and character of a disease in different places. Hippocrates (Adams, 1849) stated the principles of geographic pathology more than two thousand years ago; August Hirsch (1883-1886) crystallized them in his three-volume work of 1860; and the Second World War brought renewed
appreciation of the practical value of geographic pathology (Simmons et al., 1944). Modern travel (Lafontaine, 1964) has made the health problems of individual countries the problems of the world, with a resulting resurgence of interest in the geography of disease (May, 1958, 1961), at national (United States, National Academy of Sciences, 1962) and international (Doll, 1959) levels.

Although infectious disease has been the chief concern, other mass diseases and injuries are studied advantageously in this way. To determine what diseases occur, geographic pathology uses clinical methods, gross and microscopic pathology, and, for the infectious diseases, microbiologic techniques. When the question is why the observed differences occur, in time and person as well as in place, the effort becomes specifically epidemiologic.

Early work in geographic pathology was concerned mainly with disease in relation to the physical features of the environmental complex. To determine causality, epidemiologists have to consider the biologic surroundings, especially food and food resources, and also the social environment. Both enter into cause and behavior of disease. The epidemiologist, unlike the geographic pathologist, commonly studies disease in a limited area and within a fixed population. He shares a global interest with the pathologist when he extends his observations to the broader reaches of time and place. Current studies such as the inter-American atherosclerosis project (Tejada et al., 1958), a major concern with cancer (Council for the Coordination of International Congresses of Medical Sciences, 1950), continuing efforts with communicable disease (Philip et al., 1949) and, more recently, with accidental injuries (Gordon et al., 1962b) illustrate some of the more common interests.

Serious difficulties in interpretation arise when results of independent workers are assembled in an endeavor to compare the behavior of a specified disease in separate localities. Differences in host and environment expectedly produce variations in morbidity, mortality, and other characteristics. Equally significant inequalities commonly derive from dissimilar criteria for diagnosis of the disease itself and for measurement of the observed characteristics. Differences in training and methods of approach of investigators also contribute to the difficulties of interpretation. The only reasonable solution is adoption of common criteria and techniques by workers in a specified field (Acheson, 1965).

**Clinical epidemiology**

A clinical understanding of disease or injury is fundamental to all epidemiology, for disease can scarcely be studied in its group manifestations unless properly identified in individuals. One novel use of clinical methods is in the control of threatening or expected epidemics. In the European Theater of Operations during the Second World War, isolated and restricted
outbreaks of an unusually acute respiratory disease were noted by Gordon (1949) in August 1943. Middleton (1947) established clinical listening posts in selected localities throughout the command, assigning experienced physi-
cians to report changes in the prevailing clinical nature of respiratory
disease among patients admitted to hospitals. The epidemic of influenza
that came in November 1943 was no surprise; adequate facilities for medical
care and administrative control were ready. This principle, often com-
plemented by laboratory procedures, has been adapted to worldwide control of
many diseases. The results have proved its worth.

Clinical epidemiology has another and special connotation. As epidemi-
ologic interests have extended from large-scale epidemics to sporadic and
isolated foci of infection, to behavior of the individual patient in relation to
his family, his environment, and close associates, an intermediate activity has
developed, which may be termed "small-group" epidemiology. Here
investigation is by the clinician, commonly the pediatrician, rather than by
the epidemiologic specialist. This is a natural outgrowth of the interest of
practitioners in preventive as well as curative medicine.

Spence (1950), in Newcastle-on-Tyne, has expressed the need for the
clinician to extend his observations by research in family practice and by
field survey of random samples of the population. John Paul (1958) has
presented methods, principles, and sound examples of how to do it.

**Statistical epidemiology**

Irrespective of method or technique of investigation, all epidemiologic
procedure involves mathematics, sometimes no more than simple arithmetic,
in other instances intricate statistical methods to establish true correlations.
In research epidemiology, statistical procedure desirably runs throughout the
study, having a part in early design, during observations, and ultimately in
the interpretation of results.

Statistical method is often the main epidemiologic tool and is applied to
data from many sources. Trends in frequency over time, in rates of occur-
rence according to place, and in differences in kinds of persons affected are
sought (Hamer, 1906; Segi, 1960). Statistical epidemiology also considers
changes in the character of a disease and the resulting fatality, a method
used by Chapin & Smith (1932) to prove that smallpox breeds true in two
lines, the mild alastrim and the classical variola.

The sources of data include official reports of deaths and illnesses in a
community, clinical case histories, and the records of autopsies, as well as a
variety of supplementary information. Some data are collected for a specific
purpose; more are derived from periodic reports of official, voluntary,
or industrial health agencies. Sometimes they come from sources not
connected with the particular project, and commonly they have inherent
deficiencies.
A statistical approach is fruitful so long as the limitations and biases of the original data are recognized. Witts (1964) and MacMahon, Pugh & Ipsen (1960), among others, have discussed these obstacles in detail. They include differences in diagnostic standards, medical practices, and methods of reporting, from place to place and among observers. Information may not exist for specific population fractions; sources are generally inadequate for common illnesses such as acute respiratory disease, the diarrheas and the dysenteries, and the acute communicable diseases of childhood. All too often the result is a presentation with all of the statistical niceties applied to unreliable and inadequate data. On the other hand, solid observations from laboratory or field have suffered from deficiencies in statistical interpretation.

Statistical epidemiology has contributed to theoretical epidemiology through statistical models that explain epidemiologic phenomena, develop new concepts, or confirm others arising from experiment or observation. This feature was first brought to prominence by Ross & Hudson (1931) in their work on malaria, based on earlier contributions by Brownlee (1919) and by Greenwood & Yule (1914). Recent contributions include Muench’s work (1959) on catalytic models and Bartlett’s (1960) use of stochastic procedure. The characterization of these activities as arm-chair epidemiology is not always justified. Many contributors are themselves qualified field investigators using field data.

Field survey

An accumulating knowledge of epidemiologic theory and the introduction of quantitative methods have led eventually from descriptive procedure to an analytic discipline. Considerations of how disease originates in a population and why it behaves as it does are now among the chief concerns of epidemiology.

The field survey is a cross-sectional study seeking to determine the number of cases of disease, or of persons with some other attribute, present at a particular time and in relation to the size of the population from which they are drawn. Technically it is a prevalence study, and in its simplest form a determination of point prevalence, prevalence at a specified time. The objectives are various: to assess the health of a community under normal conditions (U.S. Department of Health, Education and Welfare, 1962), to determine occurrence of a particular disease (Fleck et al., 1960), or to measure the nutritional state of a population (Jelliffe & Jelliffe, 1961; Jelliffe et al., 1961).

