TECHNIQUE OF THE INJECTIONS AND DOSAGE.

The technique of the injections is very simple. A 10 c.cm. serum syringe is used, and the vein selected—usually either the median basilic or cephalic, or a prominent vein in the forearm—is made to stand out by tying a bandage round the upper arm, and by the patient clenching his fist. Tr. iodine is used to sterilize the skin. As soon as it is thought that the needle is in the vein the piston is withdrawn slightly, and a little blood is drawn up into a syringe; or, if a syringe of the solution is to be given, the barrel is removed from the needle and a few drops of blood are allowed to flow before the solution is injected. Great care should be taken that the injection is made into the vein, and not into the tissues around the vein. The tartar emetic solution is very irritating, and a few minims injected under the skin cause great pain at the time of the injection, and later on a hard, tender, brawny swelling, or even sloughing of the tissues.

The symptoms which follow the injections have varied in different cases. Three patients had no untoward symptoms—one experienced a metallic taste in his mouth for several hours, a disinclination for food and vague abdominal discomfort. Another had a severe paroxysm of coughing and vomiting, which came on immediately after the injection, and lasted about ten minutes.

DOSAGE.

We began with only half to 1 c.cm. of the 2 per cent. solution, but rapidly increased it to 3 or 4 c.cm., and repeated the injection every two or three days, adding 1 c.cm. at each injection up to a maximum of 10 c.cm. if the patient had no marked gastric disturbance, in which case the dose may be reduced slightly for a time. In no case have any serious symptoms developed, although we have repeatedly given the large dose of 20 cg.

CONCLUSIONS.

The cases we have briefly recorded are a consecutive series and so are quite unselected. The uniform and rapid improvement in all except the unfortunate patient with tuberculous affection of the lung and bowels—who, indeed, also appears to have been much benefited as regards his infection with kala-azar—is quite unique in our experience of the disease, which, in the case of one of us, extends over almost twenty years, and includes a knowledge of nearly every case treated in the Calcutta European Hospital for the last fifteen years. Numerous recoveries of patients with kala-azar under other lines of treatment have been recorded by one of us,⁸ but only in occasional cases, while in the majority of them most disheartening failures were met with. We now have no hesitation in advising the use of intravenous injections of tartar emetic as a routine method of treating kala-azar, beginning with 4 to 6 cg. on alternate days, and increasing by one or two centigrams every other day up to a maximum of 20 cg., if the patient will tolerate as much. The treatment should be continued until the temperature has remained normal for several weeks with steady gain in weight, and the parasites are found to have completely disappeared from the spleen, and the leucocytes have been raised to about the normal level. It is not necessary to continue until the spleen has been reduced to its normal size, as it will continue to shrink after the injections are omitted.

In children and nervous female patients inunctions of antimony ointment, preferably in a strength of 10 per cent., is worthy of trial, although there is not yet sufficient evidence to decide how far this much simpler measure can be relied on. If further experience shows it to be able to cure the disease it will present manifest advantages, especially in out-patient and village practice.

We believe that in tartar emetic, and probably in other preparations of antimony, a specific treatment for the terrible lingering and very fatal kala-azar has at length been found, and yet another serious tropical disease been brought within the pale of curable affections.

REFERENCES.

¹ BRITISH MEDICAL JOURNAL, July 31st, 1915, p. 197, and *Indian Medical Gazette*, July, 1915, p. 273. ² *Indian Medical Gazette*, October, 1915, p. 364. ³ *Ibid.*, May, 1915.

THE Police Commissioner of New York has established a psychopathic laboratory, under the direction of Dr. Louis Bischof of New York, for the examination of prisoners in respect of mental deficiency.

ATTEMPTS TO PRODUCE A SAFE AND EFFECTIVE BACILLUS DYSENTERIAE (SHIGA) VACCINE FOR PROPHYLACTIC PURPOSES.

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THE question of the prophylactic treatment of dysentery is of considerable importance, and the desirability of placing the subject on a satisfactory experimental basis is self-evident. As a preliminary contribution to this aspect of the question as it relates to the bacillary type of dysentery I venture to give the following general account of some experiments* carried out with *B. dysenteriae* (Shiga) at the Lister Institute of Preventive Medicine during the past year, and a summary of results so far obtained, with the conclusions drawn therefrom.

