Residual Agglutinins against Rickettsial Agents in Human Sera from Central and Eastern Turkey and their Relation to Cardiovascular Diseases*, †

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Many workers have shown that the recrudescence of rickettsial infections may cause cardiovascular diseases of all kinds. As Turkey is a region where rickettsial infections in man are endemic, the relation between such infections and cardiovascular disease has been further studied there. Sera were collected from patients with cardiovascular diseases and also from healthy people and patients with diseases not affecting the heart, who served as a control group. A considerably higher percentage of positive sera was obtained from the patients with cardiovascular diseases, particularly for R. prowazekii, R. conorii and C. burnetii. It was also found that the highest percentage of positive sera was obtained from people in the 20-40 years age-group. Antistreptolysin-O (ASO) determinations were also made and it was found that, on average, agglutinin-negative sera had the higher ASO titres.

As we have indicated previously (Payzin, 1958), rickettsial infections are not infrequent in Turkey; in some parts of the country, foci of different rickettsial infections have been found (Payzin & Akan, 1964). Rickettsial latency may therefore pose some public health problems.

Rickettsial latency is still under investigation. Harvard workers (Murray et al., 1950, 1951a, 1951b) have carried out extensive research in Yugoslavia on Brill’s disease (recrudescent typhus) and have established that its etiological agent is Rickettsia prowazekii and that the disease is a relapse of epidemic typhus. Philip (1957) has discussed masking and latency of rickettsiae in a review of the literature. It has been said that recrudescent attacks, usually of short duration, may occur at intervals for several months and possibly longer periods after the primary attack: French workers (Charmot et al., 1964; Delanoe, Martin & Chiaverini, 1961; Foliquet & Debever, 1962; Giroud, Capponi & Dumas, 1961) have stated that the recrudescence of rickettsial infections may cause cardiovascular diseases of all kinds. Information is being accumulated on vascular diseases and even on rickettsial “sclérose en plaque” (Degos, Delort & Charles, 1964; Giroud & Giroud, 1964; Lamotte et al., 1965; Le Gac & Arquie, 1964; Le Gac et al., 1964). As Turkey is a region where rickettsial infections in man are endemic, we have attempted to investigate their possible relationship with cardiovascular diseases.

MATERIAL AND METHODS

Sera

Blood was collected in centrifuge tubes and, after separation, sera were kept frozen until required. Sera from people with hepatitis were sent by mail from different hospitals for use in the leptospira agglutination test.

Hospitals in Ağrı, Diyarbakır, Erzurum, Trabzon and Van in Eastern Turkey were visited in order to collect sera from patients. Almost half of the sera collected were tested for rickettsial antibodies in the hospital laboratory on the day after collection. Sera (except those from hepatitis patients) were also collected in Ankara.

Sera were tested for rickettsial agglutinins against Rickettsia prowazekii, R. mooseri, R. conorii, Coxiella burnetii and neorickettsial antigens by a modification
of Giroud’s method that has been described in detail previously (Payzin & Akan, 1964).

The cholesterol contents of control group sera and cardiovascular disease group sera did not differ significantly, but differences were found according to locality and nutritional conditions. These results are not relevant to the present discussion and will be published elsewhere.

**Antistreptolysin determinations**

The procedure is based on the methods of Gooder (1961) and Liao (1951), with slight modifications. Details have been published in a thesis (Özsan, 1963). Antigens were prepared in our laboratory and standardized against antiserum supplied by WHO.

**Rickettsial antigens**

Some rickettsial antigens were supplied by Professor P. Giroud of the Pasteur Institute and others were prepared in our own laboratory. The strains used for antigens other than for *R. prowazekii* and *C. burnetii* were obtained through the courtesy of Professor Giroud. Eggs were inoculated at the typhus laboratory of Refik Saydam Central Institute of Hygiene, Ankara, by the kind permission of Dr A. Ari. Infected yolk sacs were kept frozen at −20°C.
for a few days until the antigens were prepared. Details of the techniques were given in a previous paper (Payzin & Akan, 1964).

RESULTS

Rickettsial agglutinins

Sera from patients suffering from diseases other than cardiovascular diseases and sera taken from people who were apparently physically normal were selected to provide a control group. Of the 404 control group sera, 229 were from people with a disease (rheumatic disorders, 56; nephritis or nephrotic syndromes, 32; hepatitis and icterus, 30; allergic disease, 27; congenital heart disease, 9; other diseases, 75). In addition, 86 sera were taken at random from the community of a semi-slum region of Ankara (Abidinpaşa region) and 89 from other normally healthy people or surgical patients. The 138 sera from people with cardiovascular disease were made up as follows: carditis, pancarditis or endocarditis, 29; myocardial infarction and coronary insufficiency, 20; mitral or aortic disease, 89. A third group, of 45 sera, was taken from people with arteriosclerotic heart disease or hemiplegia. As a fourth group, 41 umbilical (placental) cord sera were also included.

