CASE VIII.

Pte. G. Wounded September 10th; admitted September 20th. Three wounds present over lower part of sternum and one large laceration from which pus poured. Radiograph on September 24th showed three foreign bodies and gas.

Metal removed by Mr. Hewetson. Culture from pus showed *Bacillus perfringens*.

CASE IX.

Ionbardier R. Wounded in left loin on September 26th; admitted October 2nd. Radiograph on October 4th showed large piece of metal at a depth from the back of 3 in., with a halo of gas.

Removal by Mr. Furneaux Jordan on October 5th. Gas obvious at operation. Culture from bullet grew *Bacillus perfringens*.

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NOTE ON THE KEEPING QUALITIES OF
THERAPEUTIC SERUMS.

BY

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At the present time, when it is necessary to keep large stocks of therapeutic serums on hand to meet any sudden call, it becomes of importance to know the rate at which serums deteriorate.

The serum which has been the most carefully studied in this respect is antidiphtherial serum.

Diphtheria Antitoxin.

Anderson (1910) examined the question under experimental conditions and found that the average yearly loss in unitage was about 20 per cent. when the serum was kept at room temperature (70° F.-95° F.); at 15° C. the loss was about 10 per cent., and at 5° C. about 6 per cent. per annum. Dry diphtheria antitoxin kept in the dark at 5° C. remains stable for at least five and a half years. Old serums are just as good as fresh serums when the relative unitage is taken into account.

The writer (1913) also carried out some experiments in this direction. His results confirm those of Anderson inasmuch as he found that the loss during one year amounted to 7 per cent. when the serum was kept in an ice chest, and to 14 per cent. when the store temperature varied between 6° C. in winter and 16° C. in summer. At 36° C. the loss was 37 per cent. in six months and 51 per cent. in twelve months. One serum kept in bulk (100 c.cm.), instead of in the ordinary ampoules, at 36° C. lost 62 per cent. of its antitoxin in six months.

We see from these observations what we can count upon when the serum is kept under proper conditions, namely, in the dark and in the cold.

But serums on the market are not always stored so carefully, judging from tests carried out on samples of serum which have been in commerce.

In the *Journal of the Royal Army Medical Corps*, vol. iii, 1904, p. 601, are given results of an examination of some samples of diphtheria antitoxin. The interval between the tests was about fourteen months, and during that time some of the samples had made two voyages to India and back. The maximum loss in unitage was 20 per cent., and the average loss some 7 to 8 per cent.

Miller (1935), who examined eighty-two samples of returned serum, mentions that "A half-dozen packages of serum had evidently been exposed to considerable heat, as the serum in them was firmly coagulated." He concluded that—

1. The high potency serums change more rapidly than the weaker, but in neither case is the loss of potency as rapid as is usually supposed.
2. The ordinary packages of serum retain their full therapeutic value for the time limit, which is often stamped on the labels.
3. The demand frequently made for fresh antitoxin is not justified.

Kinyoun and Hitchens (1907) tested 100 serums, samples of "returned antitoxin" which had been subject to the usual commercial conditions. The time between the first and final tests varied from thirteen to twenty-seven months. The loss in unitage varied from 0 to 48 per cent.; the latter occurred in eighteen months. Many of the serums were out for more than eighteen months without losing so much.

In the following table are given the results of an examination of a number of samples of serum which had been in commerce, and which were all returned at the same time. It must be noted that the tests were not carried closer than 50 units. Thus a 450 unit serum might be 475 units or more, a 300 unit serum might be 340, and so on. Serum No. 4, for instance, may originally have been, say, 640, and the loss really 6 to 7 per cent., but as the serum was, for market purposes, originally reckoned as 600 units its loss in commerce was *nil*. These batches of serum were made up of bleedings which had been kept for varying periods before being mixed up to form a batch. Serum No. 7 was composed of serums which had been in store for from two to sixteen months before being mixed, and therefore the "age of serum" is put as two to sixteen months:

TABLE I.—Showing the Loss of Antitoxin which occurred in Samples of Diphtheria Antitoxin which had been in Commerce for Various Periods.

