The study of *Cancer magister* furnishes the opposite side of the picture, a species eminently successful as indicated by numbers and of large size (reaching a maximum of about 8½ inches and 3 lb.) in which form changes relatively slightly and slowly. That a high negative correlation exists between the degree of heterogony and the final size in a species will be clear to anyone examining an extensive collection of crustacea. Unusual and bizarre form can only result from markedly heterogonic growth and such species are almost invariably small. We have no data justifying speculation on the causal relation involved.

## 7473 C

## Experimental Production of "Cretinism" by Thyro-Cytotoxin.

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This work* was undertaken to ascertain if a condition simulating cretinism might be obtained by the injection of a "thyro-cytotoxic" serum into young rabbits. We were stimulated to make such a study in view of the work of Hektoen and Schulhof,[1] who were able to prepare an "antithyroglobulin", and of Portis,[2] Yates,[3] and MacCallum,[4] who studied the histology of the thyroid after injecting "thyrocytotoxic" sera.

The antigen was prepared as follows: Healthy adult rabbits were anesthetized and bled. The thyroid was then perfused *via* the carotids with normal saline solution, the thyroid being gently massaged occasionally, until the perfusate returned clear. The total time of perfusion was about 45 minutes. The thyroid was then removed, minced and ground in a mortar with prepared sand and normal saline. The suspension was permitted to stand overnight, after

* This work was started by Dr. Paul H. Kanai (who had collaborated in a previous study with Doctors Hektoen and Dragstedt[5] and whose untimely death ended his work on an attempt to produce cretinism *in utero* by thyro-cytotoxic serum. He was successful only in showing by the precipitin reaction that the antiserum passed through the placenta of rabbits).

1 Hektoen and Schulhof, *J. Am. Med. Assn.*, 1923, **80**, 386.

2 Portis, *J. Inf. Dis.*, 1904, **1**, 127.

3 Yates, *Univ. Penn. Med. Bull.*, 1903, **16**, 195.

4 MacCallum, *Med. News*, 1903, **83**, 820.

5 Hektoen, Kanai, and Dragstedt, *J. Am. Med. Assn.*, 1925, **84**, 114.

which it was centrifuged slowly to remove insoluble residue. The supernatant opalescent fluid was used as the antigen. Aseptic precautions were used throughout the various procedures.

The antigen was injected intramuscularly into healthy adult hens at 4-day intervals throughout the period of the experiment. Other hens were injected with rabbit serum, the serum of these hens serving as a "control serum". The precipitin titre was determined by the ring-test method. When the precipitin titre of the fowls reached 1 to 50,000, the serum was used for our experiments.

Litters of rabbits that numbered 7 or more were used. Injections of the "thyro-cytotoxic" serum and "anti-rabbit" serum control serum were started when the rabbits were 10 days old and were given intraperitoneally to 5 litters every fourth day and later to 3 litters every second day. Two members of the litter received the "thyro-cytotoxic" serum from the fowls treated with the thyroid, 2 received the "control anti-serum" from the fowls treated with rabbit serum, and the others were used as untreated controls.

For evidence of cretinism, the growth curve was followed, and the rate of growth of hair after shaving an area on the abdomen was observed.


A, control treated with "anti-serum"; B, "cretin" treated with "thyro-cytotoxic" serum.

Eight litters of rabbits were used. In the first 5 litters, the treated rabbits received 1 cc. of the "thyro-cytotoxic" and the "control anti-serum" sera every 4 days. After from 3 to 6 weeks it was found that the rabbits receiving the "thyro-cytotoxic" sera were growing faster than the untreated controls and than those receiving the "control anti-serum".

It was then decided to increase the dose and the frequency of injection. After some preliminary tests with larger quantities, in which 10 cc. of serum proved to be a lethal dose, we chose 3 cc. as the dose to be used in another group of young rabbits. At the first injection only ½ cc. of the "anti-sera" was given; then this dose was gradually increased to 3 cc. within 2 weeks. After this, occasionally as much as 4 cc. were given at a single dose, depending on the amount of serum obtained from the fowls at a given withdrawal of blood.

This more intensive treatment was given to 3 litters. The growth curves are shown in Fig. 1. The 6 rabbits receiving the "control anti-serum" survived throughout the period of 12 weeks. Of the 6 rabbits receiving the "thyro-cytotoxic serum", one died at 3 weeks, one at 6 weeks, 2 between 8 and 10 weeks, of respiratory infection. The remaining 2 survived the 12 week period. After withdrawal of the "thyro-cytotoxic sera" in these 2 rabbits, an increase in growth rate was evident at about 2 weeks. A marked difference in the rate of growth of the abdominal hair after shaving was observed between the rabbits treated with "thyro-cytotoxic sera" and the "treated" and "untreated" controls. The body hair of the "cretin" rabbits was lustreless and shaggy. In addition, the cretin rabbits were apathetic and their abdomens were "pot-bellied" in type. They manifested all the outward appearances of typical cretins. The thyroid glands of the rabbits in this series were examined histologically. The thyroids of the "treated" and "untreated" controls were normal. In the cretin rabbits dying prior to 12 weeks, the thyroid was so small as to be difficult to find, thus causing us to make a wide dissection of the tissue and a careful histologic examination of the mass. In one of the 4 animals, a small macroscopic gland was found which on histologic examination showed the gland to be slightly more cellular than normal. In another, no thyroid tissue was found on one side, but on the other the thyroid appeared normal histologically. In the other 2, except for the small size, the thyroid appeared normal histologically.

