In a typical experiment 17 mm^3 of gas was produced in 7.5 hours in the presence of M/1600 SA as compared with 190 in the control and 172 in M/1600 SA + M/200,000 PAB. The corresponding growth figures are 5.2, 25.3 and 25.4 bacteria $\times 10^8$ per ml respectively.

Conclusions. The results of this study show that SA inhibits the respiration and growth of *Esch. coli* in a synthetic medium with glucose as the source of energy under either aerobic or anaerobic conditions and that PAB also exerts its anti-SA activity under anaerobic conditions. Growth and respiration are inhibited to somewhat the same extent as previously reported by Sevag[8] for *Streptococcus pyogenes* and Type I *Diplococcus pneumoniæ*. SA appears to inhibit respiration somewhere between the original dehydrogenase system and the final H-acceptor, possibly inhibiting a H-carrier system as well as the decarboxylase system. It is not evident whether the decreased rate of respiration is due to inhibition of growth or vice versa although Wyss[9] reports that respiration is inhibited by isomers of SA which do not exhibit "chemotherapeutic activity."

[8] Sevag, M. G., and Shelburne, M., *J. Bact.*, 1942, **43**, 447.

[9] Wyss, O., Strandskov, F. B., and Schmelkes, F. C., *Science*, 1942, **96**, 236.

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Alteration of Crystalline Androsterone and Dehydroisoandrosterone by Alcohol as a Vehicle.

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Investigations were undertaken to evaluate the respective merits of alcohol and oil as vehicles for androgenic assays after having observed[1] that the degree of response of the chick's comb to androsterone, dehydroisoandrosterone,* and varying mixtures of these compounds was modified by the vehicle in which they were dissolved. Klempner, Frank and Hollander[2] noted a greater increase in the weight of chicks' combs treated with crystalline androsterone dissolved in 95% alcohol over that resulting from the use of an oily solution. They consequently recommended alcohol as the vehicle of choice. The enhancement of tissue response was ascribed by them to either better absorption of the hormone from the alcoholic solution or to an increased rate of evaporation of the vehicle. Our results indicate that changes occur in such alcoholic solutions, making this vehicle unsatisfactory for biological assays of these androgens.

Experimental. In these investigations we employed the chick-comb-weight method, as previously described.[3] Although several workers[4,5,6] have questioned the value of the chick as a test animal, our extensive experience during the past 4 years, with routine and experimental androgenic assays, has shown

[1] Grauer, R. C., Starkey, W. F., and Saier, E., studies in progress.

* The androsterone and dehydroisoandrosterone used in these studies were kindly supplied by the Ciba Pharmaceutical Products through the courtesy of Dr. Ernst Oppenheimer.

[2] Klempner, E., Frank, R. T., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 633.

[3] Starkey, W. F., Grauer, R. C., and Saier, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 649.

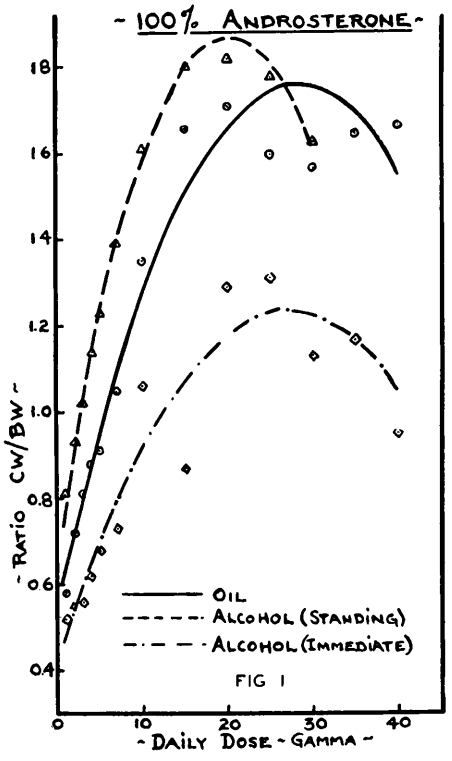
[4] Duff, P. A., and Darby, H. H., *Endocrinology*, 1941, **28**, 643.

[5] McCullagh, D. R., and Guillet, R., *Endocrinology*, 1941, **28**, 648.

[6] Hoskins, W. H., Beach, G. W., Coffman, J. R., and Koch, F. C., *Endocrinology*, 1941, **28**, 651.

