



Mitotic activity in the anterior pituitary of immature thyroidectomized rats
by C William Reiquam

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Zoology
Montana State University
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Abstract:

Most workers are agreed that there is an inter-relationship between the pituitary body and other hormone-producing organs of the body. This study attempts to show some of the trends in cellular changes in the anterior lobe of the immature rat pituitary resulting from thyroidectomy.

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It appears evident from this collection of data that the great majority of chromatophobes, basophiles and acidophiles in all the animals used in this study were arrested in the metaphase of their mitotic cycle at the time of killing.

Correlations of data from this study and findings of other workers presents a problem because of the lack of sufficient data. Various problems of pituitary body functions have been studied but under such a variety of conditions that the total collection of data is not sufficient to be conclusive in most cases.

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OF IMMATURE THYROIDECTOMIZED RATS

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ABSTRACT

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INTRODUCTION

According to Severinghaus (1933) early workers on the anterior lobes of the pituitary were interested mainly in the anatomy and the unique embryology of the structure. Early studies of the pituitary by Flesch (1884) and Schönnemann (1892) indicated the presence of the three well established cell types: acidophiles, basophiles and chromatophobes. Most of the succeeding studies have been concerned with the variations of these cells as to number, shape, granules, and position in relation to disturbed physiological functions. Still more recently experimentation has been on the study of these organ components in relation to the endocrine functions of the pituitary.

Pituitary histology and cytology have been investigated most commonly after splenectomy, castration and thyroidectomy. Pomerat (1941) in his study of rats castrated between the ages of 21 and 44 days, found that castration increases mitotic activity in the basophile cells of the anterior lobe, the rate rising proportionally as the interval between castration and autopsy increases. He also reports that the number of dividing acidophiles does not change significantly as a result of castration, while 10-day post-castrates show reduction in mitotic rate among chromatophobes.

Guyer and Claus (1937) report much the same structural and physiological changes in anterior lobe - increased and vacuolated basophiles and increased potency of transplanted substance in stimulating ovarian size and sexual precocity - occur in cancerous individuals as those following castration in either male or female.

In the case of splenectomy, Edwards and Wright (1937) found that

in animals killed 14 days after operation there appeared to be numerous mitotic activity in the basophiles.

Wolfe (1938) and his coworkers have studied the pituitary changes induced by variations in hormone supply. Their reports indicate frequent mitosis among acidophile and chromatophobe cells of the anterior lobe.

Most observers are agreed that thyroidectomy affects the pituitary. Studies made by Bryant (1930) indicate a great reduction in the number of acidophiles in the thyroidectomized rabbit. Also he reports a marked hypertrophy of chromatophobes and a loss of differential character in basophiles.

Investigations made on the pituitary have been made primarily on the rat though Bryant's (1930) work on the rabbit, Dawson and Friedgood's (1938) differentiation of two classes of acidophiles in the female rabbit and cat, Dawson's (1940) work on the African lung fish, and Hartman (1946) and coworkers' very recent study of the dog pituitary with special reference to a fourth cell type, all indicate a wide range of study in pituitary functions. In addition, pituitary studies on the guinea pig by Kirkman (1937), on the normal fowl from embryo to old age by Payne (1946), and data collected by Smith and MacDowell (1930) on a hereditary anterior pituitary deficiency in the mouse, indicate some other of the more important contributions to the field of pituitary body study.

General correlations of these data would seem impractical now because of the relative shortage of information on each form studied. Too, in studies as these, variations in species may demonstrate differences in the histological picture, the handling of the animals, or modifications of their natural habitats may modify the end result. According to McClung

(1929) it has been found in the case of the albino rat that if animals are gentled they exhibit very different reactions from those which have not been subjected to this preliminary training. While these differences are apparently largely physiological, they must have structural bases.

Thus only through the accumulation of a quantity of carefully controlled experimentations and carefully collected data can the general relationships of mitotic activity of the anterior pituitary to various body functions be made predictable.

MATERIALS AND METHODS

The animals under investigation were albino white rats of the Sprague-Dawley strain from the rat colony of the Department of Home Economics at Montana State College. The animals were fed on a diet of Purina dog chow. Both food and water were available constantly.

Twenty-seven immature female rats were used as experimentals. These were divided into two groups - those thyroidectomized at 14 days of age and those thyroidectomized at 28 days of age. Two to four rats of each group were sacrificed at 5-day intervals.