In the absence of metabolic studies, it cannot be stated from the evidence obtained that true cretinism was produced. The small

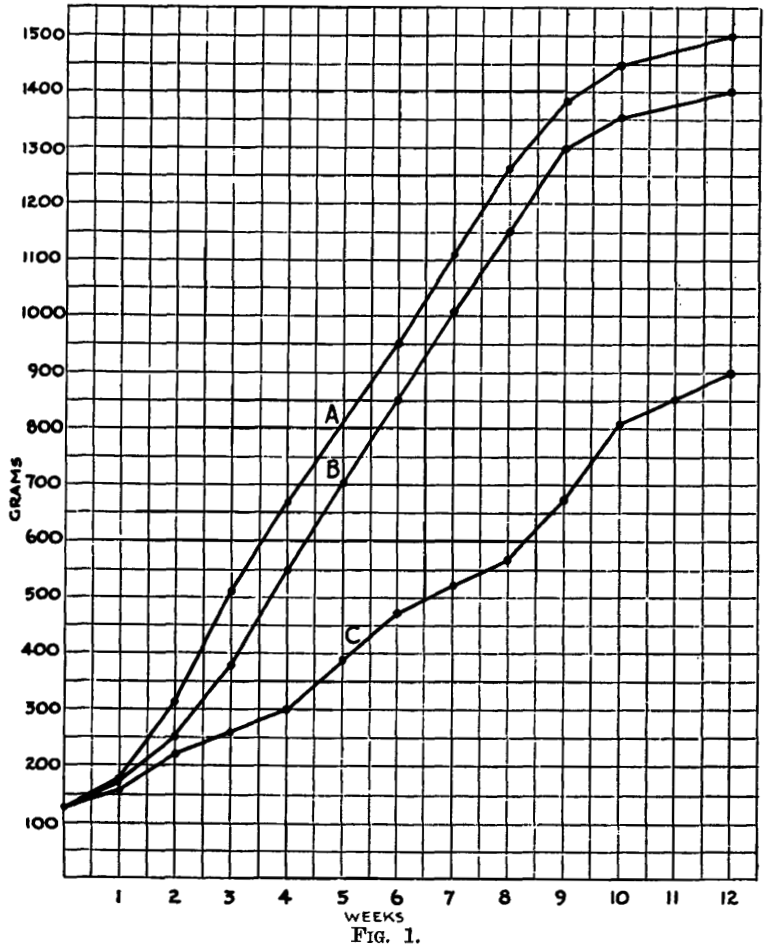


FIG. 1.

This figure shows the average growth curve of the 3 litters of rabbits referred to in the text. Curve A, the 7 untreated controls; Curve B, the 6 "treated" or "anti-serum" controls; Curve C, the 6 treated with "thyro-cytotoxic serum."

size of the thyroids of the cretin rabbits would suggest that the retarded growth of the body and of the hair was due to failure of the thyroid to develop and function normally. Before the mechanism concerned may be discussed satisfactorily, "anti-thyroglobulin" serum should be used,[6] particularly in view of Collip's observations pertaining to "anti-hormones". Since we are not in a position at this time to carry these studies further, we desire to submit the factual observations we have made.

[6] Schulhof, *Am. J. Physiol.*, 1930, **93**, 175.

*Summary.* A condition simulating cretinism was produced in six young rabbits by injecting them intraperitoneally every second day with an antiserum (3 cc.) prepared by injecting hens with the proteins of the thyroid gland that are soluble in normal saline solution.

## 7474 P

## Effect of Male Hormone Extracts, Theelin, and Theelol on the Chick Embryo.*

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This report presents the results of a preliminary investigation. Eggs from brown and from white leghorns were injected in the albumen before or on the third day of incubation with 0.1 cc. of an ethylene glycol solution of the substance to be studied. The animals surviving on the twentieth day of incubation were killed and the tissues fixed for histological study. Comparison was made with the tissues of animals injected with the solvent alone.

The results may be summarized as follows:

A. Ten standard bird units[1] of an extract from human male urine prepared by the procedure of Gallagher and Koch[2] were injected into each egg. This preparation was contaminated with 6.3 international rat units of the estrogenic substance always found in such extracts. The results on 56 animals were: Normal males 17; normal females 21; hermaphrodites 18. This diagnosis rests on the presence of a testis and either an ovary or an ovotestis. The Wolffian ducts varied greatly in size, ranging from normal (about 0.3 mm.) to 6 mm. in diameter. No relationship was apparent between the gonad and the size of the duct. The Müllerian ducts in the females likewise exhibited great variability both between individuals and between the 2 ducts of the same animal. Eight of the 17 males showed some abnormality of the Müllerian ducts.

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1 Gallagher, T. F., and Koch, F. C., *Proc. Amer, Soc. Biol. Chem.*, 1933, VIII, lxviii.

2 Gallagher, T. F., and Koch, F. C., *Endocrinology*, 1934, **18**, 107.