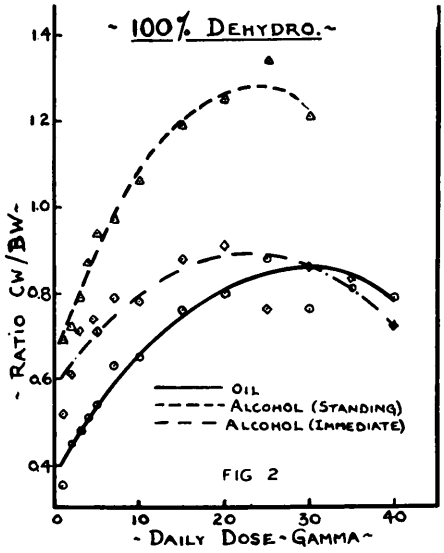
that with careful technic, standard conditions as to light and temperature, and sufficiently large groups of animals, the chick's comb will give a consistent response well within the accepted limits of biological error.

A total of 2,151 unsexed chicks was used in this study, 105 being controls treated with



the vehicle free from hormone. A constant amount of 0.01 cc of the solution containing the androgen (1 to 40 γ doses) was applied to the combs of 2-day-old chicks for 6 consecutive days, in accordance with our previous report.[3] Groups of 12 to 32 animals were used at a given dose and the response was expressed as a comb-weight (mg)/body-weight (g) ratio. From these data, suitable curves were calculated by the method of least squares. This study was primarily concerned with the use of alcohol as a vehicle; the results obtained with the compounds dissolved in oil were reviewed from a part of another study, for the purpose of comparison. These investigations were designed to determine if alcohol produced a change in the dissolved androgens, thus enhancing their activity. Two methods were employed using alcohol as the vehicle; a "standing" and an "immediate" method. The "standing method" of treatment consisted of making up the alcoholic solutions on the first day and using them throughout the 6 days of the experiment. The solutions were kept tightly stoppered and under refrigeration when not in use. The "immediate method" consisted of dissolving the crystalline hormone in 95% alcohol and applying the solution to the chicks' combs immediately. Five minutes was the maximum elapsed time from the beginning of the procedure to completion of the application of each "run." Fresh solutions were made daily.

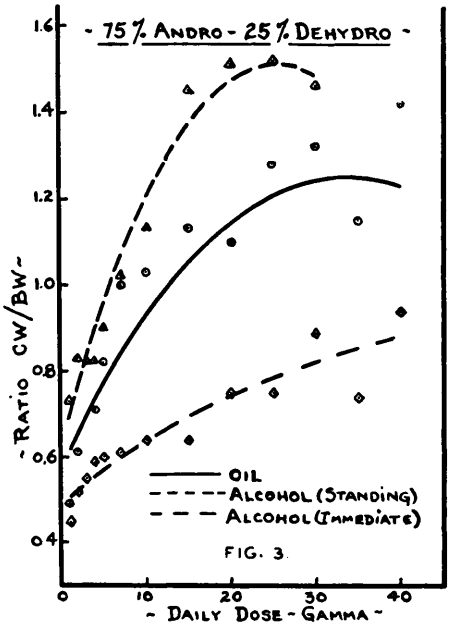
In order to determine whether prolonged contact of the androgens with alcohol resulted in some alteration of the compounds, crystalline androsterone and dehydroisoandrosterone were dissolved in 95% alcohol (USP) and the solutions allowed to stand for 6 days. The



alcohol was then evaporated at room temperature, the crystals recovered, and dissolved in sesame oil. These oily solutions were tested for androgenic activity by the chick-comb, weight method and the curves plotted in the manner previously described.

Results. With all solutions the curves were parabolic in type. The "standing method" gave a greater response than did either the "immediate method" or the oily solutions. The

25% androsterone + 75% dehydroisoandrosterone mixture (Fig. 4) showed the greatest difference between the two methods. This mixture also gave the least response when the "immediate method" was employed. The oily solutions gave greater responses than

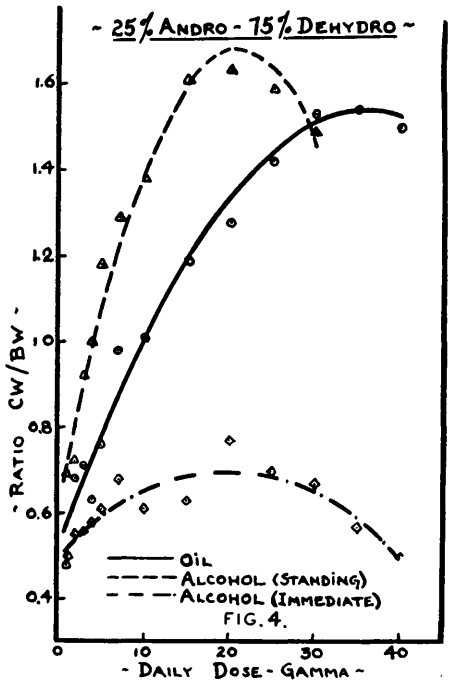


did the "immediate method" (Fig. 1, 3, 4) except in the case of the 100% dehydroisoandrosterone dissolved in alcohol which, by the "immediate method," was slightly superior to the oily solution (Fig. 2). The greatest difference in response between alcoholic (standing method) and oily solutions was noted with 100% dehydroisoandrosterone (Fig. 2).

