LETTERS TO THE EDITORS

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A New Antibiotic of Bacterial Origin

An antibiotic with therapeutic activity has been obtained from a spore-bearing bacillus of the *B. pumilis* group. The organism was isolated from soil collected in East Africa by Dr. Philip Spensley. The antibiotic is obtained in good yield from a medium containing inorganic salts, ammonium citrate, glucose and meat extract, both in shallow-layer and in aerated deep cultures.

The culture is acidified to *p*H 2·5, and ammonium sulphate added to 20 per cent concentration. The precipitate is collected by centrifugation, extracted with ethanol and the extract evaporated to dryness. The solid residue is washed with ether and with water, and the insoluble portion dissolved in ethanol and passed through a column of alumina. The active substance is obtained by evaporating the eluate to dryness and is crystallized from a mixture of acetic acid and ethyl acetate.

The antibiotic is a white crystalline solid sparingly soluble in water and turning yellow on exposure to light. It discolours at 232°, sinters at 238° and appears to melt at 252°. It has the composition C = 48·9, H = 4·7, N = 13·7, S = 16·0, O = 16·7 per cent, corresponding to a minimal formula of $C_8H_9N_3O_2S$. It does not give a ninhydrin test even after hydrolysis with hydrochloric acid, and so is unusual in being a non-peptide antibiotic from a bacterial source.

In nutrient broth it inhibits the growth of *Staphylococcus aureus* at a dilution of 1 in 20 million, of *Pasteurella muriseptica* at 1 in 50 million, of *Streptococcus haemolyticus* group *A* at 1 in 18 million and of an avirulent *Mycobacterium tuberculosis* at 1 in 200,000. It is not active against *Bact. coli*, *Shigella flexnerii* or *Candida albicans* at 1 in 25,000. It is just as active in whole blood as in broth against *S. haemolyticus*.

The substance is non-toxic. Mice survived the highest doses given, namely, 1 gm. per kgm. subcutaneously and 0·5 gm. per kgm. subcutaneously repeated daily. It is effective against hæmolytic streptococcal infection in mice. A single 2·5 mgm. intraperitoneal dose given up to five hours after the infection gave 100 per cent protection against an intraperitoneal infection of 1,000 lethal doses. Repeated 5 mgm. intraperitoneal doses of antibiotic saved all mice receiving a subcutaneous infection of 10 lethal doses. Daily 5 mgm. subcutaneous doses saved only 50 per cent of mice receiving 1,000 lethal doses of streptococci intraperitoneally. It was inactive by the oral route. The reduced effectiveness by the subcutaneous route is probably due to very slow absorption caused by its low solubility.

Larger quantities of antibiotic are being prepared and a further investigation of its chemical nature and therapeutic properties will be made. I wish to thank Mr. J. E. Lee for technical assistance.

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Feb. 24.

Quantitative Determination of Phosphatase Activity in Chick Embryo Duodenum cultured in Fluid Media with and without Hydrocortisone

Differentiation and phosphatase accumulation in the intestinal epithelium of the sixteen-day chick embryo cultured *in vitro* were accelerated by the addition of hydrocortisone to the nutritive medium[1]. When an attempt was made to culture relatively large pieces of duodenum, in order to determine phosphatase content quantitatively, it was found that the best survival and differentiation were obtained in those specimens that liquefied the plasma clot on which they were placed; such free-floating pieces maintained a normal surface configuration without any tendency to form the elaborate outgrowths that develop when a solid substrate is present. This observation has now led to the finding that as much as 25 mgm. of embryonic duodenum may be successfully cultured in 2 ml. of fluid, in a 25-ml. Erlenmeyer flask.

In the principal experiments described in this communication, the duodenal loops of sixteen-day chick embryos were excised under sterile conditions, placed in Earle's saline solution, and split lengthwise, each half being used for a single culture. Each half was cut into eight to fifteen fragments weighing 1–2 mgm. apiece, and these were placed in the medium, which was either Earle's saline or the synthetic medium No. 199 of Morgan *et al.*[2]; in some cases 1 μgm. of hydrocortisone had been added to the medium. The culture flasks were closed with tight-fitting rubber caps, and kept at 38° C. At two-day intervals, the fragments were removed from the flasks, washed in Earle's solution, and replaced in fresh medium. Cultures were maintained up to five days, by which time the donor embryos would have hatched.

Within a few hours after being cut, the fragments healed into rounded masses with the musculature inside and the villi covering most of the surface. In all test media the villi developed normally during the first forty-eight hours; but during the following day the villi in the saline alone shortened and disappeared, so that the fragments frequently came to be covered by a smooth layer of cuboidal epithelium. In the synthetic medium, similar changes occurred, though more slowly. In both media, however, when 1 μgm. of hydrocortisone was added, long villi persisted to the end of the experiments; but the epithelial cells covering these villi, though columnar, did not differentiate so far as they would have *in vivo*, seeming rather to be arrested at the nineteen-day condition.

At daily intervals some cultures were used for weight, nitrogen and phosphatase determination, or for fixation. Weighing was done on a torsion balance of 30 mgm. capacity; in this operation all fragments from one half-duodenal loop were picked up with fine forceps and placed on an aluminium weighing pan from which excess fluid was blotted up on tiny filter-paper sponges. Although it was not possible to show how the weight of any individual culture changed, comparison of the weights of terminal cultures with weights of equal numbers of uncultured sixteen-day fragments indicated that there is a steady decrease in weight, the greatest loss occurring in the first twenty-four hours. By twenty-one days the cultured fragments had apparently lost about half their initial weight. Both gross and histological