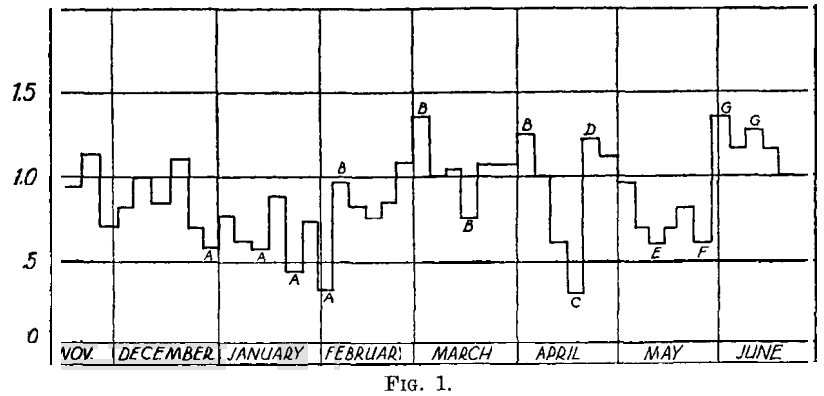

FIG. 1.

The Culture and Division Rate of *Dileptus gigas.*

A—Low division rate due to low temperature, average 15° C during periods indicated.
B—Variations due to fluctuations in temperature of artificial heating device.
C—Low division rate due to accidental exposure of culture dishes to direct sunlight.
D—High division rate due to removal of organisms to warm shaded place. Temperature average, 25° C.
D-E—Gradual drop of average temperature from 25° to 21° C.
F—Low division rate due to change of technicians during illness of author.
G—Temperature average, 28.5° C.

to indicate any "life cycle" or morphological or physiological degeneration of the experimental animals. This work thus corroborates that of earlier investigators[1-4] who used similar culture methods. More extensive work is now in progress.

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9582 P

Assay of Thyrotropic Hormone on Day-Old Chicks.

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Of the several methods employed in assays of thyrotropic hormone that utilizing the structure and weight responses of the thyroids of guinea pigs is the most commonly used.[1, 2] Day-old white leghorn chicks have been found not only to respond more sensitively than guinea pigs but also to give a relatively greater weight increase and to exhibit a more uniform thyroid structure.

1 Aron, M., *C. R. Soc. de Biol.*, 1929, **102**, 682.

2 Rowlands, I. W., and Parkes, A. S., *Biochem. J.*, 1934, **28**, 1829.

The preparation used was a partially purified alkaline extract of beef anterior pituitary.[3] Although a response is elicited by a single dose in 24 hours, 5 daily injections were found to give a maximal reaction. Further division of doses into 2 a day for 5 days gave a somewhat greater response.

One hundred and seventy-five injected chicks and 36 uninjected controls furnish the basis for the assay curve. Each sample of extract was tested on 5 chicks. At autopsy the thyroids, dissected under a binocular microscope, were weighed and body weight and sex recorded. The total dose was divided into 5 daily subcutaneous injections. It was found advisable to do the autopsy 24 hours after the last injection. Table I gives a representative series of the mean thyroid weights and total dose of extract used. Most dosages were repeated on different hatchings, with separate batches of extract. Total doses varied from 0.1 to 20.0 mg. Increases in thyroid weight were produced by increased dosages up to 10.0 mg. but beyond this there was no further increase in thyroid weight. Thus, a maximum thyroid weight is produced with 100 times the minimum stimulating dose. The larger doses produced thyroids averaging 6 to 7 times the mean weights of controls.

TABLE I.
Effect of Thyrotropic Hormone Injection on Thyroid Weight of Guinea Pigs and Day-Old Chicks.

Total Dose in mg.	Chicks 6 Days of Age			Guinea pigs Body Wt. 150-200 gm.		
	No. of Animals	Mean Thyroid Wt., mg.	Standard Error of Mean	No. of Animals	Mean Thyroid Wt., mg.	Standard Error of Mean
Controls	6	2.8	±0.5	5	22.8	±6.6
0.1	4	3.9	±0.7	—	—	—
0.4	5	5.5	±1.2	5	20.2	±3.8
1.0	—	—	—	6	31.0	±6.1
1.2	5	6.3	±0.9	—	—	—
2.0	5	7.8	±1.2	6	41.5	±6.0
4.0	5	10.0	±1.8	5	38.8	±3.2
8.0	4	14.9	±2.9	4	60.4	±7.6
10.0	5	20.1	±6.6	—	—	—
16.0	—	—	—	5	63.9	±14.8
20.0	5	20.3	±9.1	—	—	—

Thyroids of chicks receiving smaller amounts showed histological evidence of activity. The high degree of activity found in the 0.1 mg. group indicates that even lower doses are detectable histologically.

Several specimens of blood serum, urine extracts, and an extract

[3] Smelser, G. K., *Am. J. Ophthalmology*, 1937, in press.

of liver, prepared in the same manner as the pituitary substance, were ineffective, demonstrating the specificity of the chick thyroid response.

For comparison with the chick, 65 guinea pigs in groups of 5 each were injected with the same thyrotropic preparation in doses varying from 0.1 to 16.0 mg., 5 daily subcutaneous injections being given and autopsies performed 24 hours after the last injection. All animals were from the same colony and weighed from 150 to 200 gm. Table I shows that definite thyroid weight increase is not attained with less than 1.0 mg. of extract and further increase did not occur with doses above 8.0 mg. The thyroid weight increase thus reached a maximum with but 8 times the minimal stimulating dose. The larger doses produced thyroids averaging only about 3 times the mean weight of the controls. Doses too small to give a weight increase produced activation structurally, but such a dose (0.4 mg.) was at least some 4 times the amount necessary to give a comparable stimulation of chick thyroids. Normal guinea pig thyroids are more variable in weight and structure than those of baby chicks. In the latter there is not the difference in the size and morphology between the peripheral and the centrally placed acini which is characteristic of the guinea pig. Additional advantages are that the temperature of the environment is easily controlled and feeding is simple and inexpensive. An unlimited supply of chicks of standard stock, weight, and age is always available at a cost of about one-fifth that of the guinea pig.

Comparison of thyroid weights, obtained in testing unknown preparations, with values in a standard curve, yields only an approximate measure of the potency when 5 chicks are employed. However, when comparing normal and slightly stimulated glands, less variation occurs, and groups of 5 may be used. The chick thyroid weight test is not intended to supersede histological methods entirely, a combination of the two techniques being advisable. When marked thyroid hypertrophy is obtained, histological investigation may be omitted, but when questionable stimulation or none at all occurs the glands should be studied histologically.