THE SERUM TREATMENT OF EXPERIMENTAL TETANUS IN MONKEYS.

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A series of experiments undertaken for Surgeon-General Sir DAVID BRUCE'S Committee bear on one of the points dealt with in his Presidential Address, and have some interest in that connection. The question they attempt to give answer to is whether the route by which the anti-tetanus serum is introduced affects the curative efficacy. The method adopted in my experiments has been to inoculate the animal with a given dose of tetanus toxin, and after outbreak of the resulting symptoms of tetanus to inject a given quantity of antitetanus serum by one or other of the following six different routes: (1) subcutaneous, (2) intramuscular, (3) intravenous, (4) bulbar subdural, (5) lumbar subdural, (6) cerebral subdural. The animal used was the monkey. The dose of toxin was 2 mgm. per kilo. of animal. The sample of toxin used throughout was the same; it was kindly supplied by Dr. MACCONKEY of the Lister Institute; its strength, as measured by its effectiveness on mice, was that the minimal L.D. was 1 mgm. per kilo. mouse. Control experiments on monkeys shewed that the minimal L.D. of this toxin for the monkey, when the toxin was inserted into muscle, is close below .25 mgm. per kilo. monkey, the animal after that dose dying of general tetanus in nine days. The dose of this toxin employed in the series of experiments for testing the relative values of the above routes was, therefore, slightly more than eight times the M.L.D. for the animal, the toxin being inserted intramuscularly. The site of the inoculation was in all the experiments of the series the same, namely, into the outer head of the left gastrocnemius muscle. Following this dose, the initial symptoms

of tetanus invariably appeared in the muscles of the inoculated limb, being first noticeable usually 30-36 hours after the inoculation. The symptoms, then, with greater or less speed passed up on the same side, involving the forelimb and neck and, a little later, the jaw. The jaw was usually obviously affected by the 72nd hour after the inoculation. At periods varying from 48-96 hours after the inoculation, that is, from 12-60 hours after the outbreak of the symptoms, antitetanic serum was injected by one or other of the above-mentioned routes. The experiments were done in groups of threes for each of the three, the amounts of A.T.S. and the interval between toxin inoculation and serum administration being the same.

The net results of the series of experiments were as follows :—

Route of A.T. serum injection.	No. of exp'ments.	No. of deaths.	No. recovered.
Subcutaneous ...	25	23	2
Intramuscular ..	25	22	3
Intravenous ...	25	18	7
Lumbar subdural ...	25	11	14
Bulbar subdural ...	20	7	13
Cerebral subdural ...	10	10	0

The injection of the serum when subcutaneous was always in the flank of the side corresponding with the limb shewing the initial symptoms; and when intramuscular, was always into the hamstring muscles of that limb. For lumbar subdural injection a limited laminectomy was always performed, the theca exposed, and the injection needle carefully introduced through it. In five of these experiments the needle-point was intentionally thrust slightly into the spinal cord itself, an injury which caused no obvious symptoms except a slight local twitching at the time. By bulbar subdural injection is meant injection through the atlanto-occipital membrane, which was carefully exposed by operation before the introduction of the needle. Cerebral subdural injection was always done in the parietal region after turning back the temporal muscle and trephining in that situation.

It will be seen from the tabular statement that the results as regards effect upon the issue of the case are fairly unequivocally in favour of the spinal and bulbar intrathecal routes as against any of the others.

NOTES ON THE OCCURRENCE OF *B. TETANI* IN WOUNDS.

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The following is an account of some of the results obtained during the course of an investigation into the occurrence of *B. tetani* in the wound exudate of 252 cases which were not shewing any symptoms of tetanus intoxication.

The work was carried out at the suggestion of the War Office Committee for the Investigation of Tetanus, to whom a preliminary report of the main results was made in October, 1916. I am indebted to the Committee for their kind co-operation in arranging facilities for me in regard to obtaining material and in many other details.

The cases were chosen at random from four different hospitals in the London area between the dates of August 17th and September 28th, 1916, the only common factor in the selection being that the wounds were classed by the surgeons and sisters under the general heading of "septic wounds." The cases were all receiving treatment of one kind or another: eusol, hydrogen peroxide, physiological salt solution, hypertonic saline, bismuth-iodoform-paraffin paste, boric fomentations, malachite green, cyanide wool, chlorinated soda, potassium permanganate, iodine, zinc sulphate, glycerine, hypothyamine, hypochlorous acid, eucalyptus, enzymol—all figured, either singly or in various combinations, under the heading of "treatment" in the case-sheets. No attempt was made to neutralise the antiseptics used in the dressings, the samples were dealt with just as taken direct from the wounds. The period elapsing between the date upon which the man was wounded and the date upon which the sample was taken was always noted, and varied from a few days to several months.