The vaccines that have been experimented with up to the present are:

I. Heated Vaccines (at 56° C. for one hour).

- A. Ordinary vaccines (non-sensitized).
- B. Vaccines treated with normal horse serum according to the method described and advocated by Broughton-Alcock in the *BRITISH MEDICAL JOURNAL*, August 18th, 1914.
- C. Vaccines treated with diluted specific Shiga-agglutinating serum.

II. Unheated Carbolized Vaccines.

- A. Ordinary carbolized vaccines ("non-sensitized").
- C. Carbolized vaccines treated with diluted serum, obtained from known immune rabbits.

Experimental Animal.

The rabbit was chosen as the experimental animal because of its susceptibility to the Shiga bacillus and because it reacts in a characteristic manner both to the bacillus and to its toxin.

Test for Immunity.

For this I relied on the intravenous injection of living bacilli. The intravenous injection of 200 million of living bacilli (twenty-four hours culture) in 1 c.cm. of normal saline, of the strain of Shiga which was used, was known to be almost invariably fatal to the normal healthy full-grown rabbit. Of ten strong healthy control rabbits that got 250 million intravenously, only one survived. To test if the vaccinated rabbits had acquired a reasonable degree of immunity, the dose usually given was 1,000 million of living bacilli (twenty-four hours culture) in 1 c.cm. of normal saline, given intravenously.

Summary of Animal Experiments.

Four series of experiments were carried out. All vaccines were given subcutaneously. Heated vaccines were used in the first three series. These series differed from each other simply in respect of dose. Each series is further divided into three groups. In the "A" groups, ordinary (non-sensitized) vaccines were used; in the "B" groups, vaccines treated with normal horse serum; in the "C" groups, vaccines treated with diluted specific Shiga-agglutinating serum. In each case the serum was thoroughly removed by centrifugalizing and by washing. The deposit of "sensitized" bacilli was finally made into an emulsion of known strength in fresh normal saline. In Group C₁ the vaccine was sensitized and washed before it was heated, in Groups C₂ and C₃ the vaccines were first heated and then "sensitized."

SERIES I.

Group A₁—Vaccines used, ordinary (non-sensitized), heated at 56° C. for one hour.

Doses given: First, on January 14th, 200 million; second, on January 25th, 400 million; third, on February 4th, 800 million.

Of 20 rabbits that formed this group, 5 did not survive the first dose; 1 survived the first but not the second dose; 1 survived the first and second but not the third dose; 13 survived all three doses.

Each of the 13 that survived all three doses was given as a test dose 1,000 million of living bacilli (twenty-four hours Shiga culture) in

* Complete protocols of these experiments will appear, it is hoped, in a future number of the *Journal of the Royal Army Medical Corps*.

† Further experiments with unheated vaccines are now being carried out. These will include vaccines sensitized with undiluted specific Shiga serum from highly immune rabbits, prepared according to the method found successful in the case of vaccines used for therapeutic purposes by Gordon (*Proceedings of the Royal Society of Medicine*, Therapeutical and Pharmacological Section, 1913, vol. vi, pp. 153-176).

1 c.cm. of normal saline intravenously—6 on February 19th and 7 on February 23rd. Of these, 12 survived and 1 died about a month later, probably as a result of the test dose.

Group B₁.—Vaccine used, "sensitized" with normal horse serum after it had been heated at 56° C. for one hour.

Doses given: First, on January 18th, 200 million; second, on January 29th, 400 million; third, on February 8th, 800 million.

Of 23 rabbits that formed this group, 12 did not survive the first dose. In 4 of these the fatal issue was complicated by pregnancy; 1 survived the first but not the second dose; 7 survived all three doses.

Each of the 7 that survived all three doses was given as a test dose 1,000 million of living bacilli (twenty-four hours Shiga culture) in 1 c.cm. of normal saline intravenously on February 23rd. Of these, 6 survived and 1 died as a result of the test dose.

Group C₁.—Vaccine treated with dilute specific Shiga-agglutinating serum before it was heated.