No. of Batch.	Age of Serum.*	Period between First and Final Tests.	Unitage when Sent Out.	Unitage when Returned.	Loss in Unitage.
	Months.	Months.			Per Cent.
1	19-24	72	500	400	20
2	6-23	55	450	300	33
3	11	46	900	800	11
4	4-14	51	600	600	<i>Nil</i> .
5	9-13	47	550	300	45
6	3-54	49	450	300	33
7	2-16	44	550	450	18
8	3-16	41	450	350	22
9	5-13	40	450	350	22
10	7	39	1,100	600	45
11	5-9	39	450	350	22
12	6-9	36	600	400	33
13	5-6	36	450	300	33
14	4-7	33	450	350	22
15	2-9	32	450	350	22
16	10-16	25	450	400	11
17	13-16	24	450	400	11
18	6-12	29	600	450	25
19	4-7	29	550	350	36
20	7-13	28	400	300	25
21	10-14	27	450	400	11
22	13-20	18	450	350	22
23	11-16	23	500	450	10
24	7-16	22	500	450	10
25	11-22	22	600	450	25
26	12-16	19	450	300	33
27	7-16	19	400	350	12
28	10	21	500	350	30
29	13-24	17	550	400	27
30	11-14	17	500	400	20
31	14-22	9	450	400	11

* That is, period between the date of collection and the date of first testing and sending out into commerce.

The average period during which these serums were on the market is about two and a half years, and the average loss in unitage during that period is about 23 per cent., or about 10 per cent. per annum. Nothing is known about the conditions under which these serums were stored while in commerce, but it is obvious that the rates of deterioration varied greatly. If we compare the losses in Nos. 19, 20, and 21, and also those in 26 and 27, we must conclude that unless we know how serum has been stored the putting on the label of a time expiry limit tells us nothing, and may lead to a false sense of security.

Tetanus Antitoxin.

Kept under similar conditions this antitoxin loses its strength at about the same rate as diphtheria antitoxin.

Antiplague Serum.

Taking the antitoxin content of this serum as a criterion, it has been found that antiplague serum when stored under proper conditions changes little in the course of two years. (Cf. MacConkey, 1912.)

Antidysentery Serum.

There is no generally accepted method of standardizing this serum. In these laboratories we have for several years used as test "toxin" the dry, powdered bodies of bacilli of the Shiga type. The strength of this powder has remained fairly constant for three years— $\frac{1}{10}$ mg. suspended in distilled water or salt solution and injected intravenously usually proving fatal to a rabbit of 1,000 to 1,500 grams in four to five days. The test dose, 1–5 mg., of this "toxin" is mixed with the serum and at once injected intravenously. Tested in this way, antidysentery serum appears to retain its "toxin" neutralizing power with little change for at least one and a half years. Some serum which was sent out to India, kept there in an ice chest for one year and then returned, showed, when retested, no deterioration.

Antiscorpion Serum.

This serum preserves its property of neutralizing scorpion venom with little change for two years.

Antimeningococcic Serum: Antistreptococcic Serum.

We cannot say how long these serums retain their value, as there is no method accepted as reliable for standardization purposes. A paper on the standardization of anti-meningococcic serum has appeared in the *Journal of Immunology*, August, 1916, but this journal has not been available as yet.

General Remarks.

It is specially to be noted with regard to diphtheria antitoxin and tetanus antitoxin that the loss which occurs on keeping is in the number of units only. The *quality* of the antitoxin remains the same, but the *quantity* is less. This decrease can easily be remedied by giving larger doses.

It would add greatly to our knowledge if, when either antiplague serum or antidysentery serum is used, a note were made of the distinguishing mark on the bottle. If this were always done and mentioned in reports we should in time possibly be able to establish a relationship between the quality of the serum and the therapeutic effect.

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ON THE CHEMICAL FACTORS INVOLVED IN THE GROWTH OF THE MENINGOCOCCUS.

BY

DOROTHY JORDAN LLOYD, D.Sc.

(Report to the Medical Research Committee, from the Biochemical Laboratory, Cambridge.)

THE WRITER, in a recent number of the *BRITISH MEDICAL JOURNAL*,¹ stated that "the meningococcus needs for its metabolism (a) some digested protein, and (b) a comparatively high concentration of the vitamins or accessory growth hormones." The evidence in support of this generalization is given in full in the current number of the *Journal of Pathology and Bacteriology*, vol. xxi, p. 113, and is summarized below.