The exudate from the wound was collected upon a sterile swab or in

a sterile pipette, care being taken to get a representative sample. The material was emulsified in sterile saline and inoculated into culture media. Various media were used, but the main line of the investigation was carried out by using an autoclaved chopped-meat medium, the recipe of which is added at the end of the paper. The emulsified wound material was inoculated into such a meat-tube, and the tube was placed for fifteen minutes in a water-bath at 80° C. This heating gets rid of the non-sporing organisms; it seems also to throw out *B. perfringens* in a proportion of the cases, though not by any means in all. After heating, the meat-tube is cooled in water and placed in the anaerobic cylinder. On certain occasions, when there was an excessive number of tubes to handle and the anaerobic cylinders were overcrowded, another method was adopted. One or two cubic centimetres of sterile liquid paraffin were run into the tube of meat medium, which was then steamed for an hour or longer; after cooling, the material from the wound was inoculated into the tube, which was placed in the bath at 80° C. for fifteen minutes, and thereafter incubated at 37° C. without any further precautions. The paraffin seal to the meat-tubes is not really necessary and may be omitted, but care should always be taken to see that the meat-medium tubes are steamed shortly before use. These aerobic methods are very convenient and are quite satisfactory, especially for mixed cultures; nevertheless, for prolonged work and for further subcultures, an anaerobic apparatus of some kind is desirable.

The meat cultures were incubated for four or more days, and examined microscopically for the presence of anaerobic organisms. In the cases in which these organisms had developed, subcultures were made into broth and egg-broth, which were incubated anaerobically for seven days or longer. These subcultures in broth or egg-broth were used for inoculation into mice and guinea-pigs, and in every case a control animal received an equal dose to which had been added thirty U.S.A. units of tetanus anti-toxin,

The presence of *B. tetani* was held to be demonstrated when—

- (1) The characteristic tetanus reaction had been produced by the inoculation of the culture into an animal.
- (2) When the control animal had been protected by the addition of thirty U.S.A. units of tetanus antitoxin to the dose of culture injected.

No attempt was made to obtain pure cultures of the organism. *B. tetani* was always found to be accompanied by one or more other anaerobes, and the unravelling of these mixed cultures would have rendered the handling of any sufficient number of cases quite impossible. It will be seen in the sequel that, under the particular conditions obtaining, the exhibition of tetanus toxin was not prevented by the presence of the other anaerobes in the cultures.

The wound exudate from 252 cases of patients shewing no symptoms of tetanus intoxication was examined, and, in addition, the exudate from 45 cases from patients suffering from declared tetanus was also investigated.

I. THE 252 CASES SHEWING NO SYMPTOMS OF TETANUS.

The cases were divided into three classes according to the cultural results :—

- (1) Those in which organisms of the “drumstick” type with round endspores were cultivated along with other anaerobes. These amounted to 64 cases, *i.e.*, 25·5 per cent.
- (2) Those in which anaerobes of various kinds not including any types shewing round endspores were cultivated. These amounted to 105 cases, *i.e.*, 41·6 per cent.
- (3) Those in which there was no growth of anaerobes. These amounted to 83 cases, *i.e.*, 32·9 per cent.

From the above it will be seen that the total percentage of cases in which anaerobes of one kind or another were grown is 67·1.

It was not possible, owing to practical considerations, to carry out a toxicity test with everyone of the 169 anaerobic cultures obtained from the whole series under investigation. The following tests were, however, made :—

Sixty cultures belonging to class 1 (*i.e.*, those cultures shewing round endspores) were tested for toxicity, and in nine a tetanus reaction was produced in the animal injected. That is to say, that tetanus toxin was developed in 15 per cent. of the cases shewing organisms with

round endspores. These positive cultures fulfilled the criteria laid down above:—

- (1) The characteristic tetanus reaction was produced by the inoculation of the culture into an animal.
- (2) The control animal was protected by the addition of 30 U.S.A. units of tetanus antitoxin to the dose of the culture injected.

And, in addition, the cultures had revealed organisms with round endspores.

