ON THE RELATION BETWEEN TOXIN PRODUCTION AND PROTEIN SYNTHESIS BY CORYNEBACTERIUM DIPHTHERIAE AND THE IRON CONTENT OF THE CULTURE MEDIUM

by

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In a recent paper BRANDWIJK, TASMAN and VAN RAMSHORST (1) have pointed to the fact that for the purification of diphtheria toxoid it is essential that the ratio of non specific protein/specific antigen in the raw material is as low as possible. This involves in practice the production of diphtheria toxin which contains next to the toxin s.s. a minimum of non specific proteins. C. diphtheriae synthesizes during its development the latter proteins. The ratio of specific to non specific proteins depends on various factors among which may be cited: the strain used, the culture medium and the incubation time. In the following we shall make clear that also the iron content of the medium plays an important rôle.

In the paper cited it has been shown that during the development of the culture the production of specific protein (toxin) does not keep pace with that of non specific proteins, the formation of the former initially occurring at a greater rate. So it is profitable to harvest the culture at an earlier date than this is normally done. The optimal incubation time was determined at 5—6 days. In these experiments the „Pope medium” (12) was used as this was normally used in the National Institute for Public Health. The strain we used was a sub-culture of the well-known „P.W.—8” strain (6), obtained from the Wellcome Research Laboratories at Beckenham, labelled „C.N. 2000”.

The importance of iron in the production of diphtheria toxin has since long been recognised. POPE (11) is among the first who have
shown the connection between toxin production and iron content of the medium. He found a maximal yield of toxin at about 0.5 \( \gamma \) Fe per ml broth. His culture medium, however, proved to be rather iron-tolerant, a variation of the iron content between fairly wide limits (0—0.8 \( \gamma \) per ml) not perceptibly influencing the yield of toxin.

PAPPENHEIMER and JOHNSON (10) have investigated this question more thoroughly, using the medium of WADSWORTH and WHEELER (13), the main constituent of which is Difco-proteose-peptone. This medium appeared to be actually more "iron-sensitive", so that the latter investigators found a fairly sharp iron optimum at about 0.16 \( \gamma \) Fe per ml. Also in his later investigations, where a gelatine hydrolysate medium was applied, PAPPENHEIMER (7) found a same optimal concentration for this element. In 1937 HETTSCHE (2) published a paper bearing on this subject in which, however, no fresh aspects were presented. NORLIN in his thesis (5) reported that an important part of the iron added was taken up by the bacteria. In 1947 an investigation of PAPPENHEIMER (8) appeared in which the question was taken up anew and a quantitative relationship was established between the following three values: iron concentration of the medium, toxin and porphyrin production. He arrived at the following conclusion: for every 4 atoms of iron which occurred in the medium over and above the optimum for the toxin production, 4 molecules of porphyrin and 1 molecule of toxin are complex bound. This "bound toxin" can no longer be detected.

The most probable interpretation of these facts would be in diphtheria toxin being the "protein moiety" of an iron containing respiration enzyme. According to PAPPENHEIMER and HENDEE (9) this respiration enzyme would be identical with cytochrome-b.

Finally a paper of HOLT (3) may be cited, who also for his medium (casein hydrolysate) established a fairly sharp optimal iron concentration, which was determined for each fresh batch of broth.

All these investigations have been chiefly influenced by the wish to obtain maximal toxin yields (Lf/ml), whilst the "quality" of the toxin is not considered. As is well known this quality is expressed by the number of flocculation units per mg protein nitrogen (Lf mg P.N.).

In view of the results of BRANDWIJK, TASMAN and VAN RAMSHORST, which clearly show that the "quality" of the toxin depends a.o. on the incubation time of the culture, it seemed to us of interest