trypan blue reaction. It is not surprising, therefore, that tissue extracts may occasionally manifest an activity of this sort which cannot be accounted for by their histamine content. There seems no necessity, therefore, for postulating a specific factor in tissues which has the property of increasing capillary permeability as evidenced by the trypan blue reaction.

11976

Rapid Response of Guinea Pig Thyroid to a Single Injection of Thyrotropic Hormone.

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Microhistrometric studies[1] of the guinea pig thyroid after 3 daily subcutaneous injections of thyrotropic hormone have been published. It had been assumed that 3 or 4 days were required for significant histologic change to occur. Loeb[2] had noted early hypertrophy one day after an intravenous injection. Aron[3] also reported that changes were evident in less than 12 hours. Janssen and Loeser[4] described structural changes in 2 hours after a single injection of 20 guinea pig units. Kummer[5] also described changes apparent at 2 hours which were more pronounced at 4 hours. Williams,[6] by a method of continuous *in vivo* observation, detected a cycle of activity that reached a maximum within 24 hours. Okkels,[7] in his classical monograph of 1934, states "that 30 minutes after intraperitoneal injection in the guinea pig of Evans' Pituitary Extract structural changes appear in the thyroid cells. The colloid is not yet affected but the thyroid cells develop a tremendous swelling, presumably like a gel-sol transformation of the cytoplasm." A chronological and quantitative representation of the changes occurring in the guinea pig thyroid after a single subcutaneous injection of the thyrotropic hormone is desirable.

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1 Rawson, R. W., and Starr, Paul, *Arch. Int. Med.*, 1938, **61**, 726.

2 Loeb, L., and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

3 Aron, M., *Rev. Frac. d'Endocrinol.*, 1930, **8**, 472.

4 Janssen, S., and Loeser, A., *Arch. f. Exp. Path. u. Pharmakol.*, 1931, **163**, 517.

5 Kummer, H. J., *Endokrinologie*, 1938, **20**, 326.

6 Williams, R. G., *Am. J. Anat.*, 1937, **62**, 1.

7 Okkels, H., *Skan. Arch. f. Phys.*, 1934, **69**, 97.

Method. One hundred seventeen young, 200-300 g male guinea pigs were used; of these 26 served as controls. Male animals were used to obviate any effect of the estrous cycle upon the thyroid. In order to standardize the conditions of the experiment as much as seemed feasible, all of the experimental animals were submitted simultaneously to a single subcutaneous injection of 0.03 cc of Antuitrin-T.* and groups of animals—both experimental and control—were subsequently sacrificed at short predetermined incremental intervals. The chronological periods subsequent to injection, and number of animals in each group were as follows: 1 hour, 8 animals; 4 hours, 19 animals; 6 hours, 12 animals; 8, 12, 16, 20, and 24 hours, 8 animals each; 32 and 48 hours, 6 animals each. Their thyroid glands were quickly but carefully removed, preserved in formalin, embedded in paraffin, stained with hematoxylin and eosin, and prepared for histological examination.

Utilizing the method of Rawson and Starr—which simply involves the accurate measuring and recording of the height of a typical epithelial cell in 100 acini, from which measurements the arithmetical mean acinar cell height is determined—a quantitative record of the changes in mean acinar cell height at chronological intervals during the 48 hours subsequent to a single injection was obtained.

Results. Of the 26 control animals the average mean acinar cell height was found to be 4.45 micra with a range of plus or minus 1.5 micra. The mean acinar cell heights of groups of animals killed at 1, 4, 6, 8, 12, 16, 20, 24, 32, and 48 hours after injection were found to be 5.13, 5.34, 5.37, 7.57, 7.74, 8.44, 7.86, 8.49, 7.92, 7.58, respectively. The most striking change observed was an enormous increase in cell height by 8 hours; the maximum response in this series was obtained at 16 hours, the acinar epithelium nearly doubling in height; and the mean acinar cell height was still markedly increased over the control value at 48 hours. (Fig. 1.) The trend of the graph is corroborated by statistical analyses; for since the significant critical ratio is 2, it is evident that all increases in mean acinar cell height including and subsequent to the 4-hour period are statistically significant; that the hypertrophy is marked at 8 hours, maximal at 16 hours, and maintained at least 48 hours following a single subcutaneous injection of thyrotropic hormone.

Discussion. In the interpretation of the experimental response, the individual variability of the animals utilized was considered. Prior to the 8-hour period, not all animals responded significantly; however, at 8 hours and thereafter all values obtained are well above

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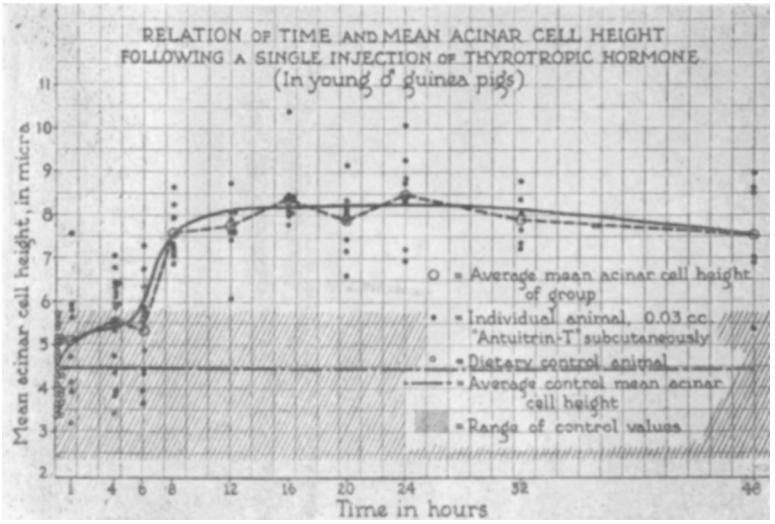

the range of the control group. Reference to the graph indicates that some animals respond more quickly, others more tardily; that some maintain the response to stimulation for prolonged periods subsequent to injection, while in others recession occurs more promptly.

The relation between mean acinar cell height and dosage of Antuitrin-T was determined,[8] and it was found that the maximal mean acinar cell height was attained with a 0.03 cc of Antuitrin-T subcutaneously, further increase in dose having little effect. It was noted, too, that the group average obtained with that dose approximates 7.8 micra, while the maximum group response in the present series lies between 7.5 and 8.5 micra; however, that response was obtained after 3 daily subcutaneous injections, killing the animals on the fourth day; and it is evident that the same height of response may be attained in 8 to 16 hours following a single subcutaneous injection. Careful study of the amount, condition, and staining reaction of the colloid in these glands indicates that it underwent alterations associated with the changes in mean acinar cell height in response to stimulation.

Summary and Conclusions. A single, subcutaneous injection of thyrotropic hormone will result in a significant increase in mean acinar cell height within 8 to 16 hours, which is comparable to that heretofore reported in 72 hours following a series of daily injections. This measured response persists at least 48 hours following injection.

[8] Starr, P., and Roskelley, R., *Am. J. Phys.*, 1940, **130**, 549.