Thirteen controls were sacrificed at ages corresponding to the ages of the experimentals.

Thyroidectomy was accomplished by anesthetizing each rat with ether from a saturated wad of cotton suspended in a glass jar. Complete anesthetization commonly was accomplished in three to five minutes after which the animal was stretched out on the table and with the aid of rubber bands secured ventral side up. The neck region was bathed in alcohol and a longitudinal incision was made midway between the lower portion of the

jaw bone and the sternum. Lymph nodes, connective tissue and muscle were pushed aside to expose the trachea where the thyroid was found - one lobe on each side of the lower larynx with a ventral isthmus joining the two. Later, in rats found to be not completely thyroidectomized, the thyroid tissue remaining was a tag located dorsal to the trachea so that at the time of the operation it would have been practically impossible to detect without serious injury to the live animal. Parathyroids were left intact to protect the animal against tetany.

After thyroidectomy the animals were placed in cages. At first the difficulty in obtaining a constant temperature of 76° to 80°F. caused several rats to die. After this was corrected, no rats succumbed from the thyroidectomy. This survival, if the rats were kept at the correct temperature, held true for both the younger animals thyroidectomized at 14 days and those thyroidectomized at 28 days.

Those animals thyroidectomized at 14 days were returned to their mothers after the operation in order that their physiological activities might not be influenced by a diet factor until the normal weaning time at four weeks.

The animals were killed between 11 and 12 a.m. or between 3 and 4 p.m. (as indicated in tables I, II, III). This was done since many reports have stressed the variability of mitotic activity at different periods of the day (Blumenfeld, 1938), and these two periods may eliminate the possible factor of diurnal rhythm in mitosis.

All experimental animals were weighed at the time of thyroidectomy and again when sacrificed. The controls were weighed when sacrificed. Note

tables I, II, III.

All animals were killed by a blow on the head. After opening the brain case close to the base of the skull with a bone forcep and carefully removing the brain, the pituitaries (included anterior lobe usually with pars nervosa and pars intermedia) were removed and fixed in a sublimate formalin fixative. The fixative consisted of a saturated solution of corrosive sublimate in .9% physiological saline (90 parts) and formalin (10 parts).

The pituitaries were left in the fixative for 24 hours and then washed in 70% alcohol for 12 to 24 hours. Three or four changes of alcohol proved satisfactory. After complete washing, the glands were dehydrated with dioxane for four to five hours. Two changes of the dioxane, dehydrated with a small amount of calcium oxide was sufficient. After dehydration the glands were placed in a mixture containing two-thirds paraffin and one-third dioxane for two hours. Paraffin with a melting point of 49-51°C. gave the best results for this infiltration process. To complete infiltration the glands were placed in pure melted paraffin for another two hours. On completion of the process they were embedded in paraffin on watch glasses coated with a very thin layer of glycerin. Paraffin with a melting point of 55-57°C. proved best for embedding. Embedding on watch glasses proved superior to the "paper boat" method in that crystallization of the paraffin when immersed in ice water was minimized and orientation of the tissue was made easier.

All pituitaries were sectioned at four microns and with the exception of the 48-day and 43-day normals (sectioned coronally) were

sectioned in the frontal plan. Approximately every twenty-fifth section was taken and mounted serially.

All pituitaries were stained by the Dawson and Friedgood (1938) modification of Heidenhain's azan method for anterior pituitary. A few modifications were necessary, however. It was found that leaving slides in the aniline blue and orange G mixture for the suggested period, greatly over-stained the cells and blotted out any differentiation. Usually from one to three hours in the solution was sufficient. Controlled destaining in 95% alcohol was then usually necessary for best results. The variation in acceptance of differential staining from slide to slide and even from section to section made uniform staining difficult. Good results could be obtained only by careful control of each step in the staining process.

Tracheal material from the experimentals was removed at killing, fixed in Bruin's fixative for 24 hours, washed in three or four changes of 50% alcohol for 12 hours, and washed for a 24-48 hour period in several changes of 70% alcohol. Each trachea was dehydrated in dioxane for four to five hours, placed in melted paraffin for two to five hours and embedded in paraffin of 55-57°C. melting point. Representative sections were cut at 8 microns, placed on slides, and stained in Delafield's hematoxylin and eosin.

Observations were made with a 90X apochromat oil immersion objective. Light was furnished by a Spencer adjustable laboratory lamp.

