luteinization has been found in some areas together with large bodies of lutein cells, some with a central cavity and with degenerating ova.

The 3 adult clitorides (7-8 mos.) which have been examined microscopically show structural modifications. A well developed *os priapi* with anterior process is present in each. This structure is not found in the normal adult female, although a homologue exists in the newborn female. Treatment with APL during the first few weeks of life has stimulated this homologue to develop into the typical male structure. There is also some stimulation of the glans epithelium in these animals in that papillae are more numerous than in the normal clitoris. In one animal differentiation of the papillae into spines has taken place. These spines are typical male structures. Some stimulation of the cavernous structures was also noted.

Both Deanesly and Hill have concluded that the androgen produced by ovaries grafted into males is not testosterone. Lamar[7] and the present authors[8] have shown that progesterone, in large amounts, is androgenic in the rat. Progesterone is presumably produced by lutein cells. It is not known whether, in the normal female rat, progesterone is produced by the luteinized granulosa or by the luteinized theca cells. At any rate, it is conceivable that the androgen produced by the ovaries of these experimental animals may be progesterone.

Summary. The administration of APL to infantile female rats causes masculinization of the clitoris consisting of gross enlargement and the development of an *os priapi.*

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Melanophore Hormone of the Pituitatry Gland and Metabolic Stimulation.*

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Collip and coworkers have recently reported metabolic stimulation in rabbits, guinea pigs, and rats occurring within a few hours following injection of certain pituitary extracts.[1, 2, 3] The substance in

7 Lamar, J. K., *Anat. Rec.*, 1937, **70**, Suppl. p. 45.

8 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrin.*, 1939, **24**, 351.

* Aided by a grant from the Otho S. A. Sprague Memorial Institute.

1 O'Donovan, D. K., and Collip, J. B., *West. J. Surg.*, 1937, **45**, 564.

2 O'Donovan, D. K., and Collip, J. B., *Endocrin.*, 1939, **23**, 718.

3 Billingsley, L. W., O'Donovan, D. K., and Collip, J. B., *Endocrin.*, 1939, **24**, 63.

the extracts producing this stimulation was called the "specific metabolic principle" of the pituitary. From the similarity in chemistry and occurrence in extracts, as well as from certain differences between these and other known pituitary hormones, it was suggested that the specific metabolic principle and the melanophore hormone might be identical. In view of the previous report of Zondek and Krohn[4] that "Intermedin" did not affect the metabolism of rabbits, it seemed worth while to study the subject further.

Accordingly, the effect on oxygen consumption of various melanophore hormone preparations was studied in rats. A modified Benedict spirometer was used to measure oxygen consumption. The rats were kept at room temperature but the metabolism was measured at 28°C. Comparable results were obtained with normal, thyroidectomized and hypophysectomized rats, and with rats recently fed as well as rats fasted 12-18 hours.

The usual procedure was to measure the basal rate in the morning, inject the extract after 2-3 hours, and measure the oxygen consumption over the following period of 8 hours without removing the rat from the machine. Using this technic, basal oxygen consumption for normal rats averaged 211 l/sq.m./day, for thyroidectomized

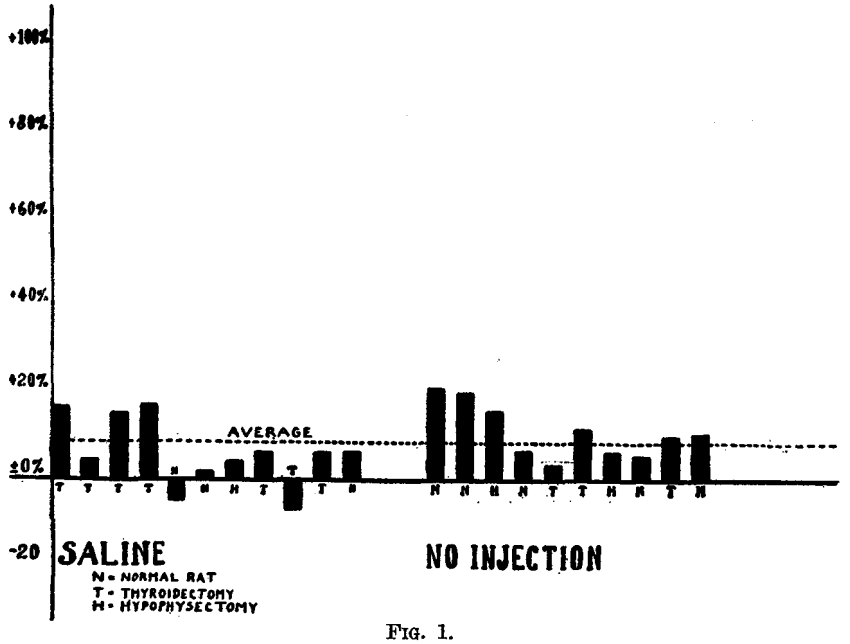

FIG. 1.

[4] Zondek, B., and Krohn, H., *Klin. Wchnschr.*, 1932, **11**, 1293.