Doses given: First, on January 26th, 200 million; second, on February 6th, 400 million; third, on February 16th, 800 million.

Of 20 rabbits that formed this group, 12 did not survive the first dose. In one pregnancy and in another pseudo-tuberculosis complicated the fatal issue; 8 survived all three doses.

Each of the 8 that survived all three doses was given, as a test dose, 1,000 million of living bacilli (twenty-four hours Shiga culture) in 1 c.cm. of normal saline intravenously on March 3rd. Of these, 7 survived, and 1 died of marasmus as a result of the dosage.

SERIES II.

In this series all the rabbits were inoculated on the same days, the doses being the same for each group—namely: First, on February 11th, 100 million; second, on February 22nd, 300 million; third, on March 4th, 900 million.

Group A₂.—Vaccine used, ordinary (non-sensitized) heated at 56° C. for one hour.

Of 6 rabbits that formed this group, 2 did not survive the first dose. In 1 of these the fatal issue was complicated by the presence of pseudo-tuberculosis; 4 survived all three doses.

Each of the 4 that survived all three doses was given as a test dose 1,000 million of living bacilli (twenty-four hours Shiga culture) in 1 c.cm. of normal saline intravenously on March 20th. All 4 survived.

Group B₂.—Vaccine heated at 56° C. for one hour, and afterwards sensitized with normal horse serum.

Of 6 rabbits that formed this group, 3 did not survive the first dose, 1 survived the first and second but not the third dose, 2 survived all three doses.

Each of the 2 rabbits that survived all three doses was given as a test dose 1,000 million of living bacilli (twenty-four hours Shiga culture) in 1 c.cm. of normal saline intravenously on March 20th. One died on the following day, and *post-mortem* examination revealed an advanced stage of pseudo-tuberculosis. The other survived.

Group C₂.—Vaccine heated at 56° C. for one hour and afterwards treated with diluted Shiga-agglutinating serum.

Of 6 rabbits that formed this group, 2 did not survive the first dose (1 of these was pregnant); 1 survived the first and second but not the third dose; 3 survived all three doses.

Each of the 3 rabbits that survived all three doses was given as a test dose 1,000 million of living bacilli (twenty-four hours Shiga culture) in 1 c.cm. of normal saline intravenously on March 20th. One became completely paralysed in its hind quarters and died on the sixteenth day after receiving the test dose. The remaining 2 survived the test dose.

SERIES III.

In this series also all the rabbits of each group were inoculated on the same days, the doses being the same for each group—namely: First, on April 28th, 25 million; second, on May 8th, 100 million; third, on May 18th, 500 million.

Group A₃.—Vaccine, ordinary (non-sensitized), heated at 56° C. for one hour.

Of 10 rabbits that formed this group, 1 did not survive the first dose. The *post-mortem* examination revealed old lesions in mesenteric glands and in liver; 1 survived the first but not the second dose—it had young after the second dose; *post-mortem* examination revealed old-standing disease of the lung; 8 survived all three doses.

Of the 8 that survived all three doses, 1 died before the test dose was due, and the cause of death in this case was complete prolapse of the womb during parturition.

Of the remaining 7, 2 got as a test dose 500 million of living bacilli in 1 c.cm. of normal saline intravenously on July 13th—1 survived and 1 died; 3 got as a test dose 1,000 million of living bacilli intravenously, 2 on June 4th, and 1 on July 13th—2 survived and 1 died as the result of this test dose; 2 got as a test dose 1,500 million of living bacilli in 1 c.cm. of normal saline intravenously—1 survived, and 1 died on the third day after this dose. The immediate cause of death was pneumonia.

Group B₃.—Vaccine heated at 56° C. for one hour, and afterwards sensitized with normal horse serum.

Of 10 rabbits that formed this group, 2 did not survive the second dose, or surviving the ten days, were too ill to get a third dose. In both "abortion" occurred; 8 survived all three doses.

