

Prolonged Injection of a Thyrotropic Extract Without Development of Refractoriness.*

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It has been uniformly reported that repeated injections of hypophyseal extracts containing the thyrotropic hormone fail to stimulate the thyroid gland beyond 4 or 5 weeks. The BMR and thyroid structure return to normal. This has been explained by Collip¹ as due to the increase of a normally present "antihormone." This theory has also been applied to the other hypophyseal hormones.

Results obtained in this laboratory with 2 different hypophyseal preparations containing the thyrotropic hormone do not support the "antihormone" hypothesis. The development of refractoriness has been found to depend on the type of extract in which the hormone is administered. The thyrotropic effect has been determined by the BMR, the microscopic structure and total iodine content of the thyroid gland. The author is very grateful to Dr. G. L. Foster of the Department of Biochemistry for his kindness in doing the iodine determinations. The determinations were made by his modification of Leipert's 1934 method.

Male guinea pigs, 250-350 gm. in weight, were used. They were maintained under constant conditions in a room in which no iodine was kept or used. The 2 preparations employed were made from beef anterior hypophyses by the van Dyke and Wallen-Lawrence Na_2SO_4 method² and by the flavianic acid method of Meyer.[†] The BMR was measured after a 13-16 hr. period of fasting in a closed system apparatus maintained at $30^\circ\text{C.} \pm 0.1^\circ$.

A. *Treatment with the Na_2SO_4 preparation.* Eleven animals

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¹ Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, 1, 28.

² van Dyke, H. B. and Wallen-Lawrence, Z., *J. Pharm. and Exp. Therap.*, 1930, 40, 413.

† A modification, devised by Dr. Meyer, of the original method published by Evans, Meyer and Simpson (*Mem. U. Calif.*, vol. 11, 1933) was used in making the flavianate extract.

The Na_2SO_4 preparation used was "Phyone" made by Wilson and Co. and provided through the courtesy of Dr. David Klein.

were injected daily subcutaneously with one cc. of the Na_2SO_4 preparation for 23-40 days. All of these showed a pronounced increase in the BMR during the first 2 weeks of treatment. Ten of these animals showed a return of the BMR to normal. Three of the refractory animals were autopsied and had structurally normal thyroids. The eleventh animal had a slightly elevated BMR on the 25th day of treatment but a normal gland at autopsy 3 days later. The findings from this group thus confirm the results obtained by Collip,¹ Friedgood² and others. No iodine determinations were made.

B. *Treatment with the flavianate preparation.* Sixty-nine guinea pigs were injected subcutaneously for 1-88 days with the flavianate preparation, 0.2-0.4 gm. equivalent of dried beef anterior hypophysis being given daily. This dosage is roughly equal in potency to one cc. of the Na_2SO_4 preparation. Forty-three of the 69 animals were autopsied by the 38th day. Thirty-nine of these gave no evidence of refractoriness. Their thyroids showed intense activation structurally and their total iodine was at the low level characteristic of the activated gland. The high BMR reached by the second week of treatment was maintained. Four of the 43 animals became refractory as revealed by a return of BMR to normal. Two of the 4 were autopsied and had thyroids with normal iodine content and structure. Of the remaining 26 guinea pigs, treatment in 19 was terminated between the 38th and 80th day. Fourteen showed a continued activation and 5 had become refractory. Treatment of the remaining 7 animals was terminated in 80-88 days. Six of these showed continued thyroid stimulation and one had developed a refractory condition.‡

C. *Controls.* There were 21 controls, 14 of which were injected daily with a flavianate preparation made from beef muscle and 7 were untreated. No difference between the 2 groups was found in the BMR or in the structure or iodine content of their thyroids, all being within the normal range.

A marked difference in the development of a refractory condition of the thyroid in prolonged administration of 2 different preparations made from the hypophyses of the beef has thus been

¹ Friedgood, H. B., *B.J.H.H.*, 1934, 54, 48.

‡ Two rabbits treated 59 days with the flavianic preparation showed marked thyroid activation structurally and a very low gland iodine content.

In 3 animals not included above, treated for 47, 60 and 85 days, there was a mild elevation of BMR and moderate gland activation but the iodine values were a low normal. It is felt that these animals were becoming refractory, with a return of the gland iodine to normal preceding the microscopic change.

found. The potency of these 2 preparations was nearly identical. These findings strongly indicate that the development of a refractory condition in prolonged treatments with preparations containing the thyrotropic principle is not due to an exhaustion of the thyroid or to the formation of an "antihormone."

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Antibody Nature of Refractoriness to Injections of Hypophyseal Extracts Containing Thyrotropic Hormone.*

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It has been shown in the preceding paper that the development of refractoriness to repeated injections of thyrotropic hormone is influenced by the type of extract in which the hormone is contained. This suggests that refractoriness is an immune response to foreign proteins and is not due to the formation of an "antihormone." If this is the explanation, preparations not inducing refractoriness would be less antigenic. This decrease in antigenicity has been tested and confirmed. No gland iodine determinations were done in this series. The extracts and methods used were the same as in the preceding paper.

A. *Effect of non-stimulating doses of the Na₂SO₄ and the flavianate preparations of beef anterior hypophyses on the development of refractoriness in the thyroid.* Two series of 6 male guinea pigs each, weighing 250-350 gm., were used. Five of the first series were given 0.1 cc. of the Na₂SO₄ preparation subcutaneously every other day for 32 days. They showed no rise in the BMR. Twenty times this amount (2 cc.) then given daily for 5 days caused no activation of the thyroid, as revealed by the BMR and biopsy specimens of thyroid. The previously untreated control showed a marked response to this large dosage. The second series was treated like the above except that the flavianate extract was used and the treatment lasted for 41 days (0.02 gm. dried gland equivalent was given at each injection). No elevation of BMR took place during this treatment. Twenty times the initial

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