The main approach in a field survey can be clinical, laboratory, or the traditional field investigation. Usually the three combine to advantage, adding precision to the study. Each has its special usefulness and, even if functioning alone, is epidemiologic so long as the information gained represents a specific field situation (Winslow et al., 1952).
Although outside its specific purposes, the field survey often leads to factors in causality, which sometimes are confirmed by subsequent laboratory study, or justify a long-term incidence study. The field survey is less exacting in time and expense than the incidence study, but the frequent attempt to use it as a shortcut is much overdone. Repeated surveys at established intervals, the determination of periodic prevalence, increase the potentiality of determining causative factors.

Field incidence study

The prospective long-term field study concentrates upon incidence rather than prevalence, and its usual objective is causality rather than distributions and behavior of a disease. When experimental principle is introduced into field study, data are collected according to a preformed plan, with conditions defined, constants established, and controls provided. The Baltimore studies on syphilis of Clark & Turner (1942) illustrate method and principle for chronic infection; the Washington studies of Rowe and co-workers (1957), for acute respiratory disease; those of Reinhard (1963) in the western Arctic, for enteroviruses; and the Cleveland family studies of Dingle and associates (1965), for the general trend of several diseases in an open community, all with an emphasis on causality. The method finds increasing application as chronic diseases and injuries replace acute infections as the dominant epidemiologic interests in industrialized countries. The inherent prolonged nature of these conditions makes long-term observation essential. The Framingham heart study (Dawber et al., 1951) is projected over 25 years. The INCAP study of the synergistic action of nutrition and infection in Guatemala (Béhar et al., 1958b; Scrimshaw et al., 1967b) covered a ten-year period. Ultimate understanding of rheumatoid arthritis, cancer, and other currently prominent problems in population pathology would seem to rest in this approach (Reed, 1949).

Experimental epidemiology

The purposeful attempt to explore general laws and principles of mass disease by direct experiment with populations of animals was a natural evolution of field study of humans. Infectious agents peculiar to the species permit study of isolated factors in epidemic causation. Despite the important results of initial studies by Greenwood and associates (1936) and by Webster (1946), this method has had little recent attention except for Schneider's work on nutrition and salmonellosis (1951) and that of Fenner (1959) with myxomatosis of rabbits.
A Concept of Epidemiology

These several approaches to epidemiology have a single purpose: to improve understanding of disease as manifested in groups of people. But they differ in specific objectives and the kind of problems they seek to solve. Each attracts both persons primarily concerned with population pathology and investigators with a primary interest in some other science. Each worker tends to see his special world most clearly and, understandably, develops at times a fragmented idea of the scope of epidemiology. And yet, all of these approaches are "epidemiology", an opinion that the roster of the American Epidemiological Society amply supports.

The unit of observation, a human population, is the common denominator. Therefore, epidemiology deals with population pathology, not clinical pathology (disease of the single person), nor microscopic pathology (disease of the cell or tissue). All population pathology relates back to the field as the source of materials for investigation, and results are interpreted in terms of the group, not the individual. If the circumstance is otherwise, the work can be medical statistics, biochemical or microbiologic research, or clinical investigation, tangential and often contributing importantly to the broad base of knowledge upon which epidemiology rests; but it is not epidemiology.

Although many workers in epidemiology are familiar enough with the broad field to be classed as epidemiologic generalists, specialization is inevitable in a field that extends through the whole of medicine and public health. Competence is often limited by interest and by training to a skill in one general activity, occasionally in several, rarely in all.

Of the nine field activities just distinguished, the first two, the search for origin and source and for administrative control of disease, are grouped as operational epidemiology, their main function being to support the practical work of public health agencies. The next four, the identification of disease agents, geographic pathology, and the clinical and statistical approaches, may be characterized as descriptive epidemiology, primarily dependent on observational methods, concerned with disease behavior, and directed toward an improved prevention. Field survey, incidence study, and experimental epidemiology constitute analytical epidemiology and are research procedures pertaining to the origin and behavior of community disease.

These are neat categories, but far from rigid. Alert observation during routine operational activities has resulted repeatedly in important contributions to causality. The descriptive approach frequently uncovers leads that permit specific investigations, and statistical and laboratory epidemiology have important and independent functions in theoretical epidemiology.

Epidemiology thus has many interlocking interests, and multiple and sometimes devious approaches. It brings together a variety of scientific disciplines and a diversity of skills and techniques. Still, the one dominant
feature, the central theme from which there is no departure, is the relation of a disease process to a population living or working in a natural environment.

For years epidemiology has recognized the potential of some unifying discipline to provide a common ground for interpretation of the biological, social, and psychological characteristics of groups of people as they relate to health and disease. Ecology, its province being the relationships of living organisms to their animate and inanimate surroundings, finds increasing application in this capacity. As a part of human ecology, medical ecology supplies the principles for understanding disease as it affects aggregates of people (Winslow et al., 1952). Because ecology is a branch of knowledge both social and biological, it serves epidemiology well, for epidemiology is an applied science (Francis et al., 1955), at times requiring such divergent skills as those of physician, mathematician, anthropologist, biochemist, or meteorologist.

**Conduct of Epidemiologic Field Studies**

**Nature of field investigation**

The essence of epidemiologic analysis is work in the field. Even with a main stress on clinical, laboratory, or statistical procedure, someone must collect the material for examination; and the integrity and completeness with which that is done determine whether the results reflect population pathology or are a contribution to some one of many allied disciplines.

Field study of disease has no special individuality, whether operational and applied, or investigative and research. It is the scientific method applied to a particular circumstance. To speak of epidemiologic method is thus as justifiable and convenient as to speak of clinical or chemical method. Although reinforced with refined clinical procedures and laboratory techniques, the everyday, house-to-house observation of earlier years continues as the fundamental feature.

Field study begins with inferences and construction of hypotheses drawn from established facts about the behavior of disease. The information is from many sources, partly from other field investigations, but more often from the broad base of knowledge that exists in the biological and social sciences. Success requires the same planning essential to any experiment or other careful observation, including statistical considerations as to frequency of event, the necessary length of observations, and the size of population required.

