because the fatty-acid composition of the fats deters the growth of moulds. The following data show the composition, and the cost of the ingredients. The prices are shown in East African cents, 100 cents being equivalent to $0.14.

Ingredient	*Amount (grams)*	*Total calories*	*Protein calories*	*Cost per 100 calories*	*Cost per gram of protein*
Ground-nuts (toasted and ground)	150	840	164	2.38	0.50
Wheat flour	50	182	20	2.74	1.00
Maize flour	100	355	37	1.82	0.55
Cotton-seed oil	25	220	—	2.50	—
Sugar	75	288	—	3.12	—

Both the biscuits and the meal made from them have been used for several months in the Group's hospital wards. The biscuits have also been given to children in a primary school near Kampala and have met with great success. Further developments must depend on the discovery of facilities for cheap production on a large scale.

Of the calories provided by the biscuits, only 11.5% are from protein. A higher percentage is desirable, especially for younger children taking the biscuit meal. One possibility lies in the use of a mixture of bean and ground-nut meals to replace some of the present ingredients without adding too much to the price.

Isolation of Rabies Virus from Insectivorous Bats in Yugoslavia

A preliminary report by MILAN NIKOLIĆ, M.D. *and* ZDRAVKO JELESIĆ, M.D., *Pasteur Institute, Novi Sad, Yugoslavia*

During the last two or three years, several cases of atypical rabies, in which the source of infection could not be established, have been reported in groups of cattle in Yugoslavia. According to Dr Manninger[a] of the Veterinary School, University of Budapest, similar cases have also been observed in Hungary. In every instance, diagnosis had to be made by means of laboratory examination.

In view of the atypical clinical picture and inexplicable mode of infection, we suspected that even in Europe bats might be vectors of rabies.[b]

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[a] Personal communication

[b] Outbreaks of rabies caused by the bite of infected bats have been known in the Americas for several years. The reader is referred to the following literature, which describes a number of these incidents: Bell, J. F., Hadlow, W. J. & Jellison, W. L. (1955) *Publ. Hlth Rep. (Wash.)*, **70**, 991; Burns, K. F., Farinacci, C. J. & Murnane, T. G. (1956) *J. Amer. vet. med. Ass.*, **128**, 27; Courter, R. D. (1954) *Publ. Hlth Rep. (Wash.)*, **69**, 9; Enright, J. B., Sadler, W. W., Moulton, J. E. & Constantine, D. (1955) *Proc. Soc. exp. Biol. (N.Y.)*, **89**, 94; Venters, H. D., Hoffert, W. R., Scatterday, J. E. & Hardy, A. V. (1954) *Amer. J. publ. Hlth*, **44**, 182.

Research was begun on the basis of this assumption, and the preliminary results of the investigation are presented in the following pages.

Bat-catching was organized in the Vojvodina district, in which cases of rabies had been diagnosed in domestic animals, and examples of the following species were obtained: *Nyctalus noctula* Schreb, *Eptesicus serotinus*, *Miniopterus schreibersi*, *Plecotus auritus*, *Myotis myotis* and *Rhinolophus ferrum equinum.*[c]

All the bats collected were numbered individually. Their brains were removed aseptically and were either prepared for immediate inoculation into white mice or preserved, for subsequent use, in a 50% glycerol-saline solution. Mice were inoculated intracerebrally with 0.03 ml of a 1:10 suspension containing penicillin and streptomycin, a test of bacteriological sterility being performed at the same time.

We have so far succeeded in isolating a pathogenic agent from 3 out of 27 insectivorous bats of the *Nyctalus noctula* Schreb species, which had been caught in a small wood not far from the Novi Sad abattoir. These bats showed no signs of illness.

In order to examine the pathogenicity and virulence of the isolated agents, various laboratory animals were used, and the following results were obtained.

White mice

Intracerebral inoculation. In the first passage, the incubation period lasted 6-10 days, but in subsequent passages it became increasingly shorter, until from the seventh passage onward it remained " fixed " at 5 days. Exceptions to this rule were incubation periods of 4 and 6 days. So far, 17 passages have been made.

The first symptoms to appear were ruffled fur, arched back and inappetence, and these were followed by rapidly increasing weakness. Total paralysis of both hind legs was noted in one mouse only. The clinical course of the illness was seldom longer than 36 hours. The LD_{50} was about 10^{-5}.

Intramuscular and subcutaneous inoculation. Infection by these routes was not always successful, and on an average the incubation period was as long as 7 days in the case of intramuscular inoculation and 9-10 days in that of subcutaneous inoculation.

