They were to be smoked in the manner customary to each individual and the subject wrote his opinion on the envelope immediately following the completion of the smoking. He or she did not look at the previous classification on the envelope before smoking, so that the opinion on every cigarette was quite uninfluenced. In these experiments also, neither subjects nor distributors knew which cigarette contained glycerine and which contained diethylene glycol; as in the preceding experiments all the results were at hand before the experimenter knew the composition of the cigarettes. The data are summarized in Table II.

Our data give no indication that cigarettes can be classified consistently as to the irritating quality of the smoke by supposedly normal humans, although Flinn's report suggests patients with various afflictions due to smoking are able to judge differences in cigarettes similar in nature to ours. In many cases the same kind of cigarette was at one time called mild and at a subsequent period pronounced irritating by the same person. We believe, therefore, that a method for determining the irritating properties of cigarettes which relies solely upon the opinions of ordinary smokers cannot be considered reliable.

The smoke of these 3 types of cigarettes increases the acidity of water to an equal extent, as determined by exact pH tests.

9210

Response of Anterior Pituitary of Immature Castrated Rat to Testosterone and Related Compounds.*

J. M. WOLFE AND J. B. HAMILTON.

From the Departments of Anatomy, Vanderbilt University Medical School, Nashville, Tenn., and Albany Medical College, Union University, Albany, N. Y.

It has been reported that injections of extracts containing the male sex hormone prevented the post-castration increase in the size and number of the basophilic cells in the anterior hypophysis of the rat.[1, 2] In later studies, Nelson and Gallagher[3] studied the action

* These studies have been aided by grants made to Vanderbilt University Medical School by the Division of Medical Sciences of the Rockefeller Foundation and the Grants-in-Aid Committee of the National Research Council.

1 Reese, J. D., and McQueen-Williams, M., *Am. J. Physiol.*, 1932, **101**, 239.

2 Nelson, W. O., and Gallagher, T. F., *Anat. Rec.*, 1935, **64**, 129.

3 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

of synthetic male factors, androsterone and related compounds, in castrated female rats. Such injections prevented castration changes in the pituitary; they also state that these injections induced the same type of degranulation as had been obtained by the injection of oestrin. Below are presented studies on the action of testosterone and its related compounds, testosterone-acetate and testosterone-propionate on the anterior pituitary of castrated immature rats.

Twenty immature rats, mostly males, castrated immediately before injections were begun, received daily injections of 500 gamma of either testosterone,† testosterone-propionate, or testosterone-acetate for 10 days; the animals were sacrificed 24 hours following the final injection. Twenty-three littermates castrated and killed at

TABLE I.
Frequency Distribution Table Showing Level of Cell Types in Injected and Non-injected Castrated Immature Rats.

Intervals in %	Frequency Distribution Control	Experimental	Mean % Control	Experimental
Eosinophiles:			%	%
30.0-34.9	9	6		
35.0-39.9	11	10	M = 36.0	M = 37.0
40.0-44.9	3	4		
Basophiles: (Granular)				
0.0- 0.9		6		
1.0- 1.9		7		
2.0- 2.9		2		
3.0- 4.9		5		
5.0- 6.9	2		M = 11.0	M = 1.7
7.0- 8.9	5			
9.0-10.9	5			
11.0-12.9	5			
13.0-14.9	3			
15.0-16.9	3			
Basophiles: (Non-gran.)				
0.0- 1.9	3			
2.0- 3.9	8	1		
4.0- 5.9	10	7	M = 3.7	M = 6.4
6.0- 7.9	2	9		
8.0- 9.9		3		
Chromophobes:				
35.0-39.9	2			
40.0-44.9	3			
45.0-49.9	8	4	M = 49.3	M = 54.9
50.0-54.9	4	7		
55.0-59.9	6	5		
60.0-64.9		4		
Mean Pituitary Wt.	5.7	4.9		

† The testosterone and testosterone propionate were furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. Testosterone-acetate was furnished by the Ciba Corporation through the courtesy of Mr. Robert Mautner.

the same time as the injected rats were used as controls. The prostate and seminal vesicles were weighed together. Their mean combined weight in the injected rats was 663 mg., the range from 279 to 1,010 mg. The mean combined weight of these organs in the control rats was 40 mg. The mean pituitary weight of the castrated control rats was slightly greater than that of the injected rats (Table I).

