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11. D. M. Bonner and A. J. Haagen-Smit, *Proc. Natl. Acad. Sci. U.S.* 25, 184 (1939).
12. C. O. Miller, *Plant Physiol.* 31, 318 (1956).

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Anterior Pituitary Preparation with Tropic Activity for Sebaceous, Preputial, and Harderian Glands

In this report, the methods of preparation and assay of a crude extract of anterior pituitary glands of hogs showing tropic effects on sebaceous, preputial, and Harderian glands in the rat are described. These effects can be called sebotropic because the ectodermal glands affected produce secretions rich in lipids.

Lasher, Lorincz, and Rothman (*1*) observed that, in mature, ovariectomized, white rats, hypophysectomy results in atrophy of cutaneous sebaceous glands and loss or greatly diminished responsiveness of these glands to the growth-stimulating effect of progesterone or testosterone. In such rats, furthermore, preparations of corticotropin, somatotropin, follicle-stimulating hormone, prolactin, pituitrin, pitressin, and chorionic gonadotropin all failed to restore responsiveness of sebaceous glands to stimulation by progesterone.

That some pituitary tropic principle is involved in the maintenance of growth responses of the preputial glands (*2*) and Harderian glands (*3*) has also been recognized. In the case of the preputial glands, this pituitary factor was recently claimed to be growth hormone (*4*), whereas, in the case of the Harderian glands, the less definite suggestion was made that growth hormone and possibly even thyrotropin were involved (*5*).

Fresh, anterior pituitary glands of hogs were dried with acetone and extracted with acetic acid by a method commonly used in the preparation of corticotropin (*6*). The remaining residue, from which corticotropin, thyrotropin, and somatotropin had been largely removed, was suspended in water, brought to *p*H 8.6 with dilute ammonium hydroxide, and filtered. The filtrate obtained was clarified with Dicalite, adjusted with dilute hydrochloric acid to *p*H 3.0, and cooled for 2 days at 8°C. The precipitate which formed was then collected, diluted with 0.5-percent phenol solution, and its *p*H adjusted to 6.8. Each milliliter of the resulting preparation (*7*) contained about 4.5 mg of nitrogen. Analysis of 0.2-ml aliquots of the final preparation for glucosamine by a method which could detect as little as 5 μg of the substance (*8*) failed to reveal its presence.

The results of experiments carried out with animals are summarized in Table 1. Hypophysectomized, castrated, male, Sprague-Dawley rats weighing between 100 and 150 g were used following postoperative recovery periods of 5 to 7 days. These animals were periodically weighed, maintained under uniform conditions, and fed *ad libitum* a diet consisting of rat food pellets, horse meat, whole-wheat bread, oranges, carrots, and canned milk. Tap water and saline were available for drinking. Hormones in the dosages indicated in Table 1 were injected subcutaneously into the lower abdominal region each day for 14 days. At the end of this period, the average cutaneous sebaceous gland volume in some rats was determined by the method of Haskin, Lasher, and Rothman (*9*), and the pairs of preputial and Harderian glands from each animal were dissected out and weighed.

It can be seen from Table 1 that sebotropic effects are not associated with the administration of either growth hormone or thyrotropin. It is also noteworthy that the response of Harderian glands to the sebotropic preparation does not require the simultaneous presence of a steroid hormone such as progesterone, as is the case with the responses of preputial (*10*) and cutaneous sebaceous glands.

The sebotropic preparation used in our experiments requires more precise characterization. There is a remote possibility that the observed effects on the growth responses of preputial, Harderian, and cutaneous sebaceous glands might have resulted from some unusual combined action of known pituitary hormones which could have been present in small amounts in our crude extract. There is also the possibility that this sebotropic extract might further be resolved into more specific components. Studies in these directions are in progress.

Besides affecting glands of the sebaceous type, our extract also appeared to influence hair follicle and surface epidermal growth and thickness. These effects, however, are based only on gross impressions, and detailed quantitation is still required.

Table 1. Effects of sebotropic preparation, somatotropin, and thyrotropin individually and in combination with progesterone on glands of sebaceous type in castrated, hypophysectomized rats. Injections were given for 14 days. Data are presented as means ± standard errors. The figures in parentheses in column 2 indicate the numbers of rats from each group which were used in the determination of data on cutaneous sebaceous glands.