Field research these days is usually a team effort, employing a variety of ancillary skills in support of a principal investigator. The community is the laboratory and, in contrast to some other approaches to population pathology, has the advantage of first-hand familiarity with the people and their
environment, and a knowledge of the strengths and weaknesses of the data collected. Modern methods of machine tabulation, computer analysis, and statistical manipulation have obvious value in dealing with long-term projects with much data, but the interpretation of results remains rooted in the value judgments of the worker in the field.

The limitation of field study to communicable disease has disappeared, although adherence to that restriction persists to an extent in clinical and basic science circles. In contrast to the lively and divergent views on scope and method, the techniques of field study have had less attention, although they are a critical and common factor. One explanation is the lack of practical experience in field operations during courses of instruction in epidemiology, of a specific effort to provide a training comparable to that of the clerkship in clinical medicine or the laboratory apprenticeship in the biological sciences. The academic epidemiologic laboratory does present principles of data collection (Anderson et al., 1962) and provides training in the analysis and interpretation of raw data, but little or no first-hand experience in the accumulation of that material. Skill in these matters usually is empirically acquired through service in a working organization. The recent establishment of a few fellowships for field training indicates the need for a more orderly development. Only too often field work is not in the best tradition of bench research, because of inadequate attention to method and materials. An all too frequent attitude is that little is demanded beyond visiting homes and talking to people, activities that require no particular skill.

Epidemiology demands more than this haphazard approach. A definite pattern is believed to permeate field endeavor, whether operational or investigative, whether a simple epidemiologic case study or an investigation of an epidemic, whether the complex prevalence survey or the still more intricate incidence investigation.

The conceptual idea

The decision to initiate a case study or to investigate an epidemic raises few questions of what to do or how to go about it. The operational procedures are a normal obligation of official health agencies, and the incentive is prescribed by regulations. In the ordinary situation, experience and tried procedures guide activities, and the objective—to institute prompt control—is definite. Occasionally, the decision for action is elective: an unusual local circumstance of no emergency nature attracts the interest of the investigator.

Help is sometimes demanded by other jurisdictions or even a foreign country when control is beyond existing resources, as in the recent cholera epidemic (SEATO, 1960) in Thailand and the Far East. Procedure is not always so simple in these situations. The cause of the trouble may be obscure, the circumstances or the size of the problem, overwhelming.
Hundreds of deformed infants were born before thalidomide was recognized as the inciting agent (Ingalls, 1962; Lenz & Knapp, 1962) and the situation remedied.

The research endeavor, the field survey or the incidence study, is of another order. Unlike operational ventures, these are voluntary undertakings, without predefined obligations. The urge is for a logical answer within the confines of practicality. Both the conceptual idea and its orderly development are important features, to be expressed finally as a hypothesis capable of test.

There are many compulsions to research, some of which lead to ideas for field study. An insistent need for better methods of prevention and control is a common incentive (Beck et al., 1962). Motivation also stems from less material things, such as a conviction that medicine should right the ecologic imbalance resulting from its success in controlling deaths by an attention to control of births. Plain academic interest, the need to know why things happen, is just as reasonable a stimulus; for the value of an established fact is not necessarily allied to its immediate usefulness. Many times the investigation is an intermediate, yet crucial, step in realizing a broader objective; the whole field of yellow fever research opened up once the rhesus monkey was established as a satisfactory laboratory animal in Lagos, Africa (Stokes et al., 1928). Occasionally, the simple challenge of the difficult provides the impetus, the sort of thing that sends men to the top of Mount Everest. Epidemiology has many such challenges (Kurland, 1958; Kurland & Westlund, 1954; Plunkett et al., 1960).

No formula exists for acquiring the critical idea. Likely ingredients are inspiration, chance, and genius, in combination or alone. As for chance, a famous dictum says that chance favors the prepared mind; and the prepared mind depends on experience and explicit knowledge in the same or a related field, searching always for analogies. Familiarity with procedures others have tried and discarded proves useful. A spirit of creativity is what really is needed, but that cannot be called up at will. In its absence, speculation may have to serve as a necessary but weak substitute.

Few ideas are born in their maturity. Studied reflection, or a period of allowing the idea to lie fallow for a time, may aid in the initial effort to form a concept. And after that, there is still work to be done in its evaluation, organization, and refinement before turning to the practical means for an answer.

First comes a statement of objectives and of the exact questions to be answered by the investigation in relation to long-term demands of the problem. This defines the dimensions of the contemplated study, its significance, and its relation to the general field of interest. Relative urgency is a consideration: whether the study must be done now to take advantage of a unique situation, or can be done equally well within the foreseeable future, or even postponed indefinitely without loss.
Finally, with objectives established and procedural methods projected, it is time to consider practical questions. Is there a suitable population available; can requirements in time, staff, and materials be met in relation to existing or potential resources? Attention turns seriously to the prospects of success in accomplishing the purpose of the proposed investigation. Scope and direction are reviewed in detail. This requires background information; to an extent it merges into preliminary planning; and this, in turn, leads to the formal design of experiment. An afternoon in the library may suffice for these purposes, or months may be necessary. The time depends on the problem, whether it is relatively familiar or a step into the unknown.

A review of the literature on what has been done tells of successes and failures, in relation to the particular problems and to the general field. Consultation with colleagues is in the normal course of events; the most valuable help often comes from a seasoned investigator, not necessarily expert in the particular field, but able to view the project with an objectivity derived from long experience in biologic research. Visits to places where similar studies are in progress are helpful.

Primarily, however, this is a time for independence of thought and action. More time than is profitable can be spent in accumulating background. Excessive delay often arises from unjustified doubts or the stifling of good ideas by conflicting opinions. There comes a time for decision. If there are serious reservations, the idea should be put aside for another day. With reasonable assurance that the argument is logical and that the operations are feasible, a hypothesis or hypotheses are formulated for test. The field study is on its way, with no uncertainty now about the next step.

Field reconnaissance

Preliminary assessment of the particular locality where the work is to be done is one principle in field investigation that should never be violated. There is no substitute for personal observation. The time necessary for reconnaissance varies with the problem, yet it is as much a part of case study and epidemic control as in the more pretentious field research of long duration.

In operational procedures, the initial survey is brief, because the investigation is of limited scope, the course of action, conventional, and the objective, definite: prompt control. An hour may suffice for a preliminary survey of poliomyelitis in a boys' camp (Rubenstein et al., 1948) and perhaps a day in scattered outbreaks, such as that of Eastern equine encephalomyelitis in Massachusetts (Farber et al., 1940). The first obligation is to establish working relations with local health and administrative authorities and, through their aid, to consult with physicians who have been seeing most of the cases.
Taking precedence over all else is a visit to the last reported patient. In a communicable disease, the object is to learn the nature of the infection, to obtain leads on lines of transmission and modes of transfer, and, if a chronic, non-infectious condition is involved, to establish clinical familiarity and to probe for possible sources of origin, which may involve anything from the habits of bus drivers to why children eat paint. Nothing must interfere with this visit, because it is there that the scent is freshest. What is found more or less establishes the plan for action.