The number of sera positive at titres of 1:20 or over in the rickettsial agglutination test and the antistreptolysin-O titres are shown in detail in Table 1. Owing to the shortage of some antigens, not all the cardiovascular disease sera were tested for antibodies to R. conorii and neorickettsiae.

Table 1 indicates that there are marked differences between cardiovascular disease sera and control group sera in their positivity for rickettsial agglutinins. The differences for R. prowazekii antigens are highly significant ($\chi^2 = 13.87$, $P = 0.0015$). Antibodies against R. mooseri and C. burnetii are found in a quite high percentage of sera, as previously reported, because of repeated infections resulting from poor environmental conditions and close association of humans with animals in village houses. The differences in the agglutinin contents of sera from the control group and sera from the cardiovascular disease group are significant for R. prowazekii, R. conorii and C. burnetii, but not for R. mooseri or neorickettsiae. Vascular disease (arteriosclerosis, hemiplegia) sera gave similar results to those of the control group, but it should be noted that the number of sera tested (45) was small. For the umbilical cord sera, the absence of R. prowazekii and R. mooseri anti-

bodies and the high content of neorickettsial antibodies are noteworthy.

It is desirable to mention here that 40% of the hepatitis sera were positive to Q-fever antigen. The R. conorii agglutinin contents of all the sera tested were unexpectedly high, especially those of the cardiovascular disease sera.

These results indicate that neorickettsial infections have occurred in Turkey. It is interesting that the sera obtained in the Abidinpaşa region of Ankara are more highly positive for neorickettsiae than sera from other areas. The small number of sera tested against neorickettsiae may be the reason for the irregularity in this column of Table 1.

The distribution of agglutinins by the age of the subject, for both the control group and the cardiovascular disease group, is shown in the figure. It is noteworthy that, except for R. conorii antibodies, the percentage of positive sera is highest in the 20-40 years age-group.

Table 2 shows the percentage of sera that are positive (titres 1:20 or higher) to one or more types of rickettsiae. When sera that are positive to more than one rickettsial antigen are taken into consideration, the percentage of positive sera is twice as high for the cardiovascular disease group as for the control group.

Owing to a shortage of antigens, only a limited number of positive sera were tested for 1:40 and higher titres. When such titres are taken into consideration, the results are as shown in Table 3.

Antistreptolysin-O contents of sera

The antistreptolysin-O (ASO) titres of the sera tested are shown in Table 1. Sera from patients with myocardial infarction or vascular disease have the lowest ASO titres in comparison with the other groups, including cord sera. Among the non-heart diseases group, those having no relation with rheumatic disorders also gave low ASO titres (on average, 145). In Table 4, ASO titres of sera that are either negative or positive to rickettsial agglutinins are compared. Unfortunately, it was not possible to determine ASO titres for all the sera. On the average, agglutinin-negative sera had the higher ASO titres.

DISCUSSION

The relationship between rickettsial infections and cardiovascular diseases has two aspects—namely, (a) cardiovascular complications or rickettsial infections during the acute phase of infections and (b) recrudescence of rickettsial infections, such as Brill’s
TABLE 2
PERCENTAGE OF SERA POSITIVE FOR ONE OR MORE TYPES OF RICKETTSIAL AGGLUTININ

<table>
<thead>
<tr>
<th>Origin of sera</th>
<th>No. of sera tested</th>
<th>Sera positive for one rickettsia</th>
<th>Sera positive for 2 or 3 rickettsiae</th>
<th>Sera positive for 4 or 5 rickettsiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Control group</td>
<td>235</td>
<td>96</td>
<td>40.9</td>
<td>21</td>
</tr>
<tr>
<td>Abidinpaşa area</td>
<td>86</td>
<td>39</td>
<td>45.8</td>
<td>13</td>
</tr>
<tr>
<td>Vascular diseases</td>
<td>45</td>
<td>8</td>
<td>17.8</td>
<td>3</td>
</tr>
<tr>
<td>All heart diseases</td>
<td>210 a</td>
<td>45</td>
<td>21.2</td>
<td>46</td>
</tr>
<tr>
<td>Children aged 0-10 years</td>
<td>36</td>
<td>7</td>
<td>18.8</td>
<td>2</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>40</td>
<td>8</td>
<td>20.0</td>
<td>2</td>
</tr>
</tbody>
</table>

a This includes additional sera tested after the Rome Congress.