The androsterone recovered after alcohol treatment for 6 days gave a much lower response than did the original material (Fig. 5). The original compound reached an optimum response at the 25-30 γ daily dose, whereas with the alcohol treated androsterone the optimum was not reached even at the 40 γ dose. The alcohol treated dehydroisoandrosterone (Fig. 6) was seen to be a more potent androgen than the original compound dissolved in the inert sesame oil. The type of response is similar to that of alcohol treated androsterone (Fig. 5), the slope of the curve continuing upward even at the 40 γ dose.

Discussion. The increasing use of alcohol as a vehicle for androgenic material warrants a note of caution. We observed[1] that varying mixtures of the biologically active androgens, androsterone and dehydroisoandrosterone, due to synergistic action, yield varying results insofar as the response of the chick's comb is concerned. From our present report, it becomes apparent that the vehicle in which these androgens are dissolved may be an additional factor in determining the nature of the response. It is therefore important for us to minimize the number of these variable factors and consequently suggest that an inert vehicle such as oil is to be preferred.

Previous workers[2,7,8,9,10] have noted that androgens dissolved in alcohol gave a greater biological response than when in oily solution, but none of them has stated the length of time



the androgens were in contact with the alcohol. The possibility that a change occurred in the compounds as a result of their prolonged

[7] Koch, F. C., *The Harvey Lectures,* 1937-38.

[8] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 633.

[9] Greenwood, A. W., Blyth, J. S. S., and Callow, R. K., *Biochem. J.*, 1935, **29**, 1400.

[10] Callow, R. K., and Deanesley, R., *Biochem. J.*, 1935, **29**, 1425.

contact with alcohol should be considered. While we have no absolute proof of chemical or physical alteration of the original androsterone and dehydroisoandrosterone due to

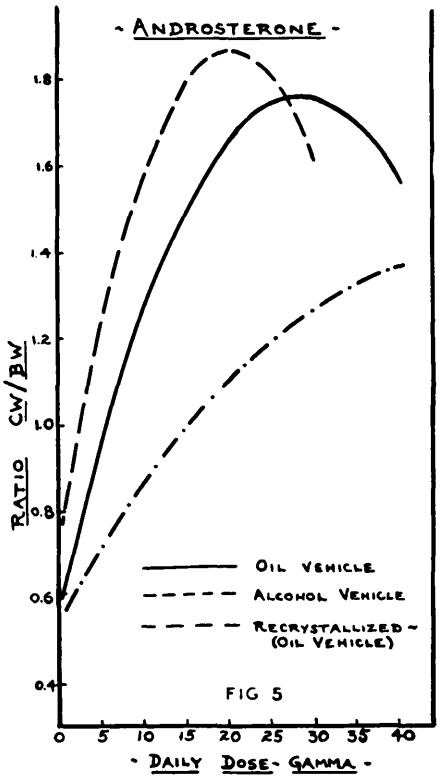


FIG 5

prolonged contact with alcohol, the quantitative biological response and the type of response curve obtained are suggestive of a difference in these compounds before and after alcohol treatment. Lack of an enhanced response by the "immediate method" of treatment indicated that the production of a more biologically active substance does not occur rapidly. If the original and alcohol treated products were the same, the response curves for both preparations should be identical when an inert vehicle such as sesame oil is employed. This, however, is not the case.

Summary. Prolonged contact of crystalline androsterone and dehydroisoandrosterone with alcohol results in an enhanced biological response as compared with the response obtained from oily solutions of these androgens, when tested by the chick-comb-weight method. When these androgens are applied to the combs immediately after going into alcoholic solution, such an enhancement does not occur.

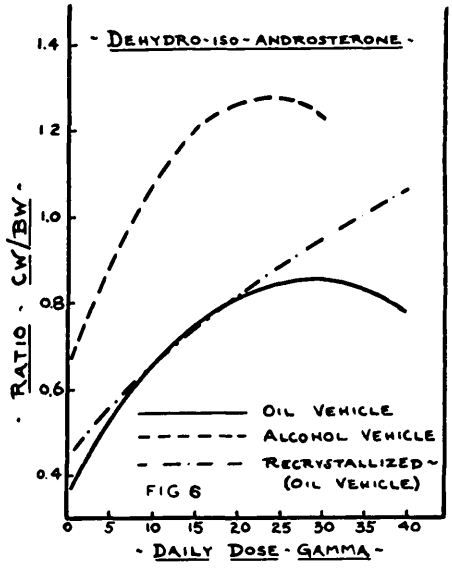


FIG 6

Recovery of the crystals from alcoholic solution, after 6 days contact, and subsequent solution in oil gives an altered biological response indicating a possible change in the character of the original materials. These observations suggest that an inert vehicle, such as sesame oil, is to be preferred to alcohol as a vehicle in assaying such androgens.