The long-term research activities, field survey and incidence study, require more detailed reconnaissance. Since the proposed investigations will extend over appreciable periods, sometimes several years, working arrangements have to be established on a fairly permanent basis. Many such studies are in foreign countries, which multiplies the need for detailed preliminary information. A week of reconnaissance is little enough, and a month or so may be necessary. Technically, now is the time to start a field diary. Details escape, especially names, titles, and addresses—information of much value in the eventual planning of the study.

Good working arrangements with local authorities are so important that the principle is to neglect no one; omissions have been the source of future difficulties or even failure of a project. It is just good sense to start with national or state health authorities and, through them, with administrative departments, but not to the neglect of local officials. There is where the work is to be done, and it is a sound investment to establish solid understanding of what is proposed, to enlist the desired co-operation, and to assure appreciation of the importance attached to the community contribution.

In regions unfamiliar to the investigator, and especially in a country other than his own, personal knowledge of people, language, terrain, and facilities within the study area enters strongly into planning. Professional colleagues are helpful but, being themselves of the culture of the country, scarcely can reflect the reactions to strangers, or the effect of customs, habits, and traditions on the results. In rural India, Gordon and Taylor (Gordon & Wyon, 1960) pitched a tent against a village wall and lived in the community for a week, spending the days in the fields with farmers or in hunting trips with villagers as guides. In the evenings, scores of men gathered around their open fire to discuss the problems of the universe. In that atmosphere, one important item was settled: work in these simple communities was feasible.

Locating a place for field headquarters is an important job because residence within the study area, whether it is rural or urban, contributes to the success of this type of study. Local transportation almost always poses a greater problem than expected. When the study area is rural with a relatively large population, substations are needed on a village basis. Residence in the more comfortable quarters of a nearby city and visits to the natives do not compensate for the advantage of becoming an accepted part of the community.
Other considerations include the available numbers, competence, and reliability of local recruits to the technical staff, with bilingual ability sometimes a feature. Local records of reported births and deaths warrant scrutiny to determine their probable reliability and completeness and the extent to which they are representative of the more readily available data for larger political units. In underdeveloped countries, the lack of large-scale maps and census data for local communities is frequently a practical problem. In such ways reconnaissance seeks to determine the probable size of the job, the critical points of attack, the needs for staff and equipment, and the special skills demanded. This preliminary information determines just how feasible is a realistic design of experiment.

Experimental design

Whether a field study is operational or analytical, emergency or long-term, the differences in people, in terrain, and in time and rate of occurrence of the events to be observed are such that field investigations differ in plan of action, even with regard to the same disease.

The several planning stages in a proposed investigation are (a) organization, including selection of staff, equipment, and supplies; (b) field test of materials and methods, to determine suitability to the immediate problem; (c) pilot study under controlled conditions for presumptive determination of expected results; (d) definitive investigation; (e) analysis and interpretation of the findings; and (f) the suggested or defined direction that subsequent investigations should take in further development of the problem. All steps may not appear to enter directly into every study plan, but only because the procedures involved are implicit, and through long experience accepted as standard operating procedure.

The construction of an ecologic model, a theoretical illustration of the relationships between host, agent, and environment, is a major feature in developing the study plan. The model's purpose is to facilitate an answer to the formulated hypothesis, through definition of the several procedural stages just presented. The plan of a field study includes technical methods and materials to be used, and statistical assurance that the answers obtained will have significance, whether positive or negative. The facts accumulated when hypotheses were formed and during field reconnaissance are the basic materials. The plan is the guide to action—the what, when, where, and how to proceed.

Epidemiologic case study, as the day-to-day work of official health agencies, has through long experience evolved a standard operating procedure, originating nevertheless in the principles just stated. The plan is thus prefabricated, and the concern is more with choice of techniques than general procedure. It is extemporaneous and follows directly from the findings of the preliminary reconnaissance.
In epidemic control, preliminary reconnaissance has determined whether the disease is familiar or obscure, whether the outbreak is large or small, of sharp evolution or protracted course. Under any circumstance, prompt decision is required on the course of action, and much depends on proper appraisal. Errors in judgment have resulted in putting up an umbrella to stop a flood or, conversely, starting to build a dam in the face of a first sprinkle. In essence, control of an epidemic involves assembling a series of case studies and determining the connection of disease in one family unit with that in another and whether the infection relates to a common or a propagated source. The investigation may end in a day or two, the situation being little more than an enlargement of a case study to be completed by the principal investigator alone; or it may last weeks and require marshalling special laboratory facilities and an appreciable field staff. Planning, therefore, centers on an assessment of the situation. The objective is clear-cut: to identify the origin of the outbreak and institute prevention and control. The urgency of the situation necessarily puts reliance on previous experience and established procedure.

Like all research endeavors, the field survey is an elective undertaking with a studied course of action. Requirements for staff and materials are complex because of longer duration and the individual nature of the study. Technical methods frequently must be tested for suitability to the particular problem and to field conditions, or new ones may have to be developed. Statistical computations, based on expected rates of occurrence, are necessary to set the length of observations and the size of population needed (Fellingham, 1966). Because interpretation is usually through comparison, either of findings determined in other regions or of behavior at other times and under different environmental circumstances, the survey is without organized controls.

The prospective incidence study, in its customary form as a true field experiment, involves a major planning endeavor. Since controls are an inherent feature, it is necessary to build the experiment on an adequate mathematical and ecologic model. Most of such studies are long-term investigations and therefore require extensive attention to staff and materials. Many are in countries foreign to the investigator, so chosen because of special conditions favoring the investigation or the wish to compare known findings in one region with those in other places. The required methods are often original, or at least untested for the purpose, or must be modified to a new situation. Consequently, few shortcuts are possible in constructing the broad plan for action. This is no place for magpie methods, for the indiscriminate collection of information.

Because of its duration, the incidence study normally functions best through an established field headquarters, with the staff living and working in the study area under more or less permanent conditions. Housekeeping as well as technical operations thus enter into planning procedure. Most
other field studies are conducted advantageously from a central base of operations or from temporary headquarters in the local area.