Rabbits

Intracerebral inoculation. The first passage was effected with a brain suspension obtained from the second mouse passage. The incubation

[c] We are indebted to Professor Beatrica Djulič of the Institute of Biology, Zagreb, for the identification of the bat species.

period lasted from 3½ to 4 days, and remained unchanged in the 20 subsequent passages. The rabbits became remarkably still, refusing all food. Their heads were bent to one side or thrown back, and their gait was unsteady. The next day they lay motionless, with the head thrown back or bent forward as far as it would go. Obvious signs of paralysis were not observed. Death occurred 1½-2 days after the onset of the illness.

Intraocular inoculation. Infection by this route was generally successful, and the clinical picture was identical with that presented by intracerebral inoculation, the incubation period lasting only 5 days.

Intramuscular inoculation. We succeeded in infecting 2 rabbits by the intramuscular route with a suspension of rabbit brain from the first passage. Both rabbits fell ill, after an incubation period of 6 days in one case and 9 days in the other. Intramuscular infection with a brain suspension from the third rabbit passage was successful in 2 out of 4 rabbits inoculated (incubation period, 9 days), but inoculations with suspensions from subsequent passages were ineffective. Nevertheless, by inoculating guinea-pigs intracerebrally with brain suspensions from the fifteenth rabbit passage (incubation period of only 2½ days), it was again possible to infect rabbits by the intramuscular route, the incubation period lasting for as short a time as 4½ days.

The clinical picture was different from that presented by intracerebrally infected rabbits. During the second day of illness, paralysis of both hind legs became apparent, with incontinence of the faeces and retention of urine, as can be observed in the typical paralytic form of street rabies.

Intravenous inoculation was unsuccessful in rabbits.

Guinea-pigs and Cats

Intracerebral inoculation. The animals fell ill 3-3½ days after being inoculated with a brain suspension from the second mouse passage. A shorter incubation period—2½ days—was achieved with the inoculation of a brain suspension from the fifteenth rabbit passage.

Two cats were fed with infected mice or with the brain of rabbits which had been used for the passage of the virus, but no symptoms were produced.

Laboratory findings

Impression smears of Ammon's horns, stained with Sellers stain, were made regularly, but in no case were Negri bodies found. For this reason, 250 preparations were sent to the Histopathology Section, Hospital for Infectious Diseases, Zagreb, for histopathological examination. Encephalitic lesions were present in every case. Although Lentz-stained prepara-

tions of Ammon's horns showed no evidence of Negri bodies, numerous intracytoplasmic and extracellular homogeneous, dark-blue bodies, 1-3 μ in diameter, were isolated in the majority of them.

In order to identify these agents, we used white mice in neutralization and protection tests with antirabies serum. In the neutralization test, groups of 4 mice were inoculated intracerebrally with 0.03 ml of 10^{-1} through 10^{-5} dilutions containing equal parts of a suspension of the bat virus and a specific antirabies serum (supplied by the Pasteur Institute, Paris). Groups of mice were similarly inoculated with 10^{-1} through 10^{-3} dilutions of a strain of Slovenian meningo-encephalitis virus and the specific antirabies serum. Controls consisted of inoculations of the respective viruses mixed with normal serum. The antirabies serum neutralized the bat virus from 10^{-2} through 10^{-4} dilutions, which killed all the controls not receiving the specific serum. The 10^{-5} dilution of the virus did not kill the mice. No neutralization of the Slovenian meningo-encephalitis virus was observed.

In a protection test using antirabies serum (produced in Kalinovice, Yugoslavia), 3 intraperitoneal inoculations of 0.5 ml each of serum were given on 3 successive days, followed by intracerebral challenge of 10^{-1} through 10^{-5} dilutions of the bat virus. The mice which received serum were protected through the 10^{-4} dilution of the bat virus, and the control mice receiving virus alone died. Again the 10^{-5} dilution of virus failed to kill the mice.

As the next step in the procedure of identification, the cross-immunity test is at present being used in a comparison of the bat virus with the " classic " fixed virus of street rabies.

In brief, our findings to date show that the neutralization and serum-protection tests have demonstrated the rabic nature of the isolated agents. The absence of Negri bodies does not invalidate this conclusion, as the existence of strains of street virus which do not produce Negri bodies is well known. However, the period of incubation presented several interesting variations. In some of the experimental animals—particularly the guinea-pigs—it was surprisingly short; while the mice took longer to develop symptoms than the larger laboratory animals. Another unexpected element was the clinical and histological evidence of meningo-encephalitis, instead of the signs of encephalomyelitis which usually accompany typical rabies.

In view of such evidence as the results of neutralization and serum-protection tests detailed above; descriptions of the strains of rabies virus with extremely short incubation periods; and the atypical clinical picture presented in cases of infection by the South American bat virus, we are convinced that the pathogenic agent isolated from our bats is in fact a strain of rabies virus, though differing in certain respects from the classic street virus.