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SECTION MEETINGS

DISTRICT OF COLUMBIA	
U. S. Public Health Auditorium	December 5, 1940
SOUTHERN	
Louisiana State University	December 20, 1940
SOUTHERN CALIFORNIA	
Occidental College, Los Angeles	December 11, 1940

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A Dosage Response Equation for Androgen Assay by the Chick Comb Method.*

FRANKLIN HOLLANDER, EMANUEL KLEMPNER AND ROBERT T. FRANK.

From the Laboratories of the Mount Sinai Hospital, New York.

In previous reports on the relation between dosage of crystalline androsterone (A)† and mean comb weight (W) of white leghorn

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† The symbols used in this report are as follows:

A = androgen activity of the portion of specimen applied to *each* chick, expressed as micrograms (γ) of crystalline androsterone.
N = number of chicks treated; sex indicated by subscripts m and f.
W = comb weight.
W^2 = comb weight squared.
B_o = initial body weight (3rd day).
B = terminal body weight (10th day).
S = standard error of estimate (a statistical measure of reliability).

A symbol in italics (*e.g.*, W) represents the mean value of this variable for a group of chicks treated similarly.

chicks, we described experiments involving 582 chicks in 5 monthly series. Each series consisted of 6 "gamma-groups" of chicks;[1, 2] *i.e.*, each bird received a total dose of 0, 10, 20, 30, 40 or 50γ androsterone in 0.35 cc of sesame oil, 0.05 cc being applied to the comb daily from the 3rd to the 9th day after hatching. The comb was excised and weighed on the 10th day.

In the work now reported the number of series was increased to 10 (1439 chicks) over a period of 12 months, but in addition to W, A, N_m, and N_t, we recorded B_o and B. We also continued to run a control (zero γ) group of chicks with each series, as a measure of

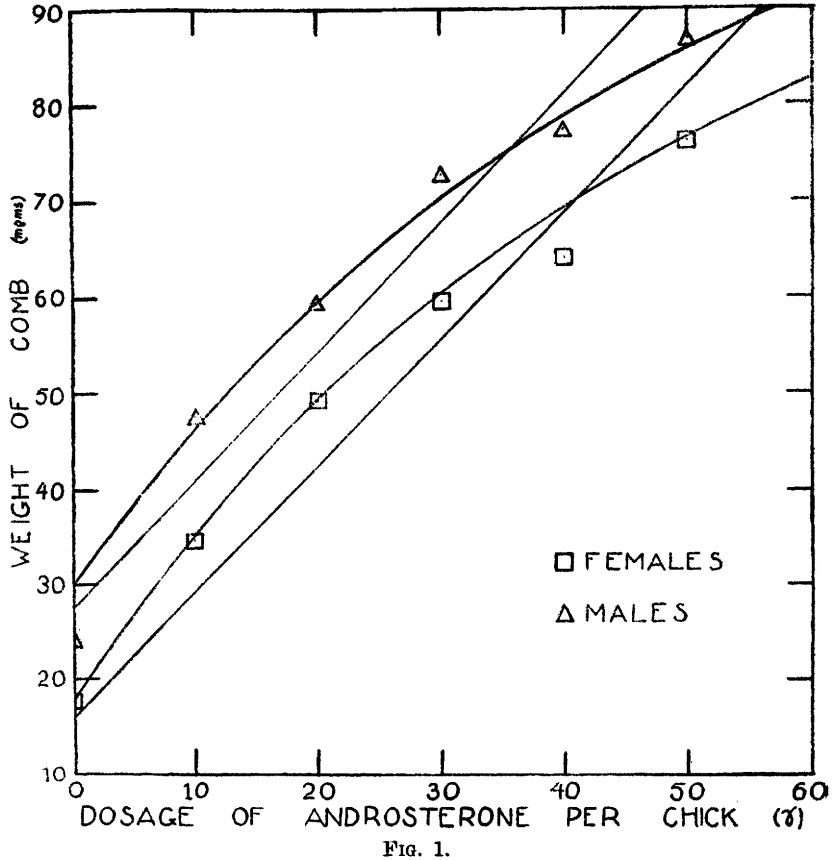

FIG. 1.