The completed design almost invariably incorporates plans for exploratory study, a pilot investigation, and the main endeavor. The principles governing each are given in the discussion to follow. If the definitive study is of short duration (a year or so), observations sometimes proceed directly from exploratory study to that objective. Today, many studies are projected over several years, and decision for a pilot study with controls, under the general conditions of the main experiment, is usually wise.

Since so much data will accumulate during the proposed investigations, a noteworthy technical consideration is to prescribe methods of assembling and recording data in a form that permits mechanical sorting and tabulation. The fundamental need in experimental design is for epidemiologic sense and experience. As Greenwood (1935) once warned, a proposed line of investigation may be logically unassailable, statistically impeccable, and biologically ridiculous.

Field organization

Aside from reconnaissance, properly a part of planning, the first step in active field operations is to institute administrative procedures, select a staff, and assemble the necessary supplies and equipment. This is the technical aspect of field study.

Little is demanded in organization of resources for case study or epidemic control because the official health agency ordinarily responsible for such activities keeps the essential equipment and supplies in readiness. Case study is a one-man endeavor; and, except for co-operation of local health personnel, so is epidemic control. If assistants are needed in large outbreaks, trained and experienced workers are available in the central agency. Beyond what is needed for clinical examination, not much field equipment is necessary. A camera is a useful item; but, in general, satisfaction and accomplishment are inversely proportional to the number of kilograms of baggage.

Preparation for the field survey or the incidence study is more detailed. In contrast to operational epidemiology, organization usually starts from scratch, requires appreciable time, and is of studied character. The base of operations ordinarily is a university, a hospital, a research institute, or an official health agency, and has several functions. It provides administrative support, is the source of staff and supplies, and furnishes central laboratory and statistical services.

Sound policy, however, separates a research study from general activities by establishing a field headquarters. The distances involved may make this necessary, especially if the study area is rural, as it so often is. But even if the research activities and administrative headquarters of a central
program for the health problem concerned are in the same city, a separate
field office for the research is highly desirable.

In many situations, the field station is both the focus of working activities
and the residence for staff. If operations involve relatively large numbers of
people and observations are continuous and repetitive over appreciable
periods, as in incidence studies, substations or local centers are established
in strategic sites, either in villages of a rural region or according to census
divisions of an urban population. In general, the substations accommodate
local workers responsible for populations of perhaps 2000 people. The
staff at field headquarters is then restricted to supervisory personnel and to
specialists concerned with the project as a whole.

Requirements for field staff are obviously a function of the problem under
study, and are further influenced by the volume and complexity of the data
to be collected. The field director, ordinarily a physician and epidemiologist,
usually needs at least three key staff members. Large-scale projects, espe-
cially those in foreign countries, require an assistant director, usually
designated from among these three, who assures satisfactory direction of the
project during vacations and other anticipated absences of the director.

The key assistants are the following:

1. A laboratory worker with qualifications suited to the problem at hand
is a primary member of the staff, since few sizable field studies function to
capacity without local as well as base laboratory facilities. This is appreci-
tated for infectious and nutritional diseases; its importance in field study of
other disease processes is increasingly recognized.

2. The statistician of a field study group departs to an extent from his
usual role as an office worker. He may be stationed wholly in the field or
divide his time between base and field station; but, under either arrange-ment, he should participate in active field work sufficiently to evaluate the quality
of record-keeping, completeness of the collected information, and the
methods used.

3. The supervisor of field workers has the task of directing the work of
the day in data collection. This is a most responsible position; and, in the
authors' experience, the public health nurse is likely to be best qualified for
it.

Recruiting general field workers for duties not requiring a physician is
often a problem. The first choice is, again, the public health nurse with
field experience; but often such nurses are not available in foreign countries.
A command of the country's language and a familiarity with the environ-
ment, aims, and outlook of the people make persons of local origin highly
desirable. Auxiliary nurses and social workers have been successful in this
work, as have school teachers or young college graduates once they have
had suitable on-the-job training. For some problems and cultures, both male and female workers are required in order to uncover the needed information.

The administrative and professional acquaintances made during reconnaissance are renewed and put on a working basis. The field laboratory and the field statistical office are established, with a clear definition of responsibilities. Some laboratory examinations can only be carried out satisfactorily in the field; others are far better accomplished at the base laboratory. Thus, the isolation and tentative identification of infectious agents commonly are done in the field, and confirmation and typing, at the base laboratory. Similarly, the statistician must supervise the recording of data and make periodic trial analyses of results in direct conference with field workers, although machine analyses and the more technical procedures are done at base headquarters.

Supplies and equipment for field operations depend on the study. When the authors' field station was in Egedesminde, Greenland, and the base laboratory in Copenhagen, Denmark (Gordon & Babbott, 1959), field facilities were necessarily elaborate. When the base was a few miles away, as in the Guatemalan studies (Scrimshaw et al., 1962), much of the work otherwise done in the field could be accomplished centrally. The guiding principle is to emphasize the practical rather than the theoretical, to add facilities as need is demonstrated, and to respect the admonition to travel lightly.

Equipment for home visits in the field is relatively simple. Large-scale maps locating all households of the study area are a primary requirement. Governmental sources occasionally have such maps, but usually the field staff prepares them. In surveys, the official census often suffices. For long-term incidence studies, however, the study group makes its own census in the course of preparing standard family folders for household visits. Record forms comply with individual features of the investigation, with special attention to exactness in terminology (Payne, 1951), to such an extent that an explanatory code stating what information is wanted and defining technical terms is required for each item of all forms put into field use.

Where house-to-house visiting is the basic procedure and the population is urban or centered in villages, as in many rural parts of the world, travel is by foot. If roads are good, there is no difficulty in getting from field headquarters to local study areas or substations; but in many places the monsoons and other seasonal difficulties require a rugged vehicle, able to travel over difficult terrain. Under certain primitive conditions, bicycle, oxcart, dog sled, and river boats have been used. Another consideration is transport of staff and supplies between field station and operating base. Provision for staff travel to conferences and for meetings of consultants in the field also becomes significant when field work is abroad or done under isolated conditions.
The time required to set up a going organization varies with the project and the plan of operations. A month may suffice; three to six months are more likely, especially in a country foreign to the investigator. It is a peculiar and yet almost invariable circumstance that this phase in the development of a field study seems to take longer than anticipated. Still, the future flow of operations and the satisfaction with the eventual results are strongly influenced by the time given to preliminary preparation.