Forty-four cultures belonging to class 2 (*i.e.*, those cultures which shewed anaerobes other than types with round endspores) were tested for the presence of tetanus toxin. In no case was a tetanus reaction produced in the animal injected.

Taking all the cultures tested, both from class 1 and class 2, together, the figures give 104 cases tested and 9 positive tetanus, *i.e.*, 8·6 per cent. This is a manifestly incorrect way of handling the figures, as in the series of 252 cases the numerical relation of class 1 to class 2 is as 25 to 41, *i.e.*, as 1 to 1·6, whereas in the sample tested the ratio of these classes is 60 to 44, *i.e.*, 1 to 0·7.

A fairer way of putting the question is to consider that the nine positive tetanus reactions were obtained upon a series of 252, which makes the percentage equal 3·5. The chance that the untested residue of class 2 would have yielded further positive tetanus reactions is small, having regard to the result of the tests actually carried out in the two classes.

Table I. gives the distribution of the cases according to the length of time elapsing since the date of wounding. The table includes up to the end of the third month. This takes in all except eleven cases where the wound was of still longer standing. It is interesting to note that the cases in which living *B. tetani* were demonstrated fall into two periods, namely, within (*a*) seven days of wounding and (*b*) between the 30th and 60th day of wounding. The period between the 30th and 60th day of wounding presents features of particular interest. (It must be borne in mind that the whole series of 252 are already selected from the bulk of wounded, in that they were chosen as being "septic wounds.") In a total of 91 cases from this period the distribution falls out as follows:—

Cases with round endsporers, *i.e.*, class 1 ... 17, *i.e.*, 18·7 per cent.

Cases with anaerobes other than round

endsporers, *i.e.*, class 2 41, *i.e.*, 45·1 „

Cases where no anaerobes could be culti-

vated, *i.e.*, class 3 33, *i.e.*, 36·2 „

The cases in which living *B. tetani* were demonstrated amount to 7, *i.e.*, 7·6 per cent. Of the cases falling into this period belonging to class 1 (*i.e.*, those shewing round endsporers) the whole 17 were tested for toxicity and produced 7 positive tetanus reactions, a percentage equal to 41·1. From class 2 (*i.e.*, those shewing anaerobes other than round endsporers) 21 cases were tested for toxicity, and produced no tetanus reaction in any of the animals injected. Now the ratio of those tested in each of classes 1 and 2 from this period is 17 to 21, *i.e.*, 1 to 1·2, which while lower than the ratio of these two classes in the entire series of 252, is nevertheless pretty near it. The ratio over the entire series is 1 to 1·6.

If the cases for the first month after wounding are taken together—they amount to exactly 100—one gets the following percentages:—

33 per cent. of cases with round endsporers.

39 per cent. of cases with anaerobes other than round endsporers.

27 per cent. of cases with no anaerobes, and in this series of 100 there are only 2 per cent. where it could be demonstrated that the patient's wound contained living *B. tetani*.

It therefore appears that, under the conditions of treatment, the second month is a period of greater danger from the point of view of tetanus being present in sufficient numbers to be demonstrable than the first month. All the patients had had a prophylactic injection of tetanus antitoxin immediately upon being wounded.

The actual time elapsing between the date of wounding and the taking of the sample in the nine cases in which tetanus was demonstrated is as follows:—

7 days in case No. 235.			45 days in case No. 73.		
8	„	146.	49	„	27.
33	„	12.	56	„	62.
37	„	123.	56	„	68.
39	„	236.			

Case no 146 had some features of particular interest—the sample was taken upon the eighth day after wounding, the patient having received one prophylactic dose of tetanus antitoxin in France. The meat culture made from the wound exudate gave a very good growth of organisms with round endspores accompanied by a rather slight admixture of another anaerobe of a proteolytic oval-spored type. The subinoculations in broth gave actively toxic cultures producing tetanus symptoms in mice and guinea-pigs, which symptoms failed to appear in the control animals receiving the same dose to which was added thirty units of tetanus antitoxin. That is to say, the wound contained an actively toxic strain of *B. tetani*. The condition of the patient was, however, good, and the wound was reported to be going on well. On the thirtieth day after wounding a second sample was taken, and investigated for the presence of anaerobes without result, the only organisms grown being cocci. The patient made an uninterrupted recovery. In this case the prophylactic dose had apparently tided the patient over without symptoms, although his wound contained a toxin-producing strain of *B. tetani*, and the organism was subsequently dealt with and eliminated during the progress of the healing of the wound.