Each dividing cell of approximately every 25th section of anterior lobe was counted in each experimental animal. The phase of mitosis and differential counts were also recorded.

The number of mitosis per section does not allow for accurate comparisons because of variation in planes at which the gland was sectioned and variations at different levels of the glands. By finding the area of the sections mitosis could be more accurately expressed in mitosis per square m.m. The area of a section was determined after measuring with a planimeter the area of the section projected at 35 linear magnifications.

Various calculations were made involving comparisons between experimentals and normals and comparisons between the two differently treated groups of experimentals. Comparisons of numbers of cells in each phase of mitosis was made between groups. Also studies were made of the percentages of cell types at specific periods after thyroidectomy.

OBSERVATIONS

A. General Considerations

The development of a technique in thyroidectomy is of interest. Several rats died within 24 hours after the operation from a paralysis beginning in the hind legs and spreading to the anterior extremities. At first this was thought to be a result of parathyroidectomy but upon adjusting the room temperature to a higher constant temperature, the rats survived.

Several rats were lost, too, in determining the best procedure for anesthetization. Occasionally throughout the entire period of thyroidectomizing the rats one would die after one or two minutes in the anesthetic. Others would require five or six minutes for complete anesthetization. Of those animals dying from either the subnormal temperature, the anesthesia or the operation itself, seven were males and only three females. Equal numbers of both sexes were used.

There appeared to be little difference in the healing capacities after the operation in either the two-week old or four-week old animals. The tracheae regions were healed on the fifth day and usually there was scarcely an indication of scar tissues between the 15th and 20th days. Sulfathiazole was sprinkled on the wound in some cases but no gross changes in healing time or in effectiveness of healing was noted.

As indicated in tables I, II and III, the normal rat weighed more than the thyroidectomized rat of a corresponding age. In the 28-day thyroidectomies the weight differences between experimentals and controls of the same age decrease as the time between thyroidectomy and autopsy increases. In the 14-day thyroidectomies the difference in weight between corresponding groups of experimentals and controls increases as the time between thyroidectomy and autopsy increases.

Experimental rats showing thyroid tissue upon tracheal observations varied in the amount present. In animal 3 nearly one-half a thyroid was found; considerable amount was also found in number 7; in rats 33, 29, 25, and 21 thyroid tissue of only a few cuboidal cells could be recognized. Those animals only partially thyroidectomized gained more weight between their operation time and autopsy than did those completely thyroidectomized.

Animals thyroidectomized at two weeks still had their eyes closed. It was noted that these animals were retarded in opening their eyes as compared to the controls of a corresponding age. The controls also had brighter eyes and showed signs of changes from the fine immature hair to adult hair before those of the thyroidectomized group. Several of these controls and experimentals were litter mates. These retardations correspond to the findings of Scov and Simpson (1945).

Table I.-Female rats thyroidectomized at 28 days.

Rat No.	Total age in days when killed	No. of sections counted	Total area in sq. m.m. of sections counted	Total mitosis	Total mitosis per sq. m.m.	Wt. in grams at operation	Wt. in grams when killed	Hour killed	Complete thyroidectomy	Vacuolated basophiles
20	33	7	5.72	20	3.5	52.2	59.7	4 pm	Yes	Many
19	33	12	7.19	67	9.3	54.5	65.2	4 pm	Yes	Few
10	33	5	3.48	23	6.6	70.5	79.2	11 am	Yes	None
16	38	7	13.13	698	53.2	78.7	100.7	11 am	Yes	None
17	38	5	5.33	469	88.0	47.5	62.8	4 pm	Yes	None
18	38	11	10.72	129	12.03	42.3	62.0	4 pm	Yes	Many
15	43	3	.788	50	63.4	74.5	101.0	11 am	Yes	None
14	43	6	9.0	800	88.8	76.0	99.5	11 am	Yes	Many
8	43	6	8.0	487	60.3	64.5	117.0	4 pm	Yes	None
7	48	8	17.6	1477	83.9	62.6	114.0	11 am	No	None
3	48	3	2.26	93	41.1	75.4	133.5	11 am	No	None
2	48	3	3.38	440	130.2	73.1	109.2	4 pm	Yes	None
1	48	14	9.52	563	59.1	70.0	96.5	11 am	Yes	None

Table II.--Female rats thyroidectomized at 14 days.

