7562 P

Protective Substances in Sera of Animals Injected with Anterior Pituitary-Like Hormone of Teratoma Testis Urine.

GRAY H. TWOMBLY AND RUSSELL S. FERGUSON. (Introduced by J. Ewing.)

From the Memorial Hospital, New York City.

Collip[1] reported observations on the results of repeated inoculations of the thyreotropic hormone, in large amounts, over prolonged periods of time into white rats. He showed that, on injection of the thyreotropic principle of the pituitary, hyperplasia of the thyroid gland occurs and the metabolic rate rises sharply. However, continued injections fail to maintain this condition and the metabolic rate returns to normal in from 2 to 3 weeks and may even become subnormal. The animals fail to show any rise in metabolism on the administration of as much as 8 times the previously effective dose. The serum of animals, thus rendered refractory, will exert a protective effect against the activity of the hormone when injected into untreated animals. It does not protect, however, from the action of desiccated thyroid tissue. Anderson and Collip[2] have prepared a potent antithyreotropic serum by repeated injections into a horse. Similar "antihormones" inhibiting the action of the anterior pituitary-like hormone of pregnancy urine have been made and reported by Selye, Bachman, Thompson, and Collip.[3]

In an attempt to confirm this work and to determine whether the same thing is true of the anterior pituitary-like hormone found in the urine of patients suffering from teratoma testis, we have injected a number of rabbits over prolonged periods of time. The anterior pituitary-like hormone was obtained according to the original method of Zondek[4] by precipitation of acidified fresh urine with 5 times its volume of 95% ethyl alcohol. The precipitate was washed 3 times with ether, dried, and stored in the ice box. Due to the difficulty of obtaining large amounts of the urine, the extracts were prepared from lots of 300 to 1000 cc. These were assayed separately for their potency by the injection of watery extracts into immature female mice. Most of the urine came from 2 patients and was found to contain 5,000 to 10,000 mouse units per liter.

---

[1] Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **1**, 28.

[2] Anderson, Evelyn M., and Collip, J. B., *Lancet*, 1934, **1**, 784.

[3] Selye, Hans, Bachman, C., Thompson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113.

[4] Zondek, Bernhard, *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Berlin, Julius Springer, 1931.

Three litter-mate female rabbits 13 weeks old, weighing 2 kilos each, were injected with approximately 100 mouse units of an aqueous extract of the powder containing the hormone, daily for a period of 3½ months. Two days after cessation of the injections the animals were bled and the serum separated. This serum was tested for its protective effect against the action of the original hormone in the following fashion: Infantile female mice, weighing 6 to 8 gm. were injected with approximately 4 mouse units of the aqueous solution of the hormone and 0.5 cc. of the rabbit serum, over a period of 30 hours (0.2 cc. of hormone solution and 0.1 cc. of serum given subcutaneously at 12 a. m., 4 p. m., 9 a. m., 12 a.m., and 4 p. m.). One hundred hours after the beginning of the injections the animals were killed and their ovaries inspected according to the usual technique of the Aschheim-Zondek test. Thirty-nine mice so treated failed to show any corpora lutea while 24 mice receiving the extract alone all showed large corpora lutea and open vaginal orifices. Thirteen mice were given the aqueous extract plus the serum of normal rabbits. The serum was obtained from 5 different animals. All the mice so treated showed corpora lutea, that is, the sera of these rabbits failed to show any protective effect against the action of the hormone such as was exhibited by the sera of the injected rabbits.

The injection of only 0.3 cc. of serum into each mouse also protected against the action of the hormone. Three mice injected with 0.1 cc. only, showed protection in one, no protection in one, and apparently no corpora lutea but an open vaginal orifice in the third. That is, there was sufficient protective substance in 0.3 cc. of the sera of the injected rabbits to counteract completely the action of 4 mouse units of A.P.L. from teratoma urine. The experiments were clear cut, none of the mice receiving half a cc. of immune serum showing corpora lutea and all of the controls showing luteinization.

A similar experiment was tried with the preparation of the A.P.L hormone of pregnancy urine made by E. R. Squibb and Sons, called by them "Follutein". This extract in glycerine contained 250 mouse units per cc. and was said to have 0.55 mg. of N. per cc. The serum of 3 female rabbits, weighing approximately 2 kilos each, was tested for any protective effect it might show against the action of the hormone, each mouse being given 0.5 cc. of serum and 5 mouse units of Follutein. Eleven mice so tested failed to show any evidence of protective substance. The rabbits were then injected daily with 75 mouse units (375 rat units) and the injections continued, with occasional interruptions, for a month. At the end of this

time samples of the serum all showed complete protective action in 0.5 cc. amounts against 5 mouse units of Follutein. The injections were continued for another 2 weeks, at the end of which time the rabbits showed multiple sores in the skin of the back, a phenomenon which had been previously noted in the animals injected with A.P.L from teratoma testis urine. All these animals continued to show a protective effect in their serum. A male rabbit injected for a month gave similar results. Twenty-seven mice tested with the sera after injection showed complete protection. Twelve control mice receiving Follutein alone all showed corpora lutea.

Cross protection experiments of this serum and the teratoma testis urine hormone were carried out. Three mice injected with the teratoma hormone alone showed corpora lutea while 9 receiving 4 mouse units of the hormone plus 0.5 cc. of serum immunized against the pregnancy hormone each showed no effect in the ovaries. Nine mice receiving four mouse units of "Follutein" plus 0.5 cc. of serum from the rabbits immunized against the teratoma hormone showed no evidence of luteinization while three control mice injected with "Follutein" alone showed corpora lutea.

7563 P

Successive Transmission of Virus of Lymphogranuloma Inguinale Through White Mice.

ARTHUR W. GRACE AND FLORENCE H. SUSKIND.
(Introduced by Paul Reznikoff.)

From the New York Hospital and Department of Medicine, Cornell University Medical College, New York.

As part of a study of the properties of the virus of lymphogranuloma inguinale white mice were inoculated intracerebrally with bacteriologcally sterile pus aspirated from an inguinal bubo and glandular material obtained from a case of lymphogranuloma inguinale. The pus and glandular material were diluted 1 in 5 with sterile distilled water and inoculated in 0.03 cc. quantities into each of 6 mice. The object of this section of the work was to ascertain whether or not the virus could be transmitted indefinitely in that manner. All of the inoculated animals died within an average of 11 days. The brain of one of these animals dying from lymphogranuloma inguinale was emulsified in 1 in 2.5 dilution of distilled water and inoculated intracerebrally in 0.03 cc. quantities into another batch of 6 white mice. All of these mice died within an