Cases Nos. 73, 27, 62 and 68 all gave cultures which were actively toxic, although the periods at which the samples were taken were respectively 45, 49, and 56 days after the date of wounding. *B. tetani* was, as usual, present with other anaerobes; the patients were shewing no symptoms of tetanus intoxication, and the surgeons did not consider that the condition of the wounds gave any cause for anxiety. To have a sufficient number of *B. tetani* organisms present in a wound for cultures to be grown from a swab or from a pipette containing not more than 0·25 to 0·5 of a c.c. of exudate, 56 days after the date of wounding, seems of necessity to imply that the organisms have been multiplying to a certain extent in the wound. The strains were capable of producing toxin as was shewn by the reaction in the inoculated animals. By the fifty-sixth day the effect of the prophylactic dose (which is usually administered within the first 24 hours from the time of wounding) would, according to all the experimental evidence, have disappeared. It would therefore seem, either that the organism in the wound was under conditions unfavourable to the production of toxin, or that the patient had produced antibodies on his own account and was able to deal with any small

quantity of toxin that was being absorbed. The evidence at present available leaves the question unsolved. Cases such as these where *B. tetani* is harboured for long periods do of course account for the late cases of tetanus which appear from time to time.

To consider now the significance of organisms with round endspores in cultures from a wound. It is perfectly clear that in itself it is not an indication that the patient is actually harbouring *B. tetani*. This is quite evident from the figures given above, and is further supported by the fact that an organism of this morphology, which differs from *B. tetani* in cultural characters and which is non-toxic, has been isolated from several of the cultures of the above series. This organism is a strict anaerobe: it is motile, it produces gas and a pink colour in meat, does not liquify gelatin, does not liquify inspissated serum, produces acid without clot in milk and ferments maltose and glucose. It is non-toxic and non-pathogenic for guinea-pigs. This organism agrees with one first isolated by Dr. MACINTOSH, a culture of which he very kindly placed at my disposal. It has also been mentioned by ADAMSON and CUTLER, and has, I believe, been recognised by many workers dealing with wound material. The fact, however, remains that it is from the group of cases containing the round endspores that the toxic cultures were obtained; one is, therefore, led to the conclusion that the particular conditions which foster round endspores as a whole also foster *B. tetani*. This conclusion is supported by a great deal of direct evidence from cultural data which cannot be gone into in the present paper. One thing, however, is strikingly shewn, namely, that the endsporing group comes up late in the mixed cultures in the meat-medium tube, its appearance being preceded by members of the proteolytic group.

This point as to the value of the round endsporer as a warning in regard to the condition of the wound will be touched upon again in dealing with the exudates from the cases of patients suffering from declared tetanus.

I do not propose to deal with the individual organisms in this paper; suffice it to say that a very considerable number of different anaerobes were found to be present, both pathogenic and non-pathogenic. Thus a strain giving all the cultural characters of the "vibron septique," and very pathogenic for guinea-pigs, was isolated from one of these cases by Miss HEMPL, of Ann Arbor University, U.S.A., who was working in the

Lister Institute at the time. Several members of the non-pathogenic proteolytic group were also obtained, so also *B. perfringens* and several oval endsporing organisms.

I would like to take this opportunity to correct an error in nomenclature in a previous paper. I identified the proteolytic organism so frequently found in wounds with the organism described by VON HIBLER as malignant œdema of KOCH. The cultures current in this country under the title of malignant œdema agreed in detail with what I was recovering from the wounds, and agreed with VON HIBLER's account, except that they were practically non-pathogenic. I am now inclined to think that malignant œdema of KOCH, as described by VON HIBLER, must be an impure culture consisting of a proteolytic sporebearer, and some one or other of the pathogenic forms. In any case I think the name is not a good one and should be given up. The proteolytic form may, I think, be considered to be *B. sporogenes* of METCHNIKOFF, but here again this is in some doubt, as METCHNIKOFF considered that his *B. sporogenes* was closely allied to *B. enteritidis sporogenes* of KLEIN; this is another of the anaerobe types that is not well-defined, and it appears to have characters in KLEIN's account that resemble *B. perfringens*. The name *B. sporogenes* has, however, been adopted by many workers for this ubiquitous proteolytic non-pathogenic form, and while it may not be entirely beyond criticism, it is a much better appellation than the one adopted by myself.

The literature of the anaerobes is full of obscurities owing to the very great difficulty of obtaining really pure cultures; this complicates the naming and placing of the forms obtained and causes considerable confusion.