Exploratory study

The purpose of this first activity in the actual collection of data is to test record forms and techniques prescribed in the experimental design. A second objective is to train staff members new to field work and, often, to research method and principle. When the project represents new or unfamiliar ground, even a seasoned staff requires specific experience.

The exploratory study ordinarily is not a feature of case study or epidemic control. Standardized procedure has developed; data forms and other materials have had previous testing through long use, and special provision commonly exists for recurring conditions such as typhoid fever or lead-poisoning. The field investigator is presumably experienced or has the guidance of those who are. The preliminary reconnaissance is the common substitute. What is elemental is to recognize the unusual epidemic, with respect to clinical behavior, causative agent, or mode of transmission. If that is the situation, individual lines of inquiry and perhaps a tentative form for recording data may be tried out on a few initial cases before proceeding with the general investigation.

In field research, a preliminary test of methods and procedures and an evaluation of staff members are close to imperative. How comprehensive this should be depends on the familiarity of the staff with the problem and their previous experience. If this is the first study in the particular area of interest, new techniques must be learned from start to finish. Certain health conditions are particularly difficult. Alcoholism, drug addiction, and behavioral abnormalities all touch intimately on the sensitivities and sympathies of the study population. Field workers familiar with organic diseases frequently have a substantial adjustment to make in accommodating to these situations. To an extent, these considerations enter into all field epidemiology. Food habits are often difficult to elicit because they are so revealing of social and economic status. The venereal diseases are an especially sensitive subject.

In major projects involving a considerable staff, it is profitable for the eventual supervisors to do this initial work themselves. In the authors' Indian studies on population dynamics in Khanna (Gordon & Wyon, 1960), the field director himself served as the local village worker in the exploratory study, with the future supervisor of village workers as his assistant. Through
personal familiarity with existing difficulties, the leaders develop an ability to direct others effectively and sympathetically.

The exploratory study may last a month. Three months are more likely, and the work may demand as much as six months. The results must be sufficiently certain to establish necessary revision of forms, procedures, techniques, and, especially, the reliability of units of measurement. Identifying errors of omission is as significant as recognizing those of commission. A further obligation is to screen the staff for competence and ability to adapt to field life.

**Pilot study**

A pilot study, the full-dress rehearsal of the main investigation, may not be necessary in a well-defined or familiar field, particularly if the investigator or others have made previous studies. A pilot study is advisable when the problem is obscure, when epidemiologic constants are ill-defined or when methods are new. A material saving in time and money often results.

The pilot study differs from the exploratory study in being an experiment with adequate controls, designed to support the validity of a concept and an experimental design. It has the advantage of a proven and experienced staff, derived from the preceding exploratory investigation. As a small edition of the projected major research, in size of population examined and in duration, it usually lasts for several months—a year if the seasonal variations in the condition studied are important.

The pilot study may identify enough flaws or difficulties to determine that the experiment is unlikely to produce a clear-cut answer; it may uncover new evidence suggesting a second pilot study from a fresh approach. It may demonstrate revisions or alterations essential to making the original investigation possible or, better still, give assurance that the plan works and can be put into operation in the definitive study as formulated. On rare occasions, the pilot study gives an answer sufficiently conclusive to obviate the need for the larger investigation.

**Definitive study**

Two factors conceivably enter into the results derived from the pilot study and the decision to continue: first, the inherent value of the plan and the methods used, and, second, the technical competence with which the work was done. More than one good plan has failed because of its implementation. At any rate, a formal revision of the experimental design, including all modifications and additions suggested by the pilot study, is the first obligation in beginning the definitive investigation. The document itself will be consulted many times, because in every lengthy investigation deviations intrude, recognizable only by reference to the recorded plan of action.
Since the definitive study covers more area and a larger population than the pilot study, the staff is necessarily increased, and recruits must be trained. This process is now much simplified since the needs of the study are more definite and an experienced nucleus of workers exists. With a judicious mixture of old and new members, field operations usually start with little delay—a desirable objective in order to avoid missing time trends in data already collected. At the beginning, efficiency may not match that of the pilot study, since some members of the original staff are commonly appointed subsequently to supervisory duties.

Once adopted, the operating procedure laid down in the revised plan should be followed scrupulously, but not pedantically. In any research, fresh leads occur that are capable of development with little added effort. Yet there is need to limit additions suggested by interests of individual staff members or, more likely, by outsiders who know the expense of such long-term investigations and realize that many field studies devoted to other purposes have the capacity to accumulate kinds of information not warranting the effort or expense of an independent study.

Commonly, interest diminishes in long-term field studies, as the same kind of data is collected over a period of years. Sometimes quality and completeness also suffer from “working for the statistician and his statistical significance when the answer is before your eyes”, as one youngster protested in frustration. Assigning such a worker the task of concurrent evaluation of results best relieves his boredom. Permission to undertake some small ancillary investigation originating with the worker himself is also good for morale. However, alterations in the general design of the study are another matter, and permissible only after strict evaluation of findings by periodic test analyses. The established goal of a definitive answer to the stated problem is not to be lost.

Staff changes are unavoidable in a study of several years’ duration. The advantages of continuous direction are so great that strong effort is warranted to maintain key personnel for the duration of active field work. This is difficult when the site is remote or isolated, as it so often is. One experienced field investigator has said that today a study group can be delivered anywhere in the world and retrieved; the trouble comes in keeping the group there. Numerous long-term field studies have suffered from a policy of short terms of service of one or two years. The need for a concern with living accommodations as well as working conditions, of opportunity for renewed professional associations, of periodic consultations, and of academic recognition and advancement during the study, stems from the considerations just stated.

Analysis and interpretation of results

The case study is ordinarily a one-day field operation, its purpose being to institute administrative measures for control. The report, therefore, is
descriptive and the language, non-technical. It should be submitted promptly. The report combines clinical and laboratory results with field observations. Its essential considerations are origin of the disease, specific etiology, causative factors other than the agent, and, if an infectious process, the mode of transmission. Recommendations for prevention and control and the recognition of actual or potential involvement of other family units are main requirements.

The report of an epidemic has the same primary features, but is necessarily more extensive. The report does not await completion of the study. There must be a prompt and decisive preliminary statement to inform the public and to direct control measures. A final report gives recommendations for long-term control and measures to prevent recurrence; it contains tabulated information on the size of the epidemic, its duration, and the classes of people affected; and it emphasizes causative factors. It is a transcript of what happened, along with a succinct summary.

