ment with the larger volume. Each of these response values has likewise been calculated as per cent of the corresponding control value and is given in column 8. The improvement in response resulting from reduction in volume of sesame oil (IR, column 9) is therefore measured by the difference of these paired percentage values. These "improvement" values are uniformly positive, corresponding to the greater response with 0.02 cc of oil than with 0.05 cc. They vary in magnitude from 43% to 108% of the corresponding control comb weight. The mean improvement is 67.9%, with a standard deviation of 5.4, and is based on a total of 710 chicks: 277 treated with the larger volume of oil, 278 with the smaller volume, and 155 untreated controls. A second group of experiments, identical with the foregoing but restricted to the dosage range 1-9 γ inclusive, has also been performed. This series employed a total of 308 chicks, of which 238 were treated and 70 were controls. The results were similar to those in the foregoing series.

It may be concluded, therefore, that this further reduction in volume of vehicle effects a further increase in the comb growth response of baby chicks. The only explanation which we can offer at the present time for this improvement is that the smaller the volume, the greater is the proportion of androgen solution utilized on the comb surface instead of being diverted to adjacent, less-sensitive head areas.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

11553

Improvement in Chick Comb Response to Androsterone Obtained with Alcohol as Vehicle.*

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In our studies of the bioassay method for androgens, utilizing the comb of the baby chick, it has appeared that further improve-

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ment in the method might be attained by utilizing some vehicle for the androgens other than sesame oil. We have been aware of the fact that an oily vehicle entails certain disadvantages: (1) It has a tendency to spread over the head feathers of the chick and thus cause a loss of variable amounts of the hormone by diversion from the comb. (2) As a solvent, oil is unsatisfactory particularly for the gummy residues frequently obtained in extracting androgens from urine. (3) The oil itself is not absorbed, as has been shown histologically by Soloway, Hansen and McCahey,[1] but the hormone is absorbed selectively by diffusion out of the vehicle instead of along with it. (4) The high viscosity of an oily vehicle is a distinct source of error in the preparation of quantitative solutions of the androgens. Other liquids which have been employed in place of sesame oil are tricaproin (Hall and Dryden[2]), 60% alcohol for estrogens (Ito, Hajazu and Kon[3]), and 96% alcohol for testosterone (Zondek and Sulman[4]). The latter also mention the possibility of using benzol, ether, benzene and acetone but report no comparative studies with these solvents. Since 95% alcohol tends to overcome the undesirable features of sesame oil enumerated above, we have compared the two vehicles with respect to the comb growth response elicited by identical doses of androsterone. The procedure was similar to that described in the preceding paper (Klempner, Hollander and Frank[5]), wherein we compared the response elicited by the same dose of androsterone in paired experiments in which the only variable was the volume of sesame oil employed. In the present investigation, however, the paired experiments differed from each other in that the first employed 0.05 cc of sesame oil and the second 0.02 cc of 95% alcohol. A comparison of the relative efficiencies of the two liquids for the purpose of these investigations would have been simplified by the use of identical volumes, but—as will be shown below—this difference in volume can be canceled out of the final result without undue complications.

The data for 9 such pairs of experiments are presented in Table I. The response in any one experiment (column 7) is measured by the difference between the mean comb weight of the group of treated

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TABLE I.
Comparison of Alcohol and Sesame Oil as Vehicle in Bioassay of Androgens by the Chick Comb Method.

Series No. (1)	Androgen dosage (γ) (2)	Vehicle (3)	No. of chicks (4)	Mean comb wt: Treated chicks W (mg) (5)	Mean comb wt: Control chicks W_c (mg) (6)	Response to androgen application (comb wt corrected for controls): (W-W_c) (7)	Response to androgen application (comb wt corrected for controls): % of W_c[1] (8)	Improvement in response (IR)[2] (9)
S39	10	Oil	18	48.1	23.6	24.5	104	+ 93
		Alcohol	17	70.2	(21)*	46.6	197	
S40	10	Oil	18	38.4	19.2	19.2	100	+155
		Alcohol	18	68.3	(37)	49.1	255	
S39	20	Oil	17	61.1	23.6	37.5	158	+152
		Alcohol	17	96.9	(21)	73.3	310	
S38	30	Oil	18	48.1	21.7	26.4	122	+182
		Alcohol	18	87.8	(25)	66.1	304	
S39	30	Oil	16	68.5	23.6	44.9	190	+134
		Alcohol	18	100.4	(21)	76.8	324	
S40	30	Oil	18	61.3	19.2	42.1	219	+172
		Alcohol	18	94.6	(37)	75.4	391	
S39	40	Oil	17	71.2	23.6	47.6	201	+167
		Alcohol	18	110.8	(21)	87.2	368	
S39	50	Oil	18	77.9	23.6	54.3	229	+206
		Alcohol	18	126.8	(21)	103.2	435	
S40	50	Oil	18	84.6	19.2	65.4	339	+140
		Alcohol	18	111.3	(37)	92.1	479	
								Mean = 155.7%
								$\sigma_M = \pm 10.1\%$

*Figures in parentheses are No. of chicks.

[1] Response as % of $W_c = \frac{W - W_c}{W_c} \times 100$

[2] Improvement (IR) as % of $W_c = \frac{W_{alc} - W_{oil}}{W_c} \times 100$

chicks (W, column 5) and that of the untreated control group in the same experiment (W_c, column 6). For comparative purposes, these "response" values are also given as per cent of the corresponding control weight (column 8). Then, the improvement in response obtained by the use of alcohol as compared with oil in any one pair of experiments is given by the difference between these percentage response values (IR, column 9). These "improvement" values are consistently in favor of the alcohol as vehicle, and vary from 93 to 206% of the corresponding control comb weight. The mean of these 9 IR values is 155.7% with a standard deviation of 10.1. This mean is based on a total of 538 chicks: 158 treated with oil, 160 treated with alcohol and 220 untreated controls.

It was mentioned above that the alcohol solutions were employed in daily volumes of 0.02 cc as compared with 0.05 cc for the sesame

oil. We have shown elsewhere (Klempner, Hollander and Frank[5]) that even with the same solvent such a difference in volume may produce a considerable increase in comb response; for 11 experiments with a range in dosage of 10-50 γ, the mean IR-value was 67.9 $\pm$ 5.4%. However, in the present investigation the mean IR value is 155.7 $\pm$ 10.1%. The difference between these two is 87.8% with a standard deviation of 11.5%. This difference is 7.6 times its standard deviation and is therefore statistically significant.

It may be concluded, therefore, that the improvement in comb growth response obtained in these experiments resulted from the substitution of alcohol for sesame oil, apart from the diminution in volume of vehicle. Such improvement may be ascribed to the rapid evaporation and absorption of the alcohol, with consequent diminution in loss by spreading to less responsive areas. It is also possible that the use of alcohol increases the rate of absorption of the androgen itself by the comb surface, but as yet we have no direct evidence of this.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

11554

Prevention of Nutritional Muscular Dystrophy in Suckling E-low Rats with Alpha-tocopherol and Related Substances.*

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That alpha-tocopherol was effective in preventing the dystrophy that appears in the suckling young of vitamin E-low mothers was first shown by Barrie,[1] Demole and Pfaltz,[2] and Goettsch and Ritzmann.[3] Goettsch and Ritzmann found that $FeCl_3$-treated wheat

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