

sterol molecule, the secondary alcohol group is intimately concerned in the activation by ultra-violet rays.<sup>3</sup>

The present communication concerns itself with the effect of irradiation on a large number of cholesterol derivatives prepared by one of us (Windaus). The following derivatives were tested: B-cholesterol, hetero-cholesterol,  $\alpha$ -phytosterol; cholesteryl-chloride, cholesteryl-amin-acetate; cholesten, pseudo-cholesten, cholesterilen, cholestan-4-7 diol, cholestan 4-on 7-ol, cholestenon; stigmasterol,  $\alpha$ -amyrol, cholesteryl-ether, cholesterol-dibromide, cholesterol-ozonide (non-irradiated and irradiated), apocholic acid.

These preparations were made with the idea of noting what rearrangement or substitution in the molecule could be made without interfering with the specific action of irradiation. Feeding tests on rats showed that none of these derivatives had acquired antirachitic potency. This result is of interest in view of the slight degree of deviation from cholesterol which has been made in some of the derivatives. Attention may be called in this connection particularly to B-cholesterol, in which there is a very slight rearrangement in the secondary alcohol, and the hetero-cholesterol, which is characterized by a shifting of the double bond. It is also of interest to note, that although it had been found previously by one of us, that cholesterol acetate, an unsaturated ester of cholesterol, developed antirachitic potency as the result of irradiation, the amin-acetate failed to become activated.

A detailed discussion of the significance of these tests of various cholesterol derivatives and allied sterols will be reported elsewhere.

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<sup>1</sup> Hess, A. F., and Weinstock, M., *J. Biol. Chem.*, 1925, lxiv, 181.

<sup>2</sup> Hess, A. F., Weinstock, M., and Sherman, E., *J. Biol. Chem.*, 1926, lxxvii, 413.

<sup>3</sup> Rosenheim, O., and Webster, T. A., *Biochem. J.*, 1926, xx, 537.

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### The Anti-Bacterial Activity of Egg-White.

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The utilization of egg-white as pabulum by bacteria is a fact of common experience. Egg-white also possesses marked anti-bacter-

ial properties for certain bacteria. This phenomena was investigated in the work reported here.

Egg-white was removed aseptically from fresh store eggs with a sterile syringe and serially diluted with meat-infusion broth pH 7.5 to 7.6. One cc. quantities of these dilutions were distributed into separate sterile small (Wassermann) test tubes and each tube, including a control tube of broth, was inoculated and well mixed with 0.1 cc. of a 24-hour broth culture of the organisms used. Similar series were made with inocula of 0.1 cc. of a 1-100 and 0.1 cc. of a 1-10,000 dilution in broth of the broth cultures. All tubes were incubated for 20 to 24 hours and then observed for inhibition of growth. In addition, loopfuls of the mixture from each tube were separately streaked out on agar plates (blood agar for streptococcus) which were incubated for 24 hours and then examined for growth. Absence of growth in the streaks on the agar plates was assumed to indicate sterilization of the inocula in the corresponding tubes.

With this technique the following results were obtained:

*B. typhosus*, *B. coli*, *V. cholerae* and *B. proteus* were unaffected by even undiluted egg-white. Slight inhibition of growth was observed in tubes containing undiluted to 1-8 egg-white, but this may be due to the relative absence of the nutriment of the broth.

*B. subtilis*, *Staphylococcus albus* and *Streptococcus hemolyticus* ("H" strain, Gay) were definitely inhibited from developing in dilutions of egg-white up to 1-8 and were killed by whole and 1-2 egg-white. These results were more clear cut with the smaller inocula.

Sarcina "A" (a strain isolated from the laboratory air) was inhibited from growing in dilutions of egg-white up to 1-12800 and was killed and dissolved by dilutions up to 1-6400.

These results practically wholly confirm previous work on this subject by Wurtz,<sup>1</sup> Laschtschenko,<sup>2</sup> Sperry and Rettger,<sup>3</sup> Gay and Morrison,<sup>4</sup> and Fleming and Allison.<sup>5</sup>

Native egg-white is quite alkaline, usually about pH 9.2 to 9.4. When diluted in broth at pH 7.5 to 7.6, as in the described experiments, the pH of the dilutions varied with the relative amounts of egg-white and broth, decreasing to the pH of the broth as a limit with the increase in dilution of the egg-white. The pH of the mixtures ceased to vary in the dilutions 1-32 or 1-64 and greater.

This factor cannot be controlled by simply adjusting egg-white to the desired pH and diluting with broth at the same pH. Such adjusted dilutions, when containing relatively large amounts of egg-white, tend to return to the original pH of the egg-white. This is

probably due entirely to the slow liberation of  $\text{CO}_2$  from the egg-white to which acid has been added.