Of the 8 that survived all three doses, each of 2 got 500 million of living bacilli in 1 c.cm. of normal saline intravenously on July 13th; both survived this test dose. Each of 4 got 1,000 million of living bacilli in 1 c.cm. of normal saline intravenously as test dose, 2 on June 4th, and 2 on July 13th; all 4 survived. Each of 2 got 1,500 million of living bacilli in 1 c.cm. of normal saline intravenously on July 13th; 1 survived and 1 died as a result of this dose.

Group C₃.—Vaccine heated at 56° C. for one hour, and afterwards treated with diluted Shiga-agglutinating serum.

Of 10 rabbits that formed this group, 1 did not survive the third dose; 9 survived all three doses.

Of the 9 that survived all three doses, each of 2 got 500 million of living bacilli in 1 c.cm. normal saline intravenously on July 13th—both survived this test dose; 1 got 750 million of living bacilli in 1 c.cm. normal saline intravenously on July 13th—survived. Each of 4 got 1,000 million of living bacilli in 1 c.cm. normal saline intravenously, 2 on June 4th, 2 on July 13th; 3 survived, and 1 died as a result of this dose. Each of 2 got 1,500 million living bacilli in 1 c.cm. normal saline intravenously as a test dose on July 13th; both survived.

In all the above three series the vaccine used had been heated at 56° C. for one hour. During the course of some complement fixation experiments, undertaken with the idea of finding some guide to the dilutions of bacilli and of specific serum which, when mixed together, would give the optimum sensitization of the bacilli, it was found that very little fixation of complement took place in any dilution if the bacillary emulsion had been first heated to 56° C. for one hour, and that none at all took place if the bacillary emulsion had been heated to 62° C. for one hour or more. For a time it was supposed that this result was in some way connected with the death of the bacilli, and acting on this hypothesis the vaccine used for Group C₁ was prepared. Later on, other experiments showed that it is the heat that destroys the property of the emulsion to act as antigen, and that emulsions of Shiga made with freshly prepared carbolized saline (0.5 per cent. carbolic in normal saline) and left until sterile, still retain that property. For the next series of experiments, therefore, the vaccines were not heated.

SERIES IV.

This series was divided into two groups of A₄ and C₄.

For Group A₄, ordinary (non-sensitized) unheated carbolized vaccine was used. For Group C₄, unheated carbolized vaccine treated, when sterile, with diluted serum from known highly immune rabbits, was used. The dosage was the same for both groups. Each group was divided into two lots. Each rabbit in lots 1 got two doses only, 50 million for the first dose, and, ten days later, 100 million for the second dose. Each rabbit in lots 2 got, in addition, a third dose, 400 million, ten days after the second dose.

Of 14 rabbits that formed group A₄, 2 did not survive the first dose. In 1 *post-mortem* examination showed that the fatal issue was complicated by pseudo-tuberculosis. The remaining 12 were divided into two lots, as above. Six rabbits (lot 1) all survived their second dose. Six rabbits (lot 2) all survived their second and third doses.

Three weeks after their last dose of vaccine, lots 1 and 2 got test doses as follows: Of lot 1, 3 got 500 million living, intravenously, and 3 got 1,000 million; all survived. Of lot 2, 3 got 1,000 million living, intravenously—all survived; 3 got 2,000 million—2 survived and 1 died as a result of this test dose.

Of 14 rabbits that formed group C₄, 3 did not survive the first dose. The remaining 11 were divided into two lots. Six rabbits (lot 1) all survived their second dose. Five rabbits (lot 2) all survived their second and third doses.

Three weeks after their last dose of vaccine the 6 rabbits of lot 1 got test doses as follows: 3 got 500 million living intravenously, and 3 got 1,000 million; all survived.

The rabbits of lot 2 did not get test doses of living bacilli, but, as above mentioned, all survived their third dose of vaccine, while of two controls that got the same dose both died.

ANALYSIS OF RESULTS.

1. Toxicity of Vaccines.

This may be measured by the percentage of rabbits that died during immunization. Eliminating those rabbits in which the fatal issue was complicated by pregnancy, abortion, or by well marked chronic disease, we find that in our attempts to immunize rabbits with heated vaccines—Series I to III inclusive—

24.2 per cent. succumbed to ordinary non-sensitized vaccines.

43.3 per cent. succumbed to vaccines sensitized with normal horse serum.