The pituitaries of all rats were studied histologically; cell counts were made on all sections studied. In the castrated control rats there was a marked increase in the size and the number of the basophilic cells (Table I). A majority of these cells were well packed with granules; the negative image of the Golgi apparatus was quite prominent, usually being more or less circular in form and situated between the nucleus and the cell membrane. A lesser number of these large basophiles appeared degranulated; in some of these cells small yellow-orange bodies, thought to be mitochondria, were observed in the blue cytoplasm. The negative image of the Golgi apparatus was large and prominent. The mean level of the eosinophiles was 36%; the range of variation was slight (Table I). The mean level of the chromophobes was 49.3% (Table I). Mitoses were found in the chromophobes and occasionally in the eosinophiles; counts revealed there was a mean of 5 per section in the chromophobes and 1 per section in the eosinophiles.

Injection of either testosterone, testosterone-acetate or testosterone-propionate suppressed the increase in the number and size of the basophiles which occurred after castration in the control rats. Furthermore, there was evident a marked degranulation in the basophiles in the injected rats; the mean level of granular basophiles was reduced to 1.7%. Degranulated basophiles were quite abundant; most of these were moderate in size. In some, small clumps of basophilic granules were found. Small yellowish-orange bodies, thought to be mitochondria, were often observed in the blue cytoplasm. The negative image of the Golgi apparatus was usually observed in these cells. Testosterone-propionate was most effective in preventing castration changes and inducing basophilic degranulation.

We were unable to detect any constant changes in the eosinophiles of the injected rats; the levels of these cells in the control and experimental groups were almost identical. In some few instances in the injected group a few eosinophiles were observed which appeared to present a reduction of granules but this finding was quite inconstant. It is possible that if we had injected larger amounts, changes might have been induced in the eosinophiles.

The percentages of the chromophobes in the injected group were slightly higher than in the castrate-control group. This was due, however, to the fact that the relative levels of the basophiles were much lower in the injected rat. As a whole, the chromophobes in the injected rats were normal in appearance; infrequently large, swollen cells with a hypertrophied negative image of the Golgi apparatus were observed. Counts of the mitotic figures revealed that they occurred in practically the same numbers as in the castrated control rats.

These studies indicate that the action of testosterone and related compounds on the anterior lobe differs from that of oestrogenic substances. The latter induce a weight increase in the pituitary, degranulation of many and often all of the basophiles and degranulation of a lesser number of eosinophiles. The chromophilic cells are reduced in percentage; the chromophobes are increased.[4] On the other hand, we have been able to detect only degranulation of the basophiles after injection of the male hormone; there is the possibility that injection of large amounts would result in changes in the anterior lobe similar to those induced by oestrin. However, other evidence indicates that the pituitary response to oestrin and to testosterone differs. It has been reported that injection of testosterone-acetate simultaneously with oestrin in either normal or castrated female rats partially suppressed the capacity of oestrin to induce pituitary hypertrophy and degranulation of the chromophilic cells.[5] These findings seem of considerable significance in view of the report[6] that injection of large amounts of the anterior pituitary-like factor (A.P.L.), simultaneously with oestrin, into either normal male or female rats partially suppressed the action of oestrin on the anterior lobe in the same manner as did the male hormone. However, A.P.L., injected simultaneously with oestrin in castrated female rats, failed to modify the response of the anterior lobe to oestrin. It is suggested that the ovary, under the stimulating influence of A.P.L., produced some factor that exerted the same suppressing effect on the action of oestrin on the anterior pituitary as did the male hormone. It is impossible to say whether this substance was actually the male hormone. However, this possibility is rendered more tenable by the finding that transplanted ovaries in castrated male mice were able to maintain the accessory reproductive organs.[7]

4 Wolfe, J. M., and Chadwick, C. S., *Endocrinology*, 1936, **20**, 503.

5 Wolfe, J. M., and Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 55 (Suppl.).

6 Wolfe, J. M., *Anat. Rec.* (in press).

7 Hill, R. T., and Gardner, W. U., *Anat. Rec.*, 1936, **64**, 21.