To control the pH factor completely when using dilutions containing large amounts of egg-white it was necessary to carry out the tests in very small test tubes tightly stoppered with sterile corks. The small free volume, above the test mixtures in such stoppered test-tubes, soon comes into equilibrium with the egg-white-broth dilution with respect to the carbonate-bicarbonate buffer system and the pH of the mixture remains constant.

*B. typhosus*, *B. coli*, *V. cholerae* and *B. proteus* have been only cursorily examined with this technique. The results indicate, however, that egg-white at different pH's has no effect on these organisms.

Detailed experiments on the anti-bacterial power of egg-white at different pH's on *Streptococcus hemolyticus* show that at pH 9.2 a dilution of 1-4 or 1-8 egg-white in broth is bactericidal and probably bacteriolytic for this organism. At pH 7.6 or pH 6.0, however, even undiluted egg-white serves as an excellent culture medium for *Streptococcus hemolyticus* "H".

In view of the extremely marked bactericidal and bacteriolytic properties of egg-white against sarcina "A" it was considered worth while to investigate more closely this phase of the problem. If an opaque suspension of this organism is mixed with a solution of egg-white whose final concentration is 1-100 and the mixture kept at 37° C. the following changes are observed: the mixture gradually becomes transparent, the color becomes yellow, the viscosity increases, the pH decreases, in 10 to 15 minutes the organisms are apparently entirely killed and dissolved, and, at the end of this time a yellow amorphous substance can be precipitated out of the solution by adding acid to a certain pH. Microscopic examination of such a test shows that the bacteria disintegrate and dissolve in a manner similar to that of the Pfeiffer phenomenon.

Further tests with sarcina "A" were carried out with a technique similar to that already described except that peptone water was used instead of meat-infusion broth, and a standard suspension of cells from a washed agar slant, in place of a broth culture. In view of the high dilutions of egg-white giving significant results it was not necessary to use the small stoppered test tubes.

Results were read in terms of the dilution of egg-white causing complete lysis of the inoculated cells after 24 hours incubation. Complete lysis was always accompanied by sterilization of the inoculum.

The thermolability of the anti-bacterial principle for sarcina "A" was investigated by heating at 65° C., 1-20 dilutions of egg-white in distilled water at different pH's. After definite intervals of time samples from each tube were removed and tested for the presence of the anti-bacterial principle. At pH 8.4 it was destroyed in 15 minutes; at pH 6.8 in 30 minutes; at pH 5.0 it was not destroyed after 60 minutes.

The effect of the concentration of salt (NaCl) on the lysis of sarcina "A" was studied by substituting different salt solutions for peptone water as the egg-white diluent. It was found that the optimum salt concentration was 0.145 N, that is, isotonic, in which the greatest dilution causing complete lysis was 1-6400. In distilled water, and isotonic saccharose solutions as well, the lytic action was suppressed to one-eighth this value; and in 0.435 N NaCl, to one-sixty-fourth.

The pH of the peptone water-egg-white dilutions was found to have a marked influence on the lysis of the sarcina organism. The peptone water was easily adjusted to different pH's by the addition of small amounts of acid and alkali. The optimum was about pH 6.0, at which a 1-12800 to 1-25600 dilution of egg-white caused complete lysis. On the acid side of this optimum pH the activity was very sharply suppressed, whereas on the alkaline side, the drop in activity was more gradual. It will be recalled that the action of egg-white against *Streptococcus hemolyticus* took place at pH 9.2 and not at all at pH 7.6 or 6.0.

The work on this problem to date has been done with chemically unaltered egg-white. Experiments are now in progress designed to isolate and purify the anti-bacterial principle and then to examine more closely its nature and activity.

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<sup>1</sup> Wurtz, M. R., *La Semaine Medicale*, 1890, x, 21.

<sup>2</sup> Laschtschenko, P., *Z. Hyg.*, 1909, lxiv, 419.

<sup>3</sup> Rettger, L. F., and Sperry, J. A., *J. Med. Res.*, 1912, xxvi, 55.

<sup>4</sup> Gay, F. P., and Beckwith, T. D., *Am. J. Hyg.*, 1922, ii, 467.

<sup>5</sup> Fleming, A., *Proc. Royal Soc.*, 1922, Ser. B., xciii, 306.

<sup>6</sup> Fleming, A., and Allison, V. D., *Brit. J. Exp. Path.*, 1922, iii, 253.

<sup>7</sup> Fleming, A., and Allison, V. D., *Lancet*, 1924, i, 1303.