Rat No.	Total age in days when killed	No. of sections counted	Total area in sq. m.m. of sections counted	Total mitosis	Total mitosis per sq. m.m.	Wt. in grams at operation	Wt. in grams when killed	Hour killed	Complete thyroidectomy	Large vacuolated Basophiles
35	19	10	2.88	1	.33	29.0	33.5	4 pm	Yes	None
34	19	8	2.84	1	.35	23.7	27.5	4 pm	Yes	None
33	19	5	2.62	6	2.3	28.5	47.5	4 pm	No	Few
32	24	4	1.74	33	18.9	24.5	32.5	4 pm	Yes	None
31	24	16	4.40	2	.45	28.0	52.0	4 pm	Yes	None
30	24	7	3.03	None	None	27.6	52.5	11 am	Yes	None
29	29	5	4.36	143	33.0	28.5	57.0	11 am	No	Many
28	29	5	3.33	100	30.0	27.0	52.5	11 am	Yes	Many
27	29	4	3.8	153	40.3	30.0	55.5	11 am	Yes	None
25	29	6	5.7	148	26.0	23.5	45.0	11 am	No	Few
26	34	3	4.59	6	1.3	25.0	53.0	11 am	Yes	Many
24	34	4	5.42	90	16.6	28.5	54.0	11 am	Yes	Few
22	34	6	7.92	12	2.7	26.0	51.0	11 am	Yes	Few
21	34	5	4.50	1	-	25.0	60.0	11 am	No	Many

Table III.-Controls.

Ret No.	Total age in days when killed	No. of sections counted	Total area in sq. m.m. of sections counted	Total mitosis	Total mitosis per sq. m.m.	Wt. in grams when killed	Hour killed	Complete thyroidectomy
19N	19	5	2.12	1	.47	44	11 am	Few
24N ¹	24	5	4.84	6	1.24	64	11 am	Very few
24N ²	24	3	2.48	45	18.2	62	11 am	None
29N ¹	29	4	4.43	145	33.4	73	11 am	None
29N ²	29	5	2.85	87	30.5	64	11 am	Few
33N ¹	33	5	5.06	18	3.6	85	11 am	Few
33N ²	33	6	3.37	36	10.7	87	11 am	None
34N	34	4	2.24	36	16.1	93	11 am	None
38N ¹	38	4	2.62	3	1.1	102	11 am	None
38N ²	38	5	3.97	3	3.3	94	11 am	None
43N	43	13	8.63	13	1.5	100	11 am	None
48N ¹	48	10	3.02	8	2.6	98	11 am	Few
48N ²	48	15	11.55	11	1.0	141	11 am	None

Table IV.-Differential Cell Count.

Rat. No.	Total No. of mitosis counted	% Acidophiles	% Basophiles	% Chromatophobes	Total age in days when sacrificed	Age in days when thyroid-ectomized
1	563	32	5	63	48	28
2	440	16	19	65	48	"
3	93	41	10	49	48	"
7	1477	14	7	79	48	"
8	487	19	2	79	43	"
10	23	35	13	52	33	"
14	800	29.66	.34	70	43	"
15	50	9		91	43	"
16	698	11	1	88	38	"
17	469	15	1	84	38	"
18	129	47	7	46	38	"
19	68	65	3	32	33	"
20	20	15	20	65	33	"
21	12	50		50	34	14
22	1		1 cell		34	"
24	90	33	3	64	34	"
25	148	48	3	49	29	"
26	6	83		17	34	"
27	153	31		69	29	"
28	100	48	1	51	29	"
29	143	31	2	67	29	"
30	None	-	-	-	24	"
31	2	None	None	2 cells	24	"
32	33	33	9	58	24	"
33	5	80		20	19	"
34	1	-	-	1 cell	19	"
35	1	-	-	1 cell	19	"
19N ¹	1		1 cell	1 cell	19	
24N ¹	6	18		82	24	
24N ²	45	9	1 cell	92	24	
29N ¹	145	19		81	29	
29N ²	87	43		57	29	
33N ¹	18	67		33	33	
33N ²	36	54		46	33	
34N	36	44	1 cell	56	34	
38N ¹	3	1 cell		2 cells	38	
38N ²	3	1 cell		2 cells	38	
43N	13	33		67	43	
48N ¹	8	50		50	48	
48N ²	11	45		55	48	