Relation between mean comb weight and dosage of androsterone. Combined data for 698 male and 741 female chicks (10 series).

[1] Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.

[2] Frank, R. T., Klempner, E., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 853.

variations in spontaneous comb growth activity associated with season and batch of chicks. Using these combined data, we investigated graphically and statistically the desirability of applying to W for any series and sex a simple correction for mean comb weight of the corresponding controls (W_c). Apart from sex difference, the improvement in the dose-response relation effected by this correction ($W - W_c$) was very small and probably negligible in the presence of corrections for B and B_o. Analysis of the data was therefore continued without such corrections. Graphs of W for each gamma-group (sexes separate) against A (Fig. 1) show that the data conform roughly to parallel straight lines, but a curvilinear fit may be closer. However, the graph for W against log A (Fig. 2) fits no better than the linear relation; this is confirmed by the identity of the corresponding correlation coefficients. Hence, adjustment for curvature must be made by the use of terms of the second order (see below).

Since the deviations of individual points in Fig. 1 from their respective lines result only in part from random variations in comb growth, we next tried to reduce these deviations by correcting for specific, uncontrollable variables. Employing the various zero-order correlation coefficients as criteria, we found that considerable improvement in reliability of the assay (*i.e.*, in S) can be effected by the use of individual chick data (both sexes combined), corrections for B_o and B, N_m and N_f, as well as W^2. Various functions of B_o and B and their squares afforded no significant improvement; likewise, a correction term for spontaneous comb growth (W_c) yielded such slight additional improvement in the presence of the other factors as to be negligible. Thus, instead of a simple linear relation between only 2 variables (W and A), we have evolved a single dosage-response equation which contains 7 variables without any bias in estimating their relative importance (*i.e.*, by multiple correlation):

$$A = 1.061W - 0.0043W^2 - 0.397B_o - 0.267B + 14.75\frac{N_m}{N_m + N_f} + 18.54\frac{N_f}{N_m + N_f}$$

It is our practice to express W in milligrams, B_o and B in grams, and A in micrograms (γ).

Under our working conditions, such an assay possesses a coefficient of multiple correlation, $R_{\gamma.W,B,etc.}$, of ± 0.78 in the dosage range 0–50γ; S (for a single chick) is $\pm 10.9\gamma$. For 16 chicks this reliability measure becomes $\pm 2.7\gamma$, corresponding to a coefficient of variation (S expressed as percent of the mean γ-value) of $\pm 11.3\%$. Hence, of any *large* series of assays, practically none should differ