TABLE 3
PERCENTAGE OF SERA POSITIVE AT TITRES OF 1:40 AND HIGHER FOR RICKETTSIAL AGGLUTININS

<table>
<thead>
<tr>
<th>Origin of sera</th>
<th>Percentage of sera positive for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R. prowazekii</td>
</tr>
<tr>
<td>Control group</td>
<td>12.0</td>
</tr>
<tr>
<td>Cardiovascular disease group</td>
<td>16.8</td>
</tr>
</tbody>
</table>
disease, or cardiovascular diseases, after a period of latency.

Cardiovascular lesions and nephritis due to acute rickettsial infections are well known and have been described in medical textbooks (e.g., Bourdellès & Aujaleu, 1948). Similar complications have been recorded during laboratory infections (e.g., Payzin & Göksel, 1945). Choroidoretinitis (Giroud & Roger, 1955), subacute endocarditis (Marmion et al., 1960), pericarditis (Horeo et al., 1962), myocardial infarction (Delanoe, Martin & Chiaverini, 1962) and many other kinds of cardiovascular disease have been reported, mainly by French and Romanian workers (Binde et al., 1952; Delanoe, Martin & Chiaverini, 1961; Folquet & Debever, 1962; Giroud, Capponi & Dumas, 1961; Giroud et al., 1959; Nicolau et al., 1962a, 1962b; Worms et al., 1951).

The recrudescence of previously acquired *R. prowazekii* infection as Brill’s disease and in other forms has been reported in many countries (e.g., Murray et al., 1952; Žirković, Simic & Curin, 1962). Chronic forms of Q fever with relapse have also been observed in many cases. Žirković and co-workers (1962), in Yugoslavia, have periodically examined, by means of the complement-fixation test, the sera of 50 people who had previously had typhus and have observed serological relapses in fever cases.

The persistence of rickettsial antibodies for a definite, but relatively long, period is well known (Murray et al., 1952). As we have previously mentioned (Payzin & Akan, 1964), serological surveys in Lebanon, Romania and Saudi Arabia have shown that 3.1%–32% of the sera obtained from people in normal health contained rickettsial antibodies. Battaglia & Lancerini (1961), in Italy, found that 17% of 1000 human sera tested and 23.4% of bovine sera were positive for Q-fever antibodies in 1:16 CF titres. Similar findings have been reported elsewhere (Bazex et al., 1963; Giroud et al., 1962; Lipp et al., 1962; Terzian & Gaon, 1956).

Giroud et al. (1962), Payzin (1953) and Payzin & Akay (1952) have reported the existence of *C. burnetii* and neorickettsiae in *Boophilus, Rhipicephalus* and *Ornithodoros* ticks collected in Central and Eastern Turkey. Some of the strains had very low virulence and poor antigenicity (Giroud et al., 1962; Payzin, 1958).

The high incidence of agglutinins in the sera we have studied may be the result of inapparent infection with strains of low virulence or of unrecognized diseases caused by normal strains. Owing to the close contact between peasants and animals in rural areas of Turkey, the results obtained are not surprising.

Our data show that there is a correlation between antibody titre and the existence of heart disease. So far, we have titrated 78 sera from heart disease patients for rickettsial antibodies at titres of 1:20 to 1:320 in the microscopic slide agglutination (MAS) test; 15 sera were found to be positive, with titres between 1:80 and 1:320. Two of these sera were from patients with atrioseptal heart defects and had

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### Table 4

| Origin of sera | Positive | | Negative | | |
|----------------|----------|------------------|----------|------------------|
|                | No. of sera tested | Average ASO titre (Todd units) | No. of sera tested | Average ASO titre (Todd units) |
| Umbilical cord | 10 | 263 | 32 | 295 |
| Children aged 0-10 years | 16 | 320 | 36 | 352 |
| Control group | 65 | 192 | 35 | 253 |
| Heart disease | 53 | 189 | 17 | 230 |
| Vascular disease | 13 | 148 | 12 | 135 |
| Cumulative | 157 | 212 | 132 | 258 |
| Abidinpaşa region | 59 | 232 | 22 | 363 |