TABLE I.

Number of days since date of wounding.	No anaerobes grown.	Anaerobes other than round endspores.	Round endspores present but cultures not toxic.	Round endspores present including <i>B. tetani</i> , cultures toxic.	Total.	Percentage of cases with round endspores.
Within 7 days of wounding ...	3	9	8	2	22	45°/o
Between 7th and 15th day ...	11	15	7	0	33	21·2°/o
Between 15th and 30th day ...	14	15	16	0	45	35·5°/o
Between 30th and 60th day ...	33	41	10	7	91	18·7°/o
Between 60th and 90th day ...	18	19	10	0	47	21·2°/o

TABLE II.

PERCENTAGES OF THE DIFFERENT CLASSES OF CASES IN THE
FIRST AND SECOND MONTHS OF WOUNDING.

	No anaerobes grown.	Anaerobes other than round endspores.	Round endspores present but cultures not toxic.	Round endspores present including <i>B. tetani</i> cultures toxic.	Actual number of cases examined.
During first month	28°/o	39°/o	31°/o	2°/o	100
During second month	36°/o	45°/o	18°/o	7·6°/o	91

II.—EXUDATES FROM CASES OF DECLARED TETANUS.

To pass now to the consideration of the exudates from cases of declared tetanus. I am indebted to the military surgeons and officers in charge of a large number of different hospitals for the samples sent and I wish to take this opportunity of thanking them for their assistance in this matter.

A total of 45 cases was dealt with culturally; they were all from patients who, in the opinion of the medical officers in charge, were or had recently been suffering from tetanus intoxication. As the material came from a large number of different sources and often from a distance, there was more variation in the nature of the samples than in those of the previous series. In addition, the data as to the period of the disease at which the sample was taken, the treatment in use, etc., were sometimes rather scanty; in several cases the patient was convalescent or indeed had recovered entirely before the material was sent to me. The relevant information was got together in as many cases as possible, but there are nevertheless some gaps which could not be avoided.

The whole series of 45 was dealt with culturally in the manner described above. Eight of these failed to produce the growth of any anaerobes; 37 cases gave cultures containing anaerobes of one kind or another. It is interesting to note that 29, or 78·3 per cent. of all these cases that gave growth, shewed the round endsporing type. Now I am not prepared, even in toxic cultures derived from cases of declared tetanus, to consider that a round endsporer must necessarily be *B. tetani*. What is important is to note that the condition of wound favourable to the production of a case of tetanus will give cultures with round endspores in at least 78 per cent. of the cases. The criticisms expressed above as to the slight irregularities in the collection of the samples and the occurrence of convalescents in various stages of recovery must be borne in mind in this connection. They tend to lower the percentage of cases with round endspores. The connection of the appearance of organisms with round endspores in the culture from the exudate and the wound condition favourable to the persistence of *B. tetani* was already shewn in the earlier part of the paper; the complementary nature of the two sets of data is obvious.

In 22 cases, out of a total of 45, part of the exudate was inoculated

directly into a mouse without cultivation; in only two cases was there a positive tetanus reaction produced, namely in Nos. 1 and 121. No. 1 shewed toxin also in the subsequent cultures; No. 121 failed to produce growth upon anaerobic cultivation. In six of the cases in which direct inoculation failed to produce a tetanus reaction, an actively toxic culture was obtained upon cultivation of the same sample. One is therefore led to conclude that a direct inoculation of wound exudate into a single mouse is not of much use as a means of determining if *B. tetani* is present or not.

A group of 23 cases, out of a series of declared tetanus, were dealt with in exactly the same way as the indifferent cases described in the first part of the paper, and were tested for toxicity by inoculation of the culture into animals. Twelve of these produced toxic cultures; in four of these the exudate was obtained from patients who ultimately succumbed to the disease. Out of the 11 cases where toxin was not exhibited in the cultures, it was found upon examining the data available that seven were definitely convalescent after treatment with antitoxin when the sample was taken: one was recorded as being a case where the symptoms were slight, and in the three remaining I could not obtain the detailed history of the case.

Certain of the cases tested were of particular interest.

Case 33.—Develops tetanus in the seventh week from date of wounding; receives energetic treatment with antitoxin, receiving a total of 57,000 U.S.A. units in three days. The sample taken the day after treatment was discontinued, developed no toxin upon culture.

Case 211.—Develops tetanus in the second week; he receives a total of 45,000 units of antitoxin in three days. The sample taken two days after the treatment was discontinued, developed no toxin upon culture.