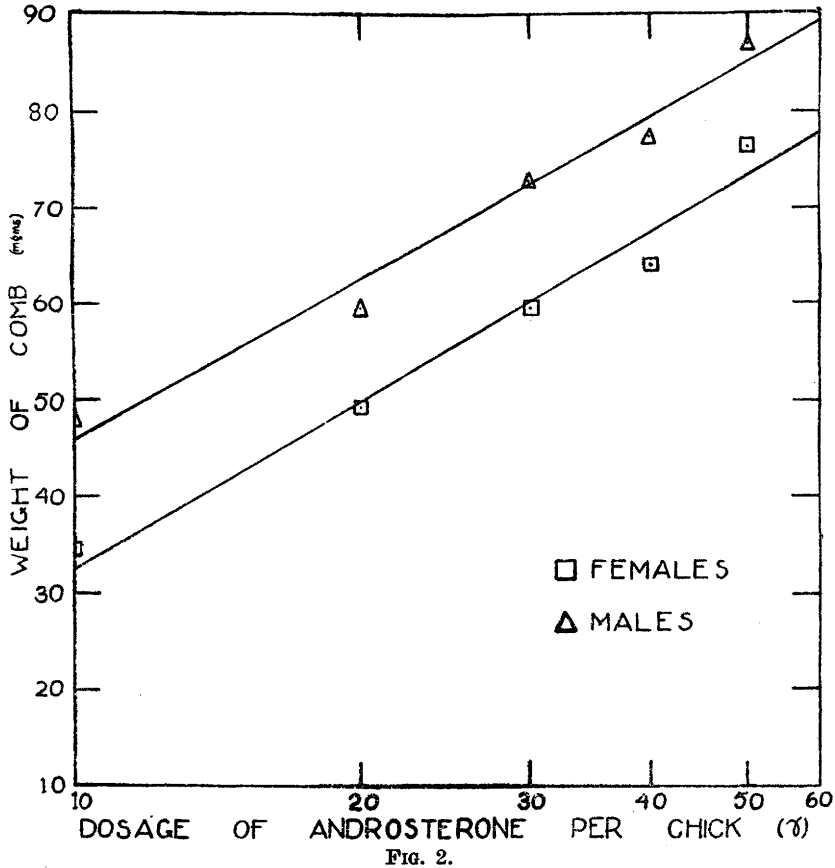

FIG. 2.

Relation between mean comb weight and log-dosage of androsterone. Combined data for 568 male and 575 female chicks (10 series).

from its "true" value by more than $\pm 8.1\gamma$ (or 34%) *provided these assays and the experiments upon which the dosage-response equation was based constitute fair samples of the same total population.* In contrast with this, we may cite the reliability measures of our earlier procedure based on $(W - W_c)$, the mean comb weight for each gamma-group (in contradistinction to comb weights for individual chicks) adjusted only for a simultaneous control group. For all 60 gamma-groups involving 1439 chicks in 10 series (6 gamma-groups per series), the correlation coefficients were ± 0.81 (males) and ± 0.88 (females), and the corresponding S-values for a gamma-group containing 16 chicks were $\pm 10.0\gamma$ and $\pm 8.2\gamma$. The root mean square of these S-values (9.1γ) is 3.4 times as great as the analogous value (2.7γ) found for assays based on our dosage-response equation,

which demonstrates the improvement in reliability effected by adjustments with the several correction terms W^2, B, etc. Reducing the dosage range to 0-40γ decreases S to ±2.2γ, but since this corresponds to a coefficient of variation of 11.6% (compared with 11.3% for the longer range) it affords no actual improvement in reliability.

Concerning a unit of androgen activity for urine, we may define the *androsterone equivalent per liter* of any specimen as the number of tenths of a milligram (or hundreds of gammas) of crystalline androsterone which yield the same *adjusted* comb growth response as one liter of the specimen. Detailed statistical reports on this work are now in preparation.

11870

Leucocytosis Induced by Injection of Kaolin into Cisterna and Ventricles of Brain.

G. ROSENOW. (Introduced by B. Kramer.)

From the Department of Laboratories, The Jewish Hospital of Brooklyn, N. Y.

As has been shown in previous studies[1, 2] neutrophilic leucocytosis can be induced in rabbits by mechanical lesions in certain parts of the diencephalon, especially the hypothalamic region; the production of leucocytosis by bacterial proteins can be inhibited by the previous administration of narcotics such as luminal which incapacitates, functionally, the parts in question.[3] Thus, there seems to be no doubt that the diencephalon plays an important role in the regulation of the composition of the peripheral blood.

The nature of this regulation is not yet clear. It seems likely that the cerebrospinal fluid might be involved in the induction of cerebral leucocytosis.

Therefore, studies were now undertaken to determine whether a partial block in the flow of the cerebrospinal fluid would produce any characteristic changes in the number of leucocytes and the blood picture. I used the intracisternal or intraventricular injection of a suspension of kaolin in order to obtain stasis in the cerebrospinal fluid over a long period. This method was inaugurated for other

1 Rosenow, G., *Z. f. d. ges. exp. Med.*, 1929, **64**, 452.

2 Rosenow, G., *Z. f. d. ges. exp. Med.*, 1929, **65**, 557.

3 Rosenow, G., *Verhandl. d. deutsch. Gesellsch. f. inn. Med.*, 1931, **43**, 75.