B. Effect of Thyroidectomy

In this study a total of 6000 mitosis was counted in 27 experimental animals. This count included only those cells in the anterior lobe. Four and four-tenths per cent of these 6000 mitosis occurred in the basophiles, 22.9% in the acidophiles and 72.7% in the chromatophobes. The distribution of mitotic levels in basophiles showed very little activity beyond the metaphase where 70% of the mitosis was stopped at the time of killing. In the dividing acidophiles were the following mitotic levels: 23% in prophase; 67% in metaphase; 8.5% in anaphase, and 1.5% in telephase. As in the acidophiles, the chromatophobe activity was arrested mostly at metaphase with 87.5% of the divisions at this stage. Prophase claimed nearly 10% and as may be seen in figure 1, anaphase and telephase accounted for about 2%.

Of the 6000 mitosis counted some 695 were found in the fourteen animals thyroidectomized at 14 days of age. Fifty-nine and one-tenth per cent of these cells were chromatophobes which were found mostly in the metaphase stage of division. The acidophiles were represented by 39% of the total with 25% in prophase and 76% in metaphase. Only 10 dividing basophiles were found and all but one were in the metaphase of division.

A similar picture was indicated in the thirteen animals thyroidectomized at 28 days. By far the greatest mitotic activity (table IV) was found in the chromatophobes with 76.4% of the 5314 dividing cells. Eighteen and nine-tenths per cent of these dividing cells were acidophiles while 4.7% were basophiles. The chromatophobes (table IX) showed 9.1% of their mitosis in prophase; 87.6% in metaphase and the remainder in anaphase and

telephase. The acidophiles (table V) showed more cells in metaphase than any other stage with 75.6%, while 24% were found in prophase. Finally 28.6% of the basophiles (table VII) were in prophase, and 71% in metaphase.

Huge basophiles with numerous and sometimes very large vacuoles were not uncommon among the thyroidectomized animals especially those animals thyroidectomized at 14 days and sacrificed on the 10th or 15th day. No vacuoles were ever found in dividing basophiles. A few vacuolated basophiles also appeared in the 24, 29, 33, and 48-day normals. (Tables I, II, III).

In the normals from 19-32 days of age only 18% of the total mitosis was found in the acidophiles; 81% was found in the chromatophobes. Those normals from 33-48 days showed 49% of the divisions among the chromatophobes and 51% were acidophiles. The majority of all the divisions in the normals were at the metaphase stage.

A comparison of the cell count variations in the anterior pituitary at given periods after thyroidectomy for both experimental groups and controls is indicated in figure 2. It indicates a steady increase in general mitosis for 28-day thyroidectomies with the corresponding normals showing a decrease and then leveling off at 15 and 20 days. Fourteen-day thyroidectomies indicate an increase in both experimentals and controls up to 15 days when both drop considerably.

The animals thyroidectomized at 14 days show more mitotic activity in the normals than in the experimentals at each interval of observation except at the 15-day period where about 2 mitosis per square m.m. more in the experimentals than in the normals are indicated (figure 2). Whether this relationship is significant or not cannot be ascertained from the data

at hand.

It is evident from figure 3 that the chromatophobe mitosis per square m.m. is considerably smaller in the animals operated upon at 14 days than those of the 28-day group. Yet the 24, 29 and 34-day normals present more chromatophobes per square m.m. than the normals of 38, 43 and 48-day. In the acidophiles we find the normals of 24, 29 and 34 days with more mitosis per square m.m. than the 38, 43 and 48-day groups. The period of greatest acidophile division is 15 days after thyroidectomy for those operated upon at 14 days while the animals thyroidectomized at 28 days display the least acidophile mitosis at the 15-day period and rise again to a maximum at 20 days. Basophile activity seems significant only in the group thyroidectomized at 28 days and rises to the maximum at 20 days after thyroidectomy.

Chromatophobes in mitosis represent 59.4% of the mitosis in the thyroidectomized animals. In the normals 62.6% of the mitosis were chromatophobes. Acidophiles represent 35.7% in experimentals with 38.5% in normals; basophiles claim 6.3% in the experimentals with only two mitosis in the normals.

Animals 3 and 7 showing only partial thyroidectomy at 28 days, show considerably less divisions per square m.m. at the end of a 20-day period than those completely thyroidectomized. (Table I). Number 7 shows nearly twice as many mitosis than any other experimental but the area in which all counts was made is also large. As in the other animals, these partially thyroidectomized rats show chromatophobes in metaphase (table IX) as the predominant type of cell and stage of division.