Treatment	No. of Rats	Body wt. (% increase)	Preputial glands (wt. in mg/100 g body wt.)	Harderian glands (wt. in mg/100 g body wt.)	Cutaneous sebaceous glands ($mm^3 \times 10^{-5}$/100 g body wt.)
Control	21 (5)	3.0 ± 1.1	11.3 ± 0.6	82.5 ± 2.8	7.4 ± 0.8
Progesterone*	23 (8)	3.5 ± 0.9	12.2 ± 0.8	75.7 ± 2.0	4.8 ± 0.3
Sebotropic preparation†	10 (8)	8.1 ± 2.1	17.0 ± 1.7	101.5 ± 6.1	7.6 ± 1.3
Sebotropic preparation † plus progesterone*	11 (7)	3.1 ± 0.9	42.9 ± 3.1	108.8 ± 5.1	18.6 ± 3.5
Somatotropin‡	14 (6)	24.9 ± 1.6	13.3 ± 0.9	73.8 ± 2.3	6.9 ± 1.7
Somatotropin‡ plus progesterone*	21 (6)	27.1 ± 1.4	15.4 ± 1.4	75.2 ± 2.5	7.7 ± 0.5
Thyrotropin§	5	3.0 ± 1.1	11.0 ± 1.1	77.2 ± 2.6	
Thyrotropin§ plus progesterone*	18	5.8 ± 1.0	12.0 ± 1.0	82.9 ± 2.0	

* Progesterone, 1.0 mg daily (Vitarine Co. No. 4520 and Syntex Co. No. 195). † Sebotropic preparation, 0.25 ml daily. ‡ Somatotropin, 0.15 mg daily (Armour Lot Nos. R491132 and D728076). § Thyrotropin, 1 U.S.P. unit daily (Armour Lot No. M2105).

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References and Notes

1. N. Lasher, A. L. Lorincz, S. Rothman, *J. Invest. Dermatol.* 24, 499 (1955).
2. R. L. Noble and J. B. Collip, *Endocrinology* 29, 934, 943 (1941); H. Selye and E. Clarke, *Rev. can. biol.* 2, 319 (1943); B. Jacot and H. Selye, *Endocrinology* 50, 254 (1952); R. Kooij, *Arch. intern. pharmacodynamie* 94, 475 (1953); C. Huggins, F. M. Parsons, E. V. Jensen, *Endocrinology* 57, 25 (1955).
3. G. K. Smelser, *Anat. Record* 86, 41 (1943); E. E. Pochin, *Clin. Sci.* 5, 75 (1945); N. F. Boas and R. O. Scow, *Endocrinology* 55, 148 (1954); and N. F. Boas and R. W. Bates, *Endocrinology* 55, 601 (1954).
4. C. Huggins, F. M. Parsons, E. V. Jensen, *Endocrinology* 57, 25 (1955).
5. N. F. Boas and R. W. Bates, *ibid.* 55, 601 (1954).
6. E. B. Astwood *et al.*, *J. Am. Chem. Soc.* 73, 2969 (1951).
7. Kindly prepared and supplied by S. W. Hier and C. E. Graham of the Wilson Laboratories, Chicago, Ill.

8. The analysis for glucosamine was kindly carried out by D. M. Bergenstal of the department of medicine, University of Chicago. A modified Morgan-Elson method of analysis was used [W. T. J. Morgan and L. A. Elson, *Biochem. J. (London)* 28, 988 (1934)].
9. D. Haskin, N. Lasher, S. Rothman, *J. Invest. Dermatol.* 20, 207 (1953).
10. N. Lasher, in unpublished preliminary work done in the section of dermatology at the University of Chicago, had observed similar preputial gland growth-promoting activity of crude sebotropic preparations plus progesterone in mature, hypophysectomized, ovariectomized rats.

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Leukopenia: an Inherited Character in Mice

It has been shown from many hematological studies that pathological conditions, such as various types of anemia (*1*) and leukemia, are inheritable. Some of these conditions can also be experimentally produced in animals. In mice, it has been reported that blood *p*H (*2*) and leukocyte counts (*3*) are correlated with resistance to typhoid organisms and are partially correlated with longevity in rats (*4*). Inherited leukopenia, however, has not been reported in either human beings or animals.

During the course of a genetic study of variability in body size, it was discovered that the small strain of mice were leukopenic. This report presents the evidence on the inheritance of this condition (*5*).

The mouse stocks used in this study were the large and small strains and their hybrid generations of the F_1, F_2, first, second, and third backcrosses. Both large and small strains were originated by selection for body size and perpetuated by brother-sister mating. The small mice studied were produced in the 17th to 20th generations of inbreeding, and the large mice in the 3rd to 8th generations. The historical developments and some of the biological properties of both strains have been reported elsewhere (*6*).