It is found that in order to obtain primary growth of the meningococcus, either from the throat or from spinal fluid, the medium used must contain some such substance as blood, serum, milk, beaten-up eggs, or certain vegetable products, as, for example, extract of peaflour (Gordon and Hine), or starch (Vedder). The influence of these additional substances in stimulating the growth of the more difficult pathogenic organisms on the ordinary nutrient media has usually been attributed to the influence of the additional protein, but that this hypothesis is

untenable is shown by an examination of the properties of the stimulating factor present in blood. This substance is very freely soluble in 80 per cent. alcohol, it is moderately stable to heat, withstanding a temperature of 100° C. for over an hour; it possesses, moreover, the property of being absorbed with very great rapidity by filter paper. These properties suggest that the stimulating factor belongs to the same class of substances which Hopkins has shown to be a *sine qua non* in mammalian diet—namely, the accessory growth hormones, or, as they are more generally called, the vitamins. Gordon and Hine,² in their latest paper on the culture of the meningococcus, have also come to the belief that the stimulating influence of this peaflour extract is due to vitamin action, and the properties of the stimulating factor described by Shearer³ in the nasal mucus suggest that it also falls into this class.

The meningococcus, then, needs vitamins for its primary growth *in vitro*, but during the early stages of subculture this need becomes greatly diminished. After a certain number of subcultures, varying from one to ten according to the strain, the meningococcus will grow vigorously on a vitamin-free medium, provided that there is present an abundant supply of free amino-acids. The only medium, as far as the writer's knowledge goes, that fulfils these two conditions, that is, vitamin-free and amino-acid rich, is the tryptic digest of casein prepared by the method of Cole and Onslow.⁴ This medium gives very heavy growth of laboratory strains, even when sown from dilute emulsions of the coccus in Ringer's solution. Douglas's tryptic heart agar⁵ also gives good growth with laboratory strains, though not so readily as Cole and Onslow's medium. It probably always contains some vitamin. The ordinary heart-broth peptone agar does not give subculture even of laboratory strains of the meningococcus unless a solid mass of the organisms be smeared thickly over the surface of the medium. Under these conditions growth is obtained, but undoubtedly the cause of growth is that a large number of the organisms die and release a vitamin supply which stimulates the growth of the survivors. The relation between the supply of free amino-acids and vitamins in media used for subculture may be summed up as follows: In the presence of an abundant supply of free amino-acids the meningococcus will grow without the addition of vitamins; in a medium containing a poor supply of free amino-acids, vitamin must be added in order to obtain growth. This relationship suggests that the amino-acids are the essential food substances, and that the function of vitamins in accelerating growth is exerted through their effect upon protein equilibrium in the cell.

Another essential condition for the laboratory culture of the meningococcus is that the organism must be kept permanently in a moist atmosphere. Given moisture, cultures of the meningococcus on agar slopes will readily survive for three weeks or more.

The medium described in the previous paper of the writer was made by adding defibrinated blood to tryptic heart agar, steaming to coagulate the blood proteins, and finally clearing by means of the centrifuge. The transparent blood medium can also be made by taking Cole and Onslow's tryptic casein agar as a basal medium, and the clearing can be accomplished by straining through a layer of glass wool (not filter paper).

The transparent medium gives a good growth in both primary and secondary culture with many of the more delicate pathogenic organisms. Pure cultures of the following organisms have been readily obtained on it from infected swabs: The meningococcus, *Micrococcus catarrhalis*, the pneumococcus, *Streptococcus pyogenes*, *B. influenzae*, *B. acrois*, *B. diphtheriae*. It seems justifiable to extend the theory of the vitamin influence to all such "parasitic" micro-organisms. It is easy to imagine that the development of the parasitic habit may be accompanied by an increasing tendency to depend on the vitamin supply from the tissues of the host. The need of a generous vitamin supply in the primary culture of a micro-organism may be taken as an indication of the parasitic habit. The disappearance of this need in subculture may, on the other hand, indicate a reversion to a non-parasitic type of metabolism.

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