Animals 21, 25, 29 and 33 (table II) also showing only partial

thyroidectomy were operated upon at 14 days of age. Number 21, 20 days after thyroidectomy shows only one cell in division. Fifteen days after thyroidectomy number 25 shows no great difference in activity from the others in that age group as was the case of number 29. Number 33 indicates a 14% increase in mitosis per square m.m. over its experimental mates sacrificed five days after thyroidectomy. This may not be significant, however, since only one mitotic division was counted in the two completely thyroidectomized animals and six in the partial thyroidectomy.

DISCUSSION

In attempting to correlate data such as that observed in this study, with so-called normal conditions and those related findings of other workers, one should be aware of the possible variations that might come about from disturbances other than those caused by thyroidectomy. Mitotic activity in relation to age should be borne in mind. Hunt (1942) states that in general it has been found that the older the animal the less frequent are the mitosis. Blumenthal (1940) suggests a correlation of food ingestion time as a factor in determining mitotic rate. The hour of killing may likewise be considered, though this factor was given special consideration in limiting the autopsies to two periods of the day. Neither period produced more or less mitosis than the other. Variations in the mitotic rate are also attributed to the various stages of the estrous cycle. Immature rats considered in the study eliminated this problem.

These conditions are only a few of the more evident factors of influence. Perhaps in thyroidectomy it is not the absence of the thyroid

Table V.-Classification of Acidophile Mitosis.

Rat No.	Total No. in mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when sacrificed	
1	182	65 36%	117 64%	None	None	48	Age = 28 days when thyroidectomized
2	70	4 6%	63 90%	1 1.33%	2 2.67%	48	
3	38	13 34%	23 61%	2 5%	None	48	
7	219	59 27%	147 67%	12 5.5%	1 .5%	48	
8	91	34 37%	55 60%	1 1.5%	1 1.5%	43	
10	8	1 12.5%	6 75%	1 12.5%	None	33	
14	238	33 14%	203 85%	2 1%	None	43	
15	3	1 33%	2 67%	None	None	43	
16	78	16 21%	60 77%	2 2%	None	38	
17	71	13 18%	56 79%	2 3%	None	38	
18	61	16 26%	45 74%	None	None	38	
19	44	7 16%	37 84%	None	None	33	
20	3	1 23%	2 67%	None	None	33	
21	6	2 23%	4 67%	None	None	34	Age = 14 days when thyroidectomized
22	1	None	1	None	None	34	
24	30	5 17%	24 80%	1 3%	None	34	
25	71	8 11%	60 85%	3 4%	None	29	
26	5	4 80%	1 20%	None	None	34	
27	48	16 33%	31 66%	1 1%	None	29	
28	48	7 15%	41 85%	None	None	29	
29	36	5 14%	31 86%	None	None	29	
30	None	None	None	None	None	24	
31	None	None	None	None	None	24	
32	19	11 58%	8 42%	None	None	24	
33	4	1 25%	3 75%	None	None	19	
34	None	None	None	None	None	19	
35	None	None	None	None	None	19	

Table VI.-Classification of Acidophile Mitosis.

Controls						
Rat No.	Total No. ♂ in mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when sacrificed
19N	None	None	None	None	None	19
21N ¹	5	2	3	"	"	24
		40%	60%	"	"	
24N ²	4	1	3	"	"	
		33%	67%			24
29N ¹	27	14	13	"	"	
		51%	49%			29
33N ¹	12	1	11	"	"	
		8%	92%			33
33N ²	19	4	15	"	"	
		21%	79%			33
34N	16	4	12	"	"	
		25%	75%			34
38N ²	1	None	1	None	None	38
38N ²	1	1	None	"	"	38
43N	4	1	3	"	"	
		25%	75%			43
48N ¹	4	3	1	"	"	
		75%	25%			48
48N ²	5	2	3	"	"	
		40%	60%			48

Table VII.-Classification of Basophile Mitosis.

