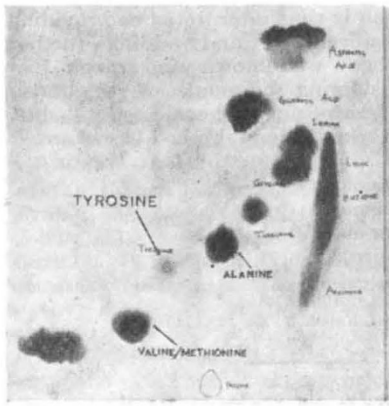

Fig. 1

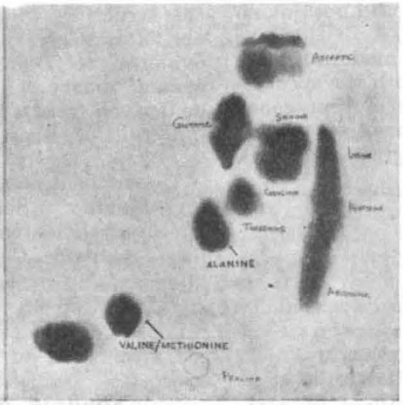

Fig. 2

gen, but were somewhat less pure in terms of Lfs./mgm. total nitrogen. The non-protein nitrogen was eliminated from both preparations by precipitation and washing with trichloroacetic acid (5 per cent) prior to hydrolysis of the protein with 6*N* hydrochloric acid for 72 hours.

Samples of the hydrolysates containing approximately 200 μgm. of nitrogen were used for the preparation of the two-dimensional chromatograms, which were run first with *n*-butanol-acetic acid and then with phenol.

Holden and Freeman[3], and also Brown[4], have shown that formaldehyde reacts with tyrosine under alkaline conditions to give a product stable to acid hydrolysis, devoid of amino-nitrogen and negative to the ninhydrin test. Therefore, a possible explanation of our observations is that the amino-group in the tyrosine present in diphtheria toxin is not involved in a peptide linkage, but is free to combine with formaldehyde during the process of 'toxoiding'. This is not, however, the only explanation for the apparent lack of tyrosine in the hydrolysed toxoid, and we are engaged in further work to find the actual mechanism involved. This will be published in detail later.

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Wellcome Research Laboratories,
Beckenham, Kent. Nov. 24.

[1] Woiwod, A. J., *Nature*, **161**, 169 (1948).
[2] Woiwod, A. J., *Biochem. J.*, **42**, xxviii (1948).
[3] Holden, H. F., and Freeman, M., *Aust. J. Exp. Biol. Med. Sci.*, **8**, 189 (1931).
[4] Brown, A. E., *J. Amer. Chem. Soc.*, **68**, 1011 (1946)

Microbiological Oxidation of Sterols

In an earlier communication[1] we stated that by cultivating *Proactinomyces roseus* in a synthetic culture medium in which the only source of carbon and nitrogen was 2 per mille pyridine, and using up the superfluous nitrogen by emulsifying 0·1 per cent cholesterol in the medium and aerating it at 34° C., in two weeks we obtained 7-oxycholesterol and 4,5-cholestonone. Later, we received a letter from Dr. G. A. D. Haslewood, of the Physiological Laboratory, Guy's Hospital, London, S.E., expressing doubt that the occurrence of 7-oxycholesterol was the result of the microbiological oxidation; in his opinion, the result was analogous to the production of 7-oxycholesterol from cholesterol in alkali medium, by aeration and elevated temperature, as shown by Bergström and Wintersteiner[2,3], in which case the micro-organism plays no part.

We have now carried out further experiments. In a synthetic culture medium at *p*H 7, *Proactinomyces* previously cultivated in a 2 per mille pyridine solution decomposes the pyridine into ammonia at 34° C. without special aeration, besides incorporating part of the nitrogen into itself. After complete destruction of the pyridine, the *p*H of the culture rises to 7·5. We then add 0·1 per cent cholesterol to the culture and again 2 per mille pyridine. At the same time we begin aeration at 34° C. Two weeks before extraction of the substances, we determined the *p*H of the cultures and found the average value to be 8·2–8·4.

Control experiments were carried out by regulating the same synthetic pyridine culture medium with ammonia to the minimum *p*H of 7·5 in one control, and in the other to the maximum *p*H value obtained at the end of the culture, that is, 8·2–8·4, and, on adding cholesterol, aerated it under the same conditions as in our chief experiment. In the control experiments we never obtained 7-oxycholesterol at these *p*H values. In a special series of experiments without micro-organisms in the synthetic pyridine culture medium described we found that, with aeration at 34° C., we obtained 7-oxycholesterol only at the outside values of 11–11·5 *p*H, and then only traces.

We must, therefore, repeat that, in the experiments originally reported[1], the 7-oxycholesterol was produced as an effect of the activity of the micro-organism.

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Hungarian Biological Research Institute,
Tihany. Nov. 22.

[1] Krámli, A., and Horváth, J., *Nature*, **162**, 619 (1948).
[2] Bergström, S., and Wintersteiner, O., *J. Biol. Chem.*, **141**, 597 (1941); **143**, 503 (1942); **145**, 309, 327 (1942).
[3] Haslewood, G. A. D., *Nature*, **154**, 29 (1944).

Complement Fixation Test in Contagious Ecthyma

During both research and routine complement fixation tests with foot-and-mouth disease virus, we attempted to develop a similar test for the condition known as contagious ecthyma of the lips, infectious or contagious pustular dermatitis or stomatitis and infectious labial dermatitis. The observations clearly indicate that this condition has an extensive geographical distribution.

The technique used was similar to that described by Aramburu[1] for complement fixation test in foot-and-mouth disease. The antigen was made from desiccated crusts from sheep infected experimentally with the virus. The scabs were thoroughly triturated with sand, suspended in 1 in 10 saline and centrifuged 10 min. at 2,500 r.p.m.; the supernatant fluid, containing the antigen, could be preserved for at least fifteen days at − 20° C. Sera were obtained both thirty-one and eight days after infection, centrifuged 15 min. at 3,000 r.p.m. and inactivated 45 min. at ~ 56° C. In our experiments the control tubes included the three types of foot-and-mouth