Case 20.—Develops tetanus in the fourth week; he receives 55,000 units in two days. The sample taken seven days after the treatment was discontinued, developed no toxin upon culture.

In these three cases at the end of treatment with antitoxin, it was not possible to demonstrate living *B. tetani* in the wound exudate. The treatment involved large doses of antitoxin.

Case 3.—Develops tetanus in the fourth week; he receives 2,500 units in four days. The sample taken four days after the treatment with antitoxin was discontinued, developed no toxin upon culture.

Case 39.—Develops tetanus in the second week ; he receives 12,000 units in four days. The sample taken two days after the treatment was discontinued, developed no toxin.

In these cases the doses of antitoxin were less massive, and here also the wound exudate at the end of the treatment failed to shew living *B. tetani*. At the termination of the treatment in the five cases cited, the patient is left in a condition which appears to place him beyond the danger of a recurrence of tetanus.

Case 220.—Develops tetanus in the eleventh week ; receives 5,000 units intrathecally in two days. The sample taken on the day the treatment is discontinued, produced a toxic culture.

Case 114.—Develops tetanus in the eighth week ; receives 16,000 units intrathecally in two days. The sample taken on the day the treatment is discontinued, produced a toxic culture.

Case 265.—Develops tetanus on the tenth week ; receives 4,500 units subcutaneously in five days. The sample taken five days after the treatment with antitoxin was discontinued, produced a toxic culture.

Case 252.—Develops tetanus in the fourth week ; the symptoms are slight. On the third day after onset of symptoms, patient gets 500 units intrathecally and 500 units intravenously ; the injections are discontinued as the patient develops pneumonia, from which he recovers. The sample wound exudate taken eighteen days after the treatment with antitoxin is discontinued, produces a toxic culture.

All the four last-cited cases recovered from the attack of tetanus.

Cases 114 and 220 do not call for any particular remark ; living *B. tetani* could be demonstrated in the wound at the end of treatment, but the samples were taken immediately upon the termination of the injection of antitoxin and the elimination of the organism might not yet have been effected. *Case 265* is interesting, as five days after the injection of antitoxin is discontinued, he still harbours living *B. tetani*. The patient recovers, but he was not definitely placed beyond the possibility of a return of the symptoms by the treatment adopted. In *Case 252* this is still more evident ; here the patient's wound still contained living *B. tetani* eighteen days after the antitoxin was discontinued. The symptoms had been suppressed, but the treatment had not eliminated the organism, and the patient was still in danger of a possible return of the disease. The importance of the condition in which cases like 265

and 252 are left at the end of the antitoxin treatment, is shewn by the history of such a case as 199. The patient has an attack of tetanus beginning on the eighth day after wounding, this is dealt with in France by the injection of tetanus antitoxin, and the patient recovers. In the ninth week after wounding, tetanus symptoms arise for the second time. He receives nine subcutaneous injections spaced over twenty-five days, the total number of units administered being 18,250. A sample of the wound exudate taken three days after the fourth injection, at a time when the patient had received 11,500 units, did not produce toxin upon cultivation.

Considering both the indifferent series and the series of declared tetanus, there seems to be a perfect graduation between the cases such as 27, 62 and 68, where living *B. tetani* can be obtained from the wound in the seventh and eighth week after wounding, but in which there are no symptoms of intoxication, through cases with slight symptoms like 252, to the rapidly fatal case like, for instance, 299, where the patient died within 24 hours of the onset of symptoms.

I wish, in conclusion, to express my thanks to Dr. HELEN CHAMBERS, Dr. ST. JOHN BROOKS, Capt. BALL, R.A.M.C., and Dr. ATKIN, for very kind co-operation in the collection of the material.

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APPENDIX.

Cooked Meat Medium.—Eight ounces of bullock's heart minced finely and then ground in a mortar; add eight ounces of tap-water and heat slowly so as to cook the meat thoroughly: add normal sodium hydrate until the mixture is markedly alkaline to litmus. Divide into tubes and autoclave at 120° C. for half-an-hour. Particular care should be taken to see that the reaction is definitely alkaline at the time of inoculation, a useful rough test is afforded by the colour of the medium, it should be brown. Care must be taken in the sterilizing of the medium; it should be borne in mind that the meat may contain many very resistant spores, and it is quite essential that the medium should be exposed to the actual temperature of 120° C. at least for half-an-hour.