Rat No.	Total No. β in Mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when sacrificed	
1	29	7 24%	22 76%	None	None	48	28 days = Aged when they were thyroidectom- ized.
2	86	21 25%	64 74%	1 1%	"	48	
3	6	3 50%	3 50%	None	"	48	
7	88	25 28%	57 65%	5 6%	1 1%	48	
8	9	2 23%	7 77%	None	None	43	
10	3	1 33%	2 67%	"	"	33	
14	1	None	1	"	"	43	
15	None	None	None	None	None	43	
16	9	4 44%	4 44%	1 12%	"	38	
17	5	1 20%	4 80%	None	"	38	
18	9	3 33%	6 67%	"	"	38	
19	2	None	2	"	"	33	
20	4	2 50%	2 50%	"	"	33	
21	None	None	None	None	None	34	14 days = Aged when they were thyroidectomized
22	"	"	"	"	"	34	
24	2	1	1	"	"	34	
25	4	None	4	"	"	29	
26	None	None	None	"	"	34	
27	"	"	"	"	"	29	
28	1	"	1	"	"	29	
29	2	"	2	"	"	29	
30	None	None	None	"	"	24	
31	"	"	"	"	"	24	
32	2	"	3	"	"	24	
33	None	"	None	"	"	19	
34	"	"	"	"	"	19	
35	"	4	"	"	"	19	

Table VIII.-Classification of Basophile Mitosis.
Controls

Rat No.	Total No. in mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when Sacrificed
19H	None	None	None	None	None	19
24H ¹	"	"	"	"	"	24
24H ²	1	"	1	"	"	24
29H ¹	None	"	None	"	"	29
29H ²	"	"	"	"	"	29
33H ¹	"	"	"	"	"	33
33H ²	"	"	"	"	"	33
34H	1	"	1	"	"	34
38H ¹	"	"	"	"	"	38
38H ²	"	"	"	"	"	38
43H	"	"	"	"	"	43
48H ¹	"	"	"	"	"	48
48H ²	"	"	"	"	"	48

Table IX.-Classification of Chromatophore Mitosis.

Rat.No.	Total No. C in Mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when sacrificed	
1	352	143 41%	204 58%	5 1%	None	48	28 days = Age when thyroidectomized
2	284	37 13%	241 85%	5 3%	1	48	
3	46	11 24%	31 67%	4 6%	None	48	
7	1170	90 7.5%	1034 89%	41 3.5%	5 .8	48	
8	386	60 13%	319 83%	7 4%	None	43	
10	12	4 33%	8 67%	None	"	33	
14	561	6 1%	544 97%	11 2%	"	43	
15	47	1 2%	45 96%	1 2%	"	43	
16	611	14 2.5%	585 96%	4 1.5%	"	38	
17	394	10 2.5%	381 97%	3 .5%	"	38	
18	60	16 27%	44 73%	None	"	38	
19	22	5 23%	17 77%	None	"	33	
20	13	5 38%	8 62%	None	"	33	
21	6	None	6 100%	None	None	34	14 days = Age when thyroidectomized
22	None	None	None	None	None	34	
24	58	4 7%	52 89%	2 4%	"	34	
25	73	4 5%	68 93%	1 2%	"	29	
26	1	None	1 100%	None	"	34	
27	105	1 1%	104 99%			29	
28	51	2 4%	49 96%			29	
29	95	13 14%	82 86%	None	None	29	
30	None	None	None	None	None	24	
31	2	1 50%	None	None	1 50%	24	
32	11	4 36%	7 64%	None	None	24	
33	1	None	1 100%	"	"	19	
34	1	1 100%	None	"	"	19	
35	1	None	None	None	100%	19	

Table X.-Classification of Chromatophobe Mitosis Controls

Rat No.	Total No. C in mitosis	Pro- phase	Meta- phase	Ana- phase	Tele- phase	Age in days when sacrificed
19N	1	None	1 100%	None	None	19
24N ¹	1	1 100%	None	None	None	24
24N ²	41	12 29%	29 71%	"	"	24
29N ¹	117	26 22%	90 77%	"	1 1%	29
29N ²	50	11 22%	39 78%	"	"	29
33N ¹	6	None	6 100%	"	"	33
33N ²	16	5 33%	11 61%	"	"	33
34N	19	1 5%	18 95%	"	"	34
38N ¹	1	1 cell	None	"	"	38
38N ²	2	None	2 100%	"	"	38
43N	9	2 23%	7 77%	"	"	43
48N ¹	4	1 25%	3 75%	"	"	48
48N ²	6	1 16.5%	4 67%	None	1 16.5%	48

that produces a mitotic variation in the pituitary but a multilateral interdependence of several organs, each contributing depressing and accelerating mitotic influences.

