each individual bearing numerous eggs, embryos or young in the brood chamber. The samples were of like size,—weighing 0.13 grams after the water had been removed as far as possible with filter paper. The animals were then placed in like amounts of 60 per cent alcohol (2 cc.) and the reagents applied. The sexual female sample developed a pronounced violet color. The parthenogenetic females showed the same color, but with much less intensity. But the males showed none of the coloration.

These results taken in connection with numerous tests made by Miss Satina on plants and the blood of certain vertebrates, show that these invertebrates give a biochemical reaction for sex comparable with that given by plants and higher animals.

220 (2743)

Studies of the formation of the streptococcus toxin.

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(Introduced by William H. Park).

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In a paper read before the American Society of Bacteriologists in December, 1924, Huntoon reported observations on the progressive formation of skin-reacting toxin in the Berkefeld filtrate from a 44 hr. culture of hemolytic streptococcus (scarlet fever). He suggested that this formation of toxin in the absence of organisms was probably due to the action of an enzyme (protease), secreted by the bacteria, in the filtrate. The following experiments were conducted to determine something of the nature of this enzyme action. The organism used was a strain of hemolytic streptococcus isolated from a scarlet fever patient and obtained from the Board of Health.

Qualitative tests on filtrates obtained by passing a 36 hr. culture (bacto-veal-horse-plasma broth) through a Berkefeld filter, and on extracts of the bacterial bodies (obtained as described below), failed to show the presence of active protease, using

as substrate either fibrin or a solution of proteose (prepared from bacto-peptone).

Mass cultures were made by growing the streptococcus on whole blood bacto-veal agar. The growth was carefully removed and dried over concentrated sulphuric acid. Three tenths gm. of dried bacteria was well ground and suspended in a M/15 Na_2HPO_4, KH_2PO_4 mixture having a pH of 7.2. This was shaken at intervals during 12 hours, and filtered through Berkefeld. This filtrate was designated "bacterial extract."

Cutaneous tests were made by intradermal injections, using the Dick technique, in the arm of an adult male subject who was skin-sensitive to the Dick toxin. After many repeated injections in this subject, it seemed that some change occurred in the skin which made the readings of the tests difficult to interpret. The later experiments, therefore, were done on infant subjects who gave positive (4 plus) reactions to the Dick test, and negative controls. The skin reactions in the various series were checked by neutralization tests with known convalescent scarlet fever sera.

Simultaneous series were run on blood broth alone, bacterial extract alone, and broth and extract mixed; unincubated, and incubated at 37°C. for 1 to 8 days; and in varying dilutions. Also, some series were done on bacterial extract incubated with solutions of serum albumin and globulin.

The results may be summarized as follows: The extract alone gave a skin reaction only in low dilution; on incubation there was a slight but progressive increase, generally to about the eighth day. Blood broth alone gave no reaction. The extract and broth together showed a progressive increase in reaction which was definitely and consistently, though slightly, greater than in the extract alone. Incubation of the extract with serum proteins gave negative results. In none of the series was the increase obtained by us comparable in magnitude to that reported by Huntoon.

It would seem that, if the increase of skin-reacting toxin in the absence of the organism is due to enzyme action, this enzyme is rather an exo-enzyme, which is not stored to any great extent in the bacteria. The increase of toxin by incubation of the extract alone, on the enzyme hypothesis, may be considered as perhaps due to protease action on small amounts of bacterial protein in

the extract. The negative results obtained with serum protein suggest either that these proteins may not be the substrate from which toxin is formed, or that some "x factor," in addition, is needed.

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The effects of radiation on calcium and phosphorus.*

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As a part of a problem on the effects of darkness and of radiation on the metabolism of normal dogs, we followed the balances and blood levels of calcium and phosphorus before and after radiation with a 25 ampere flaming arc, with a spectral energy distribution of approximately 50 per cent ultra violet, 11 per cent visible, and 39 per cent infra red. The animals were fed on a standard maintenance diet furnishing 70 calories per kilo body weight, and well balanced and complete in calcium and phosphorus. All the animals were on positive balances at the beginning of the experiments.

Under normal laboratory conditions there is a balance between the two constituents, a slight rise in serum phosphorus being accompanied by a similar decrease in the serum calcium, and vice versa. Radiation of one hour at 40 cms. (total energy equivalent to 55.44 gm. cal. per cm.^{2}) for 8 days served to accentuate this balance, there being a marked increase in the phosphorus and a corresponding decrease in the calcium during the radiation, and a return to normal soon after the radiation was stopped. On repeated exposures of the same duration, however, both constituents show almost parallel curves, a rise in phosphorus being accompanied by a simultaneous increase in calcium. Single doses of two hours for 8 days on other dogs gave results similar to those obtained on repeated exposures. Grant and Gates[1] have reported similar findings in the rabbit.

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[1] Grant and Gates, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 315.