To use an epidemiologic expression, the handling of data in long-term field research follows the principle of medical asepsis as opposed to terminal disinfection. Periodic trial tests of the findings are essential to a productive investigation. The futility of a last grand clean-up, to find out what has been discovered, has often been demonstrated. Analysis of results is more than an obligation of the statistician; in varying degree it is a responsibility of all staff members. Analysis is at three levels: the first is a concurrent function of regular field activities; the second is a periodic examination of results; and the third is the definitive analysis.

The village workers at field stations and the unit statistician at headquarters assess both records and results as they accumulate. Field records require review to ensure completeness and accuracy; once a week coincides with usual needs. Under the leadership of the director and nurse supervisor, a conference of all field workers at least once a month provides an opportunity to compare results, pool difficulties and successes, and evaluate methods of collecting data. This is desirably a technical conference, independent of administrative meetings.

To stimulate interest and facilitate the daily work, pertinent items of the study should be charted by time and place and kept current at each field station. A pin map can mark geographic distributions. A permanent records of the local health authorities. Summaries of current events likely to have influenced results require a co-operative staff effort. Ordinarily made on a monthly basis, they substitute for the daily diary recommended for the reconnaissance and organizational stages of the study, when events moved at a faster tempo.

In this part of the work, the statistician spends a prescribed time at each field station, making home visits with the field worker, and reviewing methods of collecting and recording data. Another obligation is to check, in appropriate circumstances, observations made by the study group against official
records of the local health authorities. Summaries of current events likely to have influenced results require a co-operative staff effort. Ordinarily made on a monthly basis, they substitute for the daily diary recommended for the reconnaissance and organizational stages of the study, when events moved at a faster tempo.

In any long-term study, a periodic review of accomplishment is indispensable. Depending upon the size and nature of the project, this may be an annual report or one made at shorter intervals in the brief field survey. The report assesses deficiencies in the collection of data and defects in experimental design, and identifies unproductive types of investigation. Perhaps the most important endeavor of all is the search by staff and advisory committee for new lines of inquiry, suggested by the data and developing in the course of the study.

The accumulated periodic reviews and reports set the pattern of final analysis. The first concern lies with the requirements of the stated hypothesis and the experimental design. Thereafter, the search is for ancillary information through unusual correlations to assure maximum yield from the data. Occasionally, these associated findings have equal significance with the direct objective. The work of bringing together the results of a long field study is little appreciated; in some instances, years have elapsed before it was finished. This makes formal publication of the plan of study, with major findings in preliminary form, an early obligation.

**Direction of future research**

The field study has now turned full circle, back to the point at which it began; the need is again for conceptual ideas. As the final obligation, the thoughts, the possibilities, and the suggestions for future research that have come from the study are set down in orderly fashion to ensure that subjects for future investigation are not forgotten with time.

**The INCAP Field Study of Nutrition and Infection**

The usual published report of a field experiment presents in orderly sequence an introduction, a list of materials and methods, the results attained, a discussion of the findings, and the conclusions reached. It tells little of the original incentive, the criteria for choice of method or of materials. Results follow in such logical order as to make one lose sight of dead ends, pauses for elaboration of necessary detail, or changes in emphasis as the investigation proceeded. The strategy can be traced, but the tactical development is obscure.

The purpose of this presentation would remain unfulfilled without brief consideration of these features. The illustration now presented is from
current practice; its aim is to show how principle was applied, how ecologic theory guided practice, and how action proceeded. Of necessity, this is drawn from personal experience. The choice is the field studies, at the Institute of Nutrition of Central America and Panama (INCAP), of the synergistic interaction of nutrition and infection, because these studies illustrate the long-term investigations now so much favored in field epidemiology and because the contribution of team effort in these larger endeavors becomes evident.

As so commonly happens, the conceptual idea originated from clinical observation. In this instance, independent and widely divergent sources eventually merged. Many years ago, when infectious diseases were more common, two communicable diseases frequently occurred concurrently in the same patient, usually as a result of a single exposure, their appearance being spaced by differences in incubation time. Complications in such instances were more frequent and fatality greater than the sum of expected effect from the two diseases when they occurred alone. Gordon (1932) applied the term "synergism" to this relationship, in analogy with the microbiologic designation of concerted action of two infectious agents.

Further clinical experience demonstrated a less frequent situation where concomitant infectious diseases produced the opposite effect of lower fatality, milder clinical course, and fewer complications. This was termed "antagonism"; multiple diseases sometimes seemed to hinder each other's progress. It was a far less common result than synergism. As would be anticipated, in some instances no modifying effect in either direction could be discerned.

The original concept related to infectious diseases, but the principle proved to be of broader application. A non-infectious disease with an infectious disease could induce a similar result, as could combinations of diseases unrelated to infection.

After comprehensive clinical experience in India, where coexisting diseases are commonplace, Taylor explored both the synergistic and antagonistic mass effect of concomitant diseases on general populations (Taylor & Gordon, 1953). Taylor extended these observations by laboratory experiments on the action of two associated viruses in tissue culture.

Later, in the course of field studies on population dynamics in the Punjab, Gordon, Taylor & Wyon established a prevailing synergism between acute diarrheal disease and the nutritional deficiencies of early childhood associated with weaning, and this came to be known as weaning diarrhea (Gordon et al., 1963).

Meanwhile, in Guatemala, and wholly independently, investigation of nutritional disorders among young children was taking the same direction. The Institute of Nutrition of Central America and Panama, under Scrimshaw's impetus, had long emphasized field observation in conjunction with laboratory and clinical research. The vital statistics of the country showed
a high death rate among children one to four years old, in addition to high infant mortality (Pan American Sanitary Bureau, 1964), but the recorded causes of death were suspect. Dietary surveys and clinical studies (Béhar et al., 1960) in the Guatemalan highlands established a high degree of malnutrition among rural children, especially protein deficiencies in pre-school children (Scrimshaw, 1959).

A survey of causes of death (Béhar et al., 1958a) showed that acute diarrheal disease and other infections were more often fatal in malnourished children aged one to four years than in well-nourished children (Scrimshaw, 1963). Furthermore, an infectious disease (Jelliffe, 1955), especially an acute diarrheal disorder, commonly preceded the appearance of the severe protein deficiency, kwashiorkor, by three to six weeks.

