membrane of the gut in a normal individual during complete starvation.

The results are given in a table which also includes the mean temperature and humidity on the day of each experiment. It will be noticed that the 24-hour volume of urine is less than 1,000 cubic centimetres in about half the subjects. The total nitrogen varies from 12.20 to 5.32 grammes per day, the output per kilogramme of body weight varying from 175 to 82 milligrammes. The corresponding figures for urea were 10.20 and 3.27 grammes in the one case and 150 and 52 milligrammes in the other. The different amounts of protein in the dietaries of different individuals are reflected in these figures. They are lower than the averages given in European and American text-books, in some cases markedly so, but on the other hand in only one individual was the total nitrogen as low as that found by McCay (1912) in the average Bengali (6 grammes). The creatinine nitrogen (0.67 to 0.31 grammes per day) shows a smaller variation than the urea nitrogen and is also less than in western races, but the creatinine coefficient, or amount per kilogramme of body weight per day (13.7 to 22.6 milligrammes), approximates western standards.

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## THE NATURE OF THE BACTERIAL SUBSTANCE OF AN ORAL ANTI-DYSENTERIC VACCINE.

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THIS investigation was undertaken to determine the nature of the bacterial substance contained in the antidysenteric oral vaccine of a well-known brand. The tablets were obtained from the Indian agents in the original containers similar to the one supplied to the public for prophylactic purposes. The manufacturers claim that each tablet contains 50 milligrammes of heat-killed and desiccated bacilli of Shiga, Flexner, Hiss and Strong which is equal to about 60 to 70 billions of the microbes. It is further claimed that the immunising power holds good for any type of bacillary dysentery. It is not possible to identify by chemical or physical methods, in the present state of our knowledge, the specific proteins of each species of bacterium though we can do so—fairly accurately—by their specific immunological reactions. The method of testing the agglutination reaction of an unknown bacterial emulsion with the various known antisera, as well as the preparation of an antiserum for testing against known bacteria, is too well known to be discussed here. This principle has been the underlying idea of the experiments (noted below) carried out to test the nature of the bacterial substance contained in the tablets.

### *Experiment I.*

(*a*) One tablet of the antidysenteric oral vaccine was dissolved in 40 c.cms. of normal saline bringing the strength of the emulsion to $1\frac{1}{2}$ billions of organisms per cubic centimetre (each tablet is claimed to contain about 60 billions bacilli). The following quantities were injected into a rabbit.

1st day—0.1 c.cm. of the emulsion injected subcutaneously.

4th day—0.2 c.cm. of the emulsion injected subcutaneously.

9th day—0.5 c.cm. of the emulsion injected intravenously.

14th day.—1 c.cm. of the emulsion injected intravenously.

No toxic or other untoward symptoms were noted in the animal during the course of the experiment.

Blood was collected on the 18th day; the serum was separated and put up against stock emulsions of *B. dysenteriæ* (Shiga, polyvalent, Flexner, Sonne, etc.). Dreyer's standard method was used; no agglutination was noted in any of the dilutions.

(*b*) The emulsion of the tablet was also tested with high titre Shiga and Flexner sera but no agglutination was noted even in the lowest dilution.

### *Experiment II.*

As no toxic effects were noted in experiment I, it was decided to use a stronger emulsion of the vaccine tablets.

(*a*) The emulsion was made by dissolving 1 tablet in 5 c.cms. of normal saline.

Four injections of a cubic centimetre each at intervals of 5 days were administered to a rabbit intravenously. Blood was collected 5 days after the last injection. The agglutination tests detailed in experiment I proved to

be negative in all cases. After the injections no toxic effects were noted in the animal.

*Experiment III.*

The sera obtained in experiments I and II were tested against the emulsion of the tablet described above, but no agglutination was noted.

To get some rough idea of the chemical composition some of the well-known tests for proteins and carbohydrates were applied, with the following results.

