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 antimeningococcic 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

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(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. aerolis*, *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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