39.4 per cent. succumbed to vaccines sensitized with diluted specific Shiga-agglutinating serum.

The numbers on which these percentages are based, namely, 33, 30, and 33 respectively, are small, but are approximately the same for each of the vaccines. Again, most of the deaths occurred after the first dose in Series I and II, and when the dosage was modified, as in Series III, the results were:

No death in 8 during immunization with ordinary non-sensitized serum.

No death in 8 during immunization with vaccine sensitized with normal horse serum.

1 death in 10 during immunization with vaccine sensitized with diluted specific Shiga-agglutinating serum.

With unheated vaccines only one series—Series IV—is available, and applying the same process of elimination as before, we find that

1 death in 13 occurred during immunization with non-sensitized vaccine.

3 deaths in 14 occurred during immunization with sensitized vaccine.

Toxicity may also be measured by the severity of general symptoms and by the effect on the weight of those animals that survived. Data on those points do not appear in the above summary of experiments, but it may here be stated in general terms that the bowel symptoms were most severe in Group C₁, where the vaccine used was sensitized before it was heated; that where comparatively large doses of heated vaccines were used the severer

symptoms referable to the central nervous system were most marked after "sensitized" vaccines, and that loss of weight was most marked after ordinary "non-sensitized" vaccines, but that this was less noticeable in Series IV, where the vaccines were unheated and where the dosage was less.

2. Immunity Conferred.

The fact that the numbers available for this analysis are still smaller and more unequal for each group than for the first analysis must be borne in mind when expressed in percentages. Although the conditions varied in each series, they were the same for each group of each series, so that, with the proviso made above with regard to percentages, we may quite fairly compare the "sensitized" and "non-sensitized" vaccines in respect of the immunity conferred.

First, then, considering Series I to III (heated vaccines) together, the numbers protected against the test doses given were:

- In "A" group (ordinary non-sensitized vaccine), 20 out of 23; or approximately 87 per cent.
- In "B" group (vaccine sensitized with normal horse serum), 14 out of 16; or approximately 87 per cent.
- In "C" group (vaccine sensitized with diluted specific Shiga-agglutinating serum), 17 out of 20; or 85 per cent.

But if we take Series III by itself the figures are:

- In "A" group, 4 protected out of 6.
- In "B" group, 7 protected out of 8.
- In "C" group, 8 protected out of 9.

With unheated carbolyzed vaccines, only Series IV, consisting of two groups (Group A₁, in which ordinary non-sensitized vaccine was used, and Group C₁, in which the vaccine was treated with diluted serum from rabbits known to be highly immune), is available. The number of rabbits treated is small, but the results, so far as they go, indicate that, quite apart from sensitization, unheated carbolyzed vaccines give better immunity than vaccines that have been heated at 56° C. for one hour.

Local Reaction.

In the rabbit, in doses that can safely be given, the local reactions following Shiga vaccines are quite negligible. The rabbit, therefore, is not a suitable animal in which to study and compare the local reactions of Shiga vaccines. With living Shiga bacilli the case is different, and the following experiment may be cited as showing the difference in local reaction caused by the subcutaneous inoculation of large doses of living Shiga bacilli in immune as compared with that in unprotected rabbits.

1. Two healthy unprotected rabbits (Reference Nos. 14 and 15) got subcutaneously 2,000 million and 4,000 million of living Shiga bacilli respectively. On the following day there was in No. 14 a soft, prominent swelling of the subcutaneous tissues at the site of inoculation, and the skin over the centre of the swelling was purplish-red in colour. In No. 15, the swelling at the site of inoculation was somewhat more prominent, and the skin over it was mottled purple and red in colour. No. 15 died on the fourth day, and No. 14 on the fifth day after the inoculation. In both cases the skin over the centre of the swelling had necrosed, and on cutting into the swelling a central slough about $\frac{1}{4}$ in. thick at its centre, and thinning off towards the periphery, was exposed. The area of the necrosed skin and the slough were somewhat more extensive in No. 15 than in No. 14.