The opalescent brown emulsion had a faint smell resembling that of meat extracts. The emulsion was not coagulated by heat, was negative when tested with Heller's test and did not give the biuret reaction. It was positive with Fehling's and Benedict's tests for sugars but gave no iodine reaction for starch.

*Summary of results.*

1. It is evident from the above that the bacterial substance claimed to belong to the Shiga, Flexner, Hiss and Strong types is not agglutinated by their respective high titre sera.
2. The substance itself is incapable of producing a serological response in animals and produces no toxic effects when injected intravenously in large doses in rabbits (*c. f.* Shiga emulsions).

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## APPLICATION AND USE OF LARVICIDES.

By K. BOSE,

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At the Conference held at Birnagar in February 1929 to discuss the problem of malaria in that town, I submitted a proposal for concentrating attack upon *Anopheles philippinensis* which was found to carry malarial parasites in Nature in the Nadia District. In preference to a general campaign against anophelines, Sir Malcolm Watson, who presided at the meeting, accepted the view that the breeding grounds of *A. philippinensis* should be located in co-operation with the Public Health Department, Bengal, and dealt with by larvicides, but suggested at the same time that the campaign against other anopheline species breeding in numerous other tanks and pools at Birnagar should be continued. It must be admitted that our knowledge of the carrier species at Birnagar was inadequate at that time and he was fully justified in supporting the continuance of a general campaign of oiling which had been our aim at the beginning. I have explained in my previous reports that this "general campaign" was, in practice, of a restricted nature owing partly to the public opposition and partly to the inadequacy of our funds.

As a result of a subsequent survey we found *A. philippinensis* breeding not only in certain of the tanks and pools but in extensive marshy lands both in and outside Birnagar. In view of our limited resources, we were compelled to confine oiling operations to the *philippinensis* breeding grounds only, which covered a large area, during the non-malarial season from March to the beginning of July 1929, when a general campaign was inaugurated in accordance with Sir Malcolm's advice. Three mosquito brigades had to be formed, namely, the oil brigade consisting of 3 men for dealing with numerous tanks, the Paris green brigade consisting of 2 to 3 men, according to circumstances, for treating the canal and pools, and a third brigade consisting of 3 men for making experiments with soluble cresol in marshy tracts, such as Purana Dighi.

All this expenditure proved too much for the Mandali to bear, and although we managed to carry our wider programme throughout the rains we were obliged to revert to the policy of treating the *philippinensis* breeding grounds only, from December 1929. This arrangement was followed till March 1930. The mosquito survey at Birnagar, a report of which will be found in the June number of the *Records of the Malaria Survey of India,* shows conclusively that *A. philippinensis* is the principal carrier of malaria at Birnagar, and that even if other anopheline species are subsequently found to carry the malarial parasite they must have a low infectivity.

I shall now deal with the respective merits of crude oil, Pesterine M. D. B.,* soluble cresol, and Paris green. In order to get a good spray we mixed crude oil with solar oil. The use of this larvicide was wholly superseded by Pesterine M. D. B., which gives a better film, on the recommendation of Sir Malcolm Watson in 1929. Where the breeding pools are free from vegetation it is advantageous to spray oil at the water edges. This was done every ten days. Where tanks or pools are covered with water weeds they have to be cleared every time before the application of oil for effective larval control. If such tanks are partially cleared at the edges only, the oil film cannot penetrate through the thick vegetation so that the larvæ in the rest of the water surface escape destruction. Certain devices have from time to time been adopted for dealing with anopheles breeding in the middle of a big tank (Khan Dighi), such as hanging a ball of cotton waste dipped in oil, or small gunny bags filled with saw dust soaked in oil, on a bamboo pole driven to the bed at intervals. But this contrivance was found to be laborious and could not be continued as a permanent measure without a large staff. It may be mentioned that if vegetation is completely removed from

* Pesterine M. D. B. is the particular mixture of crude oil and kerosine oil which was recommended by the Mosquito Destruction Board in the Straits Settlements.