All blood samples were obtained between 2 and 3 P.M. from the tails of mice 60 to 90 days old, except in the second backcross to the large mice. In this case, the samples were taken from mice of 1.0 to 1.5 years of age. Before the blood was taken, the mice were placed in a battery jar and heated for approximately 10 min under a 100-watt light. Each tail was washed with soap and warm water, and a prominent tail vein was incised to obtain blood for the white blood counts. The enumeration of white blood cells was carried out in duplicate in the conventional manner.

Duplicate blood smears were also prepared. They were fixed in absolute methyl alcohol and stained with Wright's and Giesma stains. The differential counts were performed by recording the cells in both longitudinal sides and diagonal cross of each slide. In most cases, 100 cells were counted in a slide. Degenerated cells were not counted.

The mean counts of leukocytes, agranulocytes, and granulocytes in each group of mice are given in Table 1. They were computed by pooling the counts in each group, since no significant difference between sexes was found. The total white-blood-cell counts averaged 8380 in the large strain and 2320 in the small strain. In other common inbred lines of mice (*7*), such low counts as were found in the small strain, have not been reported. Although the mean counts of both the agranulocytes and the granulocytes were low, the reduction in the latter was more severe than it was in the former type.

The total and differential counts of the F_1 and F_2 generations are closer to those found in the large strain than in the small strain. With the advance of backcrossing, the counts of the backcrosses to the small were shown to be approaching in magnitude those of the small strain, and the counts of the backcrosses to the large were shown to be approaching those of the large strain. But the rate of approach in the former groups was higher than that in the latter groups. Although the mean count in the B_{3S} appears to be even lower than that of its parental strain, the difference is probably not significant. Insofar as leukopenia in the small mice is concerned, the present evidence indicates that it is an inherited character and possibly determined by a small number of genetic factors.

It can be seen from the magnitudes of the standard deviations of the non-segregating groups that the variation of leukocyte counts seems to depend upon the mean count. However, as both environmental effects and technical errors tend to contribute greatly to the over-all variation in such a physiological trait, a more detailed determination of the relationship between the mean and distribution will require larger samples than we have used in the present case. Consequently, partitioning of the variances (σ^2) presently obtained into their different genetic and environmental components would not be valid. Nevertheless, since the means of the F_1 and F_2 are quite similar, their variances may be used to estimate roughly the relative magnitudes of effects of the genotype and environment. Assuming that the variance in the F_1 is an estimate of the environmental variation and that the variance in the F_2 is environmental variation confounded with genetic, the genetic contribution to the variation in the F_2 generation can be estimated by the formula

$$\frac{\sigma^2_{F_2} - \sigma^2_{F_1}}{\sigma^2_{F_2}}$$

In the present case, this estimate is approximately ½.

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Table 1. Mean total counts of leukocytes, agranulocytes, and granulocytes in each genotypic group of mice. Genotypes: S, small strain; L, large strain; B_{1S}, B_{2S}, and B_{3S} are the first, second, and third backcrosses to the small strain; B_{1L}, B_{2L}, and B_{3L} are the first, second, and third backcrosses to the large strain.

Genotype	Number of mice	Total number of leukocytes (No. × 10^3)		Agranulocytes (No. × 10^3)	Granulocytes (No. × 10^3)
		Mean	σ		
S	80	2.32	1.09	2.04	0.28
L	39	8.38	2.38	6.75	1.63
F_1	32	7.13	1.36	5.58	1.55
F_2	30	6.20	1.92	4.92	1.28
B_{1S}	17	5.71	1.93	4.52	1.19
B_{2S}	20	2.42	0.97	2.21	0.21
B_{3S}	17	1.89	0.61	1.65	0.24
B_{1L}	19	6.48	1.88	4.52	1.96
B_{2L}	27	5.93	1.87		
B_{3L}	27	6.97	2.20	5.79	1.18

References and Notes

1. H. Grüneberg, *The Genetics of the Mouse* (Nijhoff, The Hague, ed. 2, 1952).
2. J. A. Weir, *J. Infectious Diseases* 84, 252 (1949).
3. J. W. Gowen, *ibid.* 73, 40 (1943).
4. C. Reich and W. F. Dunning, *Science* 93, 429 (1951).
5. I express thanks to J. Anthony for help in the total and differential blood counts and to Joan Muneta and Shirley Sims for help in the total blood count; this work was aided by grants C-1074(C5) and C-3108 from the National Cancer Institute, U.S. Public Health Service.
6. C. K. Chai, *Genetics* 41, 158 (1956).
7. E. S. Russell *et al.*, *Proc. Soc. Exptl. Biol. Med.* 78, 761 (1951).

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