2. Two rabbits (Reference Nos. 3 and 6) that had each survived a dose of 200 million prepared vaccine on December 14th, 1914, were afterwards treated as follows. Each got on January 13th, 1915, 200 million, on January 20th 400 million, on January 27th 800 million, and on February 3rd 1,600 million of ordinary killed Shiga vaccine that had been heated at 56° C. for one hour. On May 27th No. 3 got 2,000 million and No. 6 got 4,000 million of living Shiga bacilli subcutaneously. In each case there was on the following day a soft puffy swelling of the subcutaneous tissue and some redness of the skin at the site of inoculation. Two days later the swelling was less puffy and the skin was less red. Ten days later there was still some puffy swelling in the case of No. 3 and a firm elongated swelling in the case of No. 6. The skin over the swellings was normal in appearance; the swellings themselves were much reduced in size and

later on gradually disappeared. Both rabbits remained well.

The above results suggest that the local reaction following the injection of vaccines used for therapeutic purposes in patients suffering from the disease—at all events, in chronic cases or in cases that have lasted for some considerable time—may be mild compared with that in normal persons, and workers using exclusively any particular vaccine for therapeutic purposes only may be misled as to its probable local reaction if used prophylactically in normal persons.

At the King George Hospital I understand that ordinary Shiga vaccine has been given to patients suffering from chronic dysentery of the Shiga bacillary type without its producing any local reaction, in doses that in normal persons have produced severe local reactions.

SUMMARY AND CONCLUSIONS.

1. *Toxicity.*—The toxicity of the vaccines studied has been determined by the liability of the initial dose to kill the experimental animals; and in those that survived by the severity of general symptoms, but more especially by the effect on the weight of the animals during the period of immunization.

In the series of animals in which moderate and large initial doses of vaccines were given, it was found that serum-treated vaccines were more fatal than untreated vaccines, and in the case of animals in which comparatively small initial doses were given the numbers are not sufficiently large to warrant us drawing a conclusion on this point.

With heated vaccines, bowel symptoms were most severe after the vaccine that had been treated with diluted specific Shiga-agglutinating serum before being heated. Symptoms referable to the central nervous system were, on the whole, most severe after serum-treated vaccines.

Serum-treated vaccines produced less loss of weight than untreated vaccines, but this was less noticeable in Series IV, where the vaccines were unheated and the dosage was less.

2. It has been found necessary to diminish the initial dose of vaccine in each succeeding series of rabbits, and the results obtained encourage the view that an initial dose at once relatively non-toxic and still able to immunize may be hoped for, though such a dose has not yet been established.

3. The first dose is the critical dose with all the vaccines used. Even with 50 million as a first dose, no less than four healthy animals died out of twenty-seven, while none died after 100 million given as a second dose.

4. The second dose should not be greater than twice the size of the first dose, though this ratio (1 : 2) may be exceeded in subsequent doses.

5. Heating should be avoided in the preparation of Shiga vaccines. It does not reduce their toxicity, and it destroys the antigen that calls forth the complement fixing body. Moreover, unheated vaccines may be sterilized by weak carbolic, freshly prepared, without losing the above-mentioned antigen; and these unheated carbolyzed vaccines gave in our experiments a better immunity.

6. Immunity, as tested by the power to withstand lethal doses of living bacilli given intravenously, was obtained with serum-treated and with untreated vaccines in apparently about equal degree. Whether the immunity obtained by the one is more lasting than that obtained by the other was not determined.

7. *Local Reactions.*—The rabbit is not a suitable animal on which to study and compare the local reactions caused by dead Shiga vaccines.

THE Accademia dei Lincei of Rome has awarded the King's prize of £400 for human physiology to Dr. Filippo Bottazzi, professor of physiology in the University of Naples. He is the author of a treatise on physiological chemistry and of memoirs on the metabolism of the red corpuscles, and other subjects.

It has been arranged to hold the Oxford Ophthalmological Congress as usual this year. It will assemble at Keble College on the evening of July 12th. The whole of the following day will be devoted to a discussion on the relation of ophthalmology to general medicine, to be opened in the morning by Sir William Osler and in the afternoon by Sir Anderson Critchett. Further particulars can be obtained from the honorary secretary, Mr. Bernard Cridland, Salisbury House, Wolverhampton.