solution kept at a constant temperature of 38° C. When the muscle developed a satisfactory tone and rhythm, 0.2 c.c. or 0.5 c.c. of horse serum was added to the bath at a point which permitted uniform diffusion throughout the fluid before coming in contact with the tissue. The uteri of the sensitized and X-rayed animals reacted typically with maximal response just as did the uteri of the sensitized animals not X-rayed. The tracings of the uteri removed at both intervals show no essential differences.

The information furnished by the data presented we believe to have a direct bearing on the controversial point regarding the locus of antigen-antibody union which results in anaphylactic shock. The results of our experiments indicate that the anaphylactic reaction of isolated smooth muscle is not an index of the reaction of the animal as a whole. Also it is indicated that other factors than the reaction of sensitized smooth muscle should be taken into account in the statement of a theory concerning the mechanism of anaphylactic shock.

We have now in process an investigation in which we are determining the relation between the existence of free antigen and the presence of precipitins in the circulation of animals X-rayed and not X-rayed. The results of our experiments to date indicate that free antigen remains in the serum of X-rayed animals for a much longer period than is found in animals not X-rayed. A full report of these results, together with studies on passive anaphylaxis, will be published later.

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Contribution to study of diphtheria toxin.

By **P. J. MOLONEY** and **L. HANNA** (by invitation).

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The results reported are those of experiments planned to throw further light on the mechanism of toxin production by *B. diphtheriæ*.

An extended series of test-tube experiments was carried out in which the Park 8 strain of the diphtheria bacillus was grown in broth and daily counts made of the number of viable organisms

(as estimated by the poured plate method), and at the same time the toxicity of the broth, free from organisms, was estimated.

The growth curve had the characteristics of those reported at various times in the literature for other organisms; *i.e.*, a period of logarithmic growth, and then a continued rise which in general reached a maximum in 48 hours, a sudden falling off of the number of viable organisms and then a more gradual decrease, with sometimes small increases in the number of viable organisms during the period of decrease similar to the observations of Graham-Smith[1] for staphylococcus. It is suspected that these small secondary increases may only be apparent, and are due to the method of counting. The toxicity of the broth was very low at the end of 24 hours (approx. 1 M.L.D. per 0.5 c.c.), with a small increase at the end of 48 hours and then a rapid increase (if the increase and decrease of the cells was characteristic), and after a maximum was reached a falling off in toxicity which was sometimes very sudden. This sudden falling off in toxicity has also been reported by Bunker.[2]

The hydrogen-ion concentration of the broth during growth showed an initial increase and then a gradual falling off; this agrees with the findings of Bunker[2] and Davis.[3] Bunker in his paper gives the limits of maximum toxicity at P_H 7.8 — 8.25; contrary to this, good toxin has continued to be formed at P_H 8.7

There are several ways in which the above results may be interpreted:

1. Reproduction and toxin production do not go on at the same time; a cell produces toxin when it is incapable of division; apparently it is not accumulation of toxin which inhibits reproduction since toxin—200 M.L.D's per c.c.—will support growth when replanted with *B. diphtheriæ.*

2. A non-toxic substance may be produced during the period of cell division which is transformed into toxin; or this non-toxic substance acts on some constituent of the broth and produces toxin. Non-toxic germ-free broths (after organisms had grown for less than 24 hours) which were sterilized in various ways—

[1] Graham-Smith, *Journal of Hygiene*, 1920 (19).

[2] Bunker, *Journal of Bacteriology*, 1919, iv.

[3] Davis, *Journal of Bacteriology*, 1920, v.

heating at 56° C., berkefelding, and by adding various disinfectants, phenol, gentian violet, etc.—and then allowed to incubate at 37° C. for 5–6 days, failed to show any toxin production. Some experiments by Walbum[1] are interesting in this connection. Walbum deduced the existence of what he called pro-toxin; he injected a mixture of toxin plus peptone into a guinea pig in such amount that the pig died in five days; the same amount of peptone and toxin given separately to two other guinea pigs failed to kill. The experiments hardly seem conclusive. The peptone might conceivably injure the animal so that the toxin would kill more easily. He tried no experiments with other reagents than peptone, to check this point; and no experiments were undertaken to test the specificity of any toxin which may have been formed by this mixture.

3. There may be some sort of autolytic disintegration of the cells—so far there has been no evidence of this sort of mechanism. Organisms at different stages of life activity—after growing 1–2–4–6 days—were incubated at 37° C. for 6 days with saline and with distilled water. The clear sterile liquid in these several cases showed no toxin whatever. A possible objection to these experiments is that the digestion was not carried on in a colloidal substrate.

Appropos of this were the attempts made to prevent growth by planting a very large number of organisms, but these were unsuccessful, for no matter how large a number of organisms was planted there was always some growth. However, in every case so far tried the broth planted with a very large number of organisms showed less toxin than the same broth planted with a loopful. In some cases the difference was very marked. This difference in toxicity was apparently not due to absorption of the toxin by the organism; toxin allowed to stand in the ice chest with large numbers of organisms at different stages of life activity did not show any change in toxicity.

4. During the period of cell division a substance may be produced which acting on the non-viable cells produces toxin. The following results bear on this. After growing the organisms for less than 24 hours the whole was sterilized, in some cases by heating to

[1] Walbum, *Zeit. Immunitatsforschung*, 1909 (3), originale.

56° C. and in others by adding disinfectants, and in each case allowed to incubate at 37° C. for 6 days. Sterilized thus by heat, by phenol and by gentian violet there is no evidence of toxin production. Experiments to test more thoroughly these various hypotheses are being planned. Details will be published in full elsewhere.

15 (1762)

The change in reaction of dying tissue.

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In the studies of tissue enzyme action which the senior writer has been making since 1910, there has always been the question of the stages immediately following the death of the tissue and also of the conditions of reaction of medium, which have been shown to regulate the character of the process, that is, the rate and equilibrium. An attempt was made by Strauss and Morse[1] to determine the reaction of medium in the kidney during hematogenous infarction brought on by ligation of the blood vessels and at the same time to determine whether autolysis proceeded or not. The former collaborator (D. C. S.) being called for service rendered it impossible to complete this series of studies. Earlier still[2] the Sørensen colorimetric method was employed in similar work, but the obvious difficulty of the time element involved in the dialysis inhibited very critical conclusions. Recently, Dernby[3] applied the Sørensen solutions with the Clark-Lubs indicators to the study of the problem, but the critical point regarding the inception of autolysis and the state of reaction of medium in the earliest stages was not investigated. In his third paper in the "Studies of Autolysis" [4] Bradley and collaborator found "soon

[1] Strauss, D. C., and Morse, M. W., 1917, PROC. SOC. EXP. BIOL. AND MED. 1917, xiv, 171.

[2] Morse, M., *J. Biol. Chem.*, 1916, xxiv, 163.

[3] Dernby, K. G., *J. Biol. Chem.*, 1918, xxxv, 179.

[4] Bradley, H. C., and Taylor, J., *J. Biol. Chem.*, 1916, xxv, 261.