The association of malnutrition and intestinal infection was sufficiently regular to compel intensive study of diarrheal disease and its nature. Completed in 1957, these studies demonstrated that, although Shigella was frequently present in healthy rural children (Beck et al., 1957), clinical diarrheal disease was not regularly associated with the ordinary intestinal pathogens (Pierce et al., 1962), nor wholly dependent on environmental influences of sanitation and medical care (Scrimshaw et al., 1962; Bruch et al., 1963).

In 1958, chance brought Scrimshaw, Taylor, and Gordon together; and, in a year of common effort, they reviewed in detail the evidence on synergism and antagonism in its specific relation to nutrition and infection (Scrimshaw et al., 1959). With the help of several experienced consultants to INCAP in the fields of diarrheal disease and pathology, plans followed for a field investigation in Guatemala.

Field operations began in February 1959, with a pilot study to perfect methods and procedure. This phase was brief since much of the desired information about people and living conditions had accumulated during the exploratory studies, which were more numerous and extensive than usual. The experience of the pilot study was reviewed in May, and a revised plan was put into action that month as the definitive investigation. Field operations continued during the next five years, ending in 1964.

The experimental design called for children less than five years old in one rural village to live on the customary diet of the community, which was recognized as deficient. A medical clinic was established in the community, and provision was made for the home care of illnesses. A comprehensive program of preventive medicine was introduced, including immunization against preventable diseases, control of water and food supplies, fly control, and environmental sanitation through construction of privies and attention to wastes. A second child population of a distant village was provided with supplemental foods sufficient to give an optimal diet if consumed in the quantities provided. Medical care and environmental sanitation remained that of the usual village. A third population served as a control, with medical care, preventive medicine, and nutrition remaining unchanged.
In all three populations, resident village workers made regular home visits twice a month to learn of illnesses and to promote the particular program of that village. Bacteriologic surveys of the prevailing intestinal infectious agents were made every three months. All patients with acute diarrheal disease were studied as the disease developed, and bacteriological examinations were made. The nutritional status of childhood populations was measured quantitatively at suitable intervals by determinations of height, weight, skinfold thickness, and the presence or absence of a variety of classical signs and symptoms. Cases and deaths during outbreaks of communicable disease were plotted as a function of time, place, and person.

Some of the observations on acute diarrheal disease are presented in Chapter 6. They support and extend earlier studies in India and in the Arctic. The regularity with which the syndrome was again intimately related to nutritional practices reaffirmed its earlier characterization as "weeping diarrhea". The second-year death rate has proved as useful an index of the nutritional state of pre-school children as the better known infant mortality rate for general health practices (Gordon et al., 1967). A series of outbreaks of measles (Gordon et al., 1965b; Scrimshaw et al., 1966) and chickenpox (Salomón et al., 1966) in the rural villages and scattered cases of other common communicable diseases of childhood (Salomón et al., 1968) provided a further opportunity to compare the effects of nutrition on still other infectious diseases.

The more fundamental information derives from the almost daily records of illnesses, large and small; their number and their frequency; and the effect these illnesses have on growth and development, as determined by periodic measurements of height, weight, and bone maturation. These indices of the human ecology active in the formative years of early childhood portend the human resources of the future and therefore the economy and development of the region (Scrimshaw et al., 1967a, b, c, 1968; Ascoli et al., 1967; Gordon et al., 1968; Guzmán et al., 1968).

Comment

The study of disease as it occurs in nature, with the unit of observation a human population, is the basic concept of epidemiology. Its concern is population pathology. The observational unit distinguishes this approach from investigation of disease in the individual person (clinical pathology) and from disease of cells, tissues, or organs (microscopic pathology). The study of disease in groups of people takes different forms, through laboratory experiment, clinical investigation, or field study. If the findings relate directly to a specified population, or to representative fractions thereof, all are epidemiologic. The techniques, too, are from divergent sources: microbiology, genetics, sociology, statistics, and natural history.
The work of field epidemiology takes two directions: first, the practical service rendered by physicians in health departments and clinics in tracking down communicable and non-communicable disease, which constitutes operational epidemiology; and, second, the planned investigation in search of causes, origins, and distributions of a community disease or a physiologic state which is classed as analytical epidemiology.

The broad principles of ecology govern field study. Field method is no more than fundamental scientific method, based originally, as it is, on Baconian principles, refined by Claude Bernard, and turned to a special purpose. With its fundamental concepts of the significance of time, place, and person, field epidemiology naturally involves a sophisticated understanding of disease behavior under highly different situations.

Inevitably, field investigation ranges widely in order to recognize disease in the varied environments in which it occurs; it is more than geographic pathology and includes far more than what happens under the artificial conditions of crowded metropolitan communities. In temperate zones, diphtheria is a faecal disease; in the tropics, it is a skin disorder. The unusual behavior of a disease in exotic places often contributes fundamentally to knowledge, yet the quality of available data frequently is as incredible as the places from which the information comes. There is no substitute for first-hand observations.

A community of people serves as the field laboratory. The necessity for field investigation rests in the futility of attempting to divorce the study of what happens to man from what happens to his biological, social, and physical environment. Clinical entities as distinct as scarlet fever, erysipelas, and puerperal fever have a single infectious agent and constitute an epidemiologic entity. Other epidemiologic entities, such as aseptic meningitis and acute bacterial conjunctivitis, result from a variety of infectious agents, but the diseases they produce are clinically indistinguishable. Tularemia is a specific infectious disease, produced by a single infectious agent; yet it exhibits several epidemiologic patterns, each with its characteristic distributions by season, age, sex, and geography, as determined by deer-fly, tick, and rabbit.

The opportunities for serendipity are an attraction of field work. Investigation of almost any situation brings discovery of indirectly related or even unrelated phenomena, sometimes as important as the original study itself. A study of population dynamics in India gave insight into deaths from weanling diarrhea, later to be recognized as an epidemiologic entity.

This presentation has traced field method in principle from an original conceptual idea to construction of a hypothesis, then the plan for research and the process of fulfilling that plan, and, eventually, the interpretation of results and recognition of the next objective. The development of hypotheses is the crucial part of any investigation; the soundness of the research design is the main determinant of success. For these reasons, the emphasis
here has been on principle; and, as intriguing as it is, only minor attention has been accorded technical procedure.

Epidemiology takes many turns. Its modern development brings into play an imposing variety of scientific disciplines, both biological and sociological, so that it loses something of its original connotation. The laboratory now goes to the field, as does clinical medicine; and the field turns to the laboratory for confirmation and support of findings. Activities in office and laboratory sometimes dominate the work, with the result that the investigator misses the finest part of epidemiology—the personal and intimate familiarity with people in their natural environment.