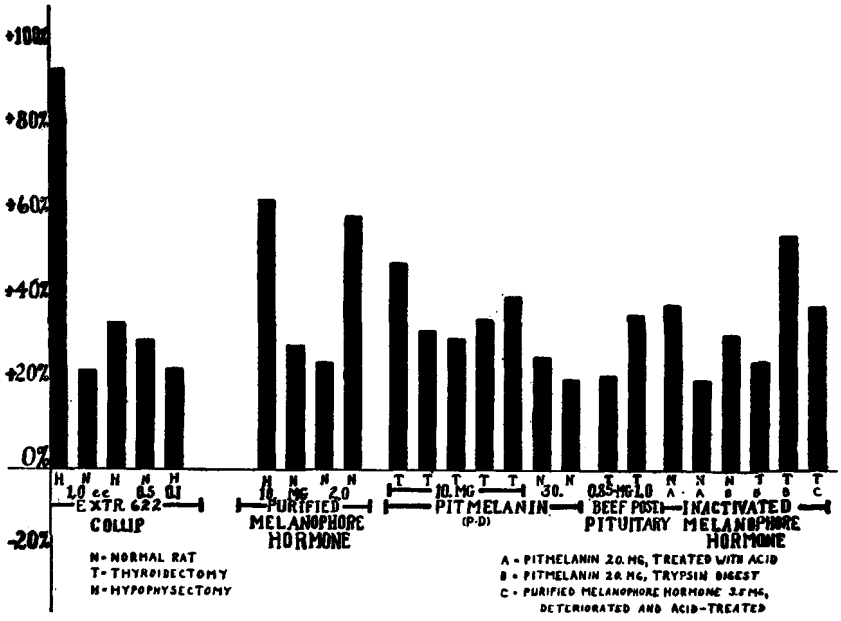

FIG. 2.

rats 160, and for hypophysectomized rats 137. Control studies are shown in Fig. 1. On the ordinate is the percentage change in oxygen consumption over the basal value. Each column represents the highest reading obtained after injection in one experiment. Fig. 2 summarizes only the positive findings with various pituitary extracts. The melanophore hormone content of each extract was determined by injection into hypophysectomized frogs which constitute a specific test object for the melanophore hormone.[5] No increase in oxygen consumption under 20% was considered significant. In 45 experiments with extracts rich in melanophore hormone a significant increase in oxygen consumption over controls was obtained in 18 experiments. No significant change was found in 27 experiments. It will be seen from Fig. 2 that a significant increase in oxygen consumption was obtained with a sample of Collip's extract 622† which is very rich in melanophore hormone. With this extract large doses, as a rule, gave a rise. With a purified melanophore hormone preparation prepared from beef posterior pituitary glands‡ and from

[5] Teague, R. S., Noojin, R. O., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1939, **65**, 115.

† Obtained through the courtesy of Dr. J. B. Collip.

‡ Supplied by the Wilson Laboratories.

whale anterior lobe,§ by a method developed in this laboratory,[6] there were 4 positive to 9 negative experiments. With "Pitmelanin",‖ a partially purified melanophore hormone preparation, there were 2 positive to 7 negative experiments. Five significant and 5 non-significant results were also obtained with Pitmelanin after alkali treatment to destroy the slight contamination with the pressor principle (checked by cat assay). U.S.P. posterior pituitary powder contains a moderate amount of melanophore hormone. On one occasion a significant rise was obtained with this, while 3 other experiments were negative. After alkali treatment to destroy the pressor principle, an increase was obtained once, no change once.

The melanophore hormone in a sample of Pitmelanin was destroyed by boiling in concentrated HCl. Twenty mg of Pitmelanin so treated, containing only a trace of melanophore hormone, produced a significant increase on 2 occasions. After destruction of melanophore hormone by trypsin digestion, a similar sample of Pitmelanin still produced a significant increase in 4 experiments, no increase in 2. However, after inactivation of the melanophore hormone by repeated exposure to ultraviolet light, no significant metabolic stimulation was obtained in any of 8 trials. Likewise no results were obtained in 2 experiments using Pitmelanin inactivated by H_2O_2. Further control experiments with beef muscle, liver and kidney extracts in acetic acid have been negative.

It may be that some uncontrolled biologic variation in the rats may account for the variation in results. There may be a prosthetic group of a complex molecule of melanophore hormone affecting frog melanophores, while another prosthetic group of the same molecule stimulates mammalian metabolism. With such a hypothesis the results of melanophore destruction experiments may be explained by assuming that the prosthetic group stimulating metabolism is not affected by acid boiling or trypsin digestion, while that affecting melanophores is altered, while ultraviolet light or oxidation may destroy both. Another explanation is that metabolic stimulation is produced by a breakdown product of the melanophore hormone. Finally, the possibility of a non-specific effect on metabolism with such concentrated extracts has not been entirely eliminiated. As with so many other problems in endocrinology, the isolation of the chemically pure hormone may be required to settle the problem.

[6] Fostvedt, G., personal communication.

§ Obtained as a result of a grant from the Rockefeller Foundation to Dr. E. M. K. Geiling.

‖ Supplied by Dr. O. Kamm of the Research and Biological Laboratories of Parke, Davis and Co.