*a A further 199 sera from normal persons, tested by Dr M. Özsan, gave an average titre of 127 Todd units, with a standard deviation of ± 65 units.*
1:80 titres for *R. prowazekii*. Giroud et al. (1965) have shown that, in rats experimentally subjected to latent *R. conorii* infection, malformation due to rickettsiae occurs; presumably, a similar consequence might be possible in human beings.\(^1\) Typical titres found in our work included 1:80 for *R. conorii* in one case of constrictive pericarditis, 1:320 for *R. prowazekii* in five cases of myocardial infarction, 1:320 for *R. prowazekii* and 1:80 for neorickettsiae in one case of arteriosclerotic heart disease, and 1:320 for *R. prowazekii* or *R. mooseri* (one case) in four cases of mitral or aortic disease.

\(^1\) Similar findings in human beings have, in fact, been reported by Cajal et al. (1962). Malformations due to *R. burnetii* were proved by the isolation of rickettsiae.

The percentage of sera found positive in this test is somewhat higher than the average obtained in the complement-fixation test. This is due to the higher efficiency of the microscopic slide agglutination test; in studies on Q fever, it was found to be ten times as sensitive as the capillary agglutination test (Babudieri, 1958). In the present work, however, difficulties were encountered as a result of the agglomeration of antigens after tests had been in progress for about three months, even though the antigens had been kept in a refrigerator.

The results of the present work support the hypothesis that rickettsial relapses may play a role in the etiology of cardiovascular diseases; more information on this relationship is clearly desirable.

ACKNOWLEDGEMENTS

We offer our sincere thanks to Professor P. Giroud of the Pasteur Institute for gifts of antigens and strains of rickettsiae and to Dr A. Ari of Refik Saydam Institute for permitting us to carry out egg inoculations there. We are also indebted to the clinicians in Agri, Diyarbakir, Erzurum, Trabzon and Van and in the medical school hospitals who gave permission for the collection of sera and made medical records available. Dr M. Özsun and Mr M. Özkul carried out some of the antistreptolysin determinations.

RÉSUMÉ

L’existence d’une relation entre les infections rickettsiennes et les maladies cardio-vasculaires a été souvent affirmée, que ces dernières surviennent au cours d’une infection aiguë ou apparaissent, après une période de latence, à l’occasion des rechutes. Les auteurs ont tenté d’établir une corrélation entre l’endémie rickettsienne telle qu’elle s’observe en Turquie et les maladies cardiovasculaires. Utilisant la méthode de Giroud modifiée, ils ont recherché des anticorps vis-à-vis de *Rickettsia prowazekii*, *R. mooseri*, *R. conorii*, *Coxiella burnetii* et des antigènes néorickettsiens, dans les séums prélevés chez 138 malades atteints d’une affection cardio-vasculaire. Parmi ces patients, on comptait 29 cas de myocardite, pancardite ou endocardite, 20 cas d’infarctus du myocarde et d’insuffisance coronarienne, 89 atteintes valvulaires. Un groupe témoin a été constitué: il comprenait 404 personnes, dont 56 souffraient de troubles rhumatismaux, 32 de syndromes néphrithiques ou néphrotiques, 30 d’hépatite et d’ictère, 27 de maladies allergiques, 9 d’affections cardiaques congénitales, 75 d’autres maladies; en outre, 86 séums ont été prélevés au hasard dans une collectivité de la région d’Ankara et 89 chez des personnes saines ou chez des malades atteints d’une affection chirurgicale. Un troisième groupe de 45 séums a été prélevé chez des cardiaques atteints de lésions d’athéroscorlose ou des hémiplegiques. Enfin, 41 séums ont été prélevés sur des cordons ombilicaux.


Le pourcentage de séums positifs a été plus élevé, sauf pour *R. conorii*, dans le groupe d’âge 20-40 ans. La proportion des séums positifs vis-à-vis de plusieurs rickettsies a été deux fois plus forte chez les sujets atteints de maladies cardio-vasculaires que dans le groupe témoin.

Les auteurs ont également tenu les antistreptolysines O dans certains séums. Ceux du groupe des maladies cardiovasculaires ont présenté les valeurs les plus faibles. En moyenne, les séums renfermant le moins d’agglutinines présentaient également les titres les plus élevés d’antistreptolysines O.

Pour les auteurs, ces résultats sont en faveur de l’hypothèse qui attribue aux rechutes d’affections rickettsiennes un rôle dans l’étiologie des maladies cardio-vasculaires, mais la nature de ces relations demande à être précisée.
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