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An Unidentified Growth Factor for Certain Strains of the Diphtheria Bacillus.

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It has been possible to grow a number of strains of the Park 8 variety of diphtheria bacillus, as well as one or 2 other unrelated strains, on media of entirely known composition.[1] Growth obtained on such media is extremely luxuriant, yet a number of the factors essential for the growth of organisms such as the staphylococci, the streptococci and the pneumococci are absent. It appeared, therefore, that such a medium might be unusually suitable for diagnostic purposes in preparing media for throat cultures to replace the ordinarily used Loeffler medium. A medium based upon Formula A in the paper referred to above has therefore been prepared, solidified by the addition of 2% agar, and used for the cultivation of organisms obtained by throat swab from a considerable number of individuals, both normal and suffering from clinical diphtheria.

The results in general, although encouraging, have been too unsatisfactory to suggest any immediate practical value for the method. The majority of normal throats yield no growth whatever after 24 hours' incubation. A certain number of cases of diphtheria, or cultures taken from the throats of individuals known to harbor the diphtheria bacilli, have given strongly positive growth of practically a pure culture of diphtheria. On the other hand, there are a certain number of normal throats which contain organisms capable of growth on this medium, a matter naturally to be expected, but there are also a number of cultures known to have been taken from positive throats which yielded no growth.

In following up this matter, it appeared that the difficulty lay in the fact that even with strains which grow well on the simple medium, a light inoculum generally fails to grow. This has been readily shown by streaking out cultures of suitable strains on agar plates prepared with such media as mentioned above. Growth is always heavy along the first streak of the inoculum, but later on—where one would expect single colonies to develop—no growth at all appears. In the course of 3 or 4 days' incubation, very frequently isolated colonies appear and grow to a good size. There is, how-

[1] Mueller, J. H., *J. Bact.*, 1938, **36**, 499.

ever, a definite and very long lag, and sometimes a complete failure to grow where the inoculum is light. A similar state of affairs appears to exist with the majority of freshly isolated strains which have been tested. They will grow with a comparatively heavy inoculum but not with a light one. Parallel streakings of the same material done in the same way on ordinary blood meat infusion agar give prompt growth, with single colonies developing over night. It is evident, therefore, that some essential factor for early growth, or for growth from small inocula, is absent from medium prepared according to the present formula.

This factor has been shown to be present in blood, where it would seem to be confined mostly to the serum. It will withstand a reasonable amount of autoclaving and of course may prove to be multiple. It has not been possible so far to show that it is identified either with Vitamin B 1 (thiamine), Vitamin B 2 (riboflavine), pantothenic acid, or mixtures of these substances. The nature of this factor is being investigated further, and the purpose of the present note is merely to indicate that the full story of the nutritional requirements of the diphtheria group of organisms is not yet completely cleared up.

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Absorption and Titration of Androgenic Hormone in Alcoholic and Oily Solutions Administered Percutaneously.

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The percutaneous resorption of oestrone in oily solutions has been described.[1] Recently one of us[1] showed that the absorption through the skin of oestrogenic hormone in organic solvents is quantitatively fully equivalent to the subcutaneous absorption of oily solutions. As organic solvents, 96% alcohol, benzol, ether, benzene, acetone and many others may be used.

Ito, Hajazu and Kon[2] reporting similar experiments emphasized particularly the ready absorption of estrogenic hormone in 60% alcohol.

Our experiments with androgenic hormone were directed towards

1 Zondek, B., *Klin. Wschr.*, 1929, 2229; *Lancet*, 1938, 1107.

2 Masao Ito, Seizi Hajazu and Turuziro Kon, *Zbl. Gyn.*, 1937, **61**, 1094.