Hunt (1942) in trying to correlate estrous cycle with mitotic activity reports no mitosis in basophiles of either mature or immature rats. As shown in table VIII this study indicates the same results in immature rats. According to Severinghaus (1933) and Pomerat (1941) repeated researches have shown that the basophile are greatly increased in the anterior lobe of the castrated rat while Bryant (1930) in his study on adult rabbit pituitaries after thyroidectomy reports a decrease in the basophile count. Experimental evidence from Pomerat (1941) makes it reasonable to assume that any increase or decrease in basophiles may be accounted for by mitosis, i.e. disregarding cell transformations. The present report shows the number of basophile mitosis increasing to 5 per square m.m. in 48 day old rats 20 days after thyroidectomy. This indicates nearly a 500% increase over the normals. Yet only 10 mitoses are found in the 14 rats thyroidectomized at 14 days; rats thyroidectomized at 28 days show 4.7% of the total dividing cells are basophiles. This may be only another indication of increased general mitotic activity in this group rather than increased basophile activity, however.

Pomerat (1941) saw basophiles with rather large vacuole in long period castrates. Evidently this same type of cell was observed in the young thyroidectomies. The recognition of a few smaller vacuolated basophiles in the normals is consistent with general consensus that infrequently vacuolated basophiles are found in the normal pituitary.

Bryant's (1930) study on pituitary mitosis in adult rats sacrificed at periods of two to four months after thyroidectomy indicates a degeneration of hypertrophied chromatophobes. He adds, "mitoses are exceptional". The present study on immature rats indicates no degeneration of chromatophobes over their short interval of observation and the mitotic activity among the chromatophobes far outnumber division figures for either acidophiles or basophiles. Though no valid comparison may be made between Bryant's work and this report, a restatement of another worker's results presents some idea of related activities and may suggest possible correlations when enough data is produced.

In 28-day thyroidectomies the chromatophobes claimed 76.4% of the total mitosis. In the normals of the same age period only 49% of the total mitosis were chromatophobes. Thus we see a possible indication of stimulated chromatophobe mitosis. Perhaps this is at the expense of the acidophiles which drop from 51% in the normals to 18.9% in the experimentals. In 14-day thyroidectomies we find 59.1% of the total mitosis among the chromatophobes and in the non-thyroidectomized group of corresponding age we find 81% of the mitosis among the chromatophobe. Here we find an indication that thyroidectomy has depressed chromatophobe mitosis, at least for the short period of this experimentation. This depression may have an interrelationship with the increase in acidophile mitosis from 18% in the normals to 39% of the total mitosis in the corresponding experimentals.

If Bryant (1930) is correct in concluding that his noted specific reaction of the chromatophobe cells to thyroid deficiency indicates that

these cells represent a type of specific secretory element in the hypophysis, then it would seem that the rats thyroidectomized at 14 days were following the trend toward the indications of his observations of mitosis being exceptional in the rabbit pituitary two to four months after thyroidectomy.

The increase in chromatophobe mitosis in the 28-day thyroidectomies suggests a possible difference in sensitivity in the effect of physiological disturbances at different ages. There is no suggestion from the available data of an explanation for the complete reversal of the chromatophobe and acidophile activities in the two age groups.

Severinghaus (1933) states that several observers report a decrease in acidophiles after castration. This decrease may have little correlation with actual mitotic activity as recent workers (Wolfe, Severinghaus, Pomerat) suggest cell transformations to account for variations in cell populations rather than an increase or decrease in mitotic activity. The observations of this study on thyroidectomy indicate that the 14-day operatives and corresponding normals rise to a peak at 15 days in acidophile mitosis. Both drop again in 20 days. In contrast, the 28-day operatives are lowest at 15 days and rise again at 20. Whether these variations are results of thyroidectomy alone or the influence of thyroidectomy together with other internal and external factors manifesting themselves at the periods of observation, is beyond the scope of this study.

At the time of killing the mitotic cycle was stopped mostly at the metaphase stage in each type of cell. Pomerat (1941) found a differential sensitivity to colchicine in which the most sensitive chromatophobes were

arrested at the metaphase. Acidophiles were less affected and basophiles were found in all stages of the division cycle. Figure 1 illustrates the predominance of metaphase with prophase claiming most of the remaining cells in this study. It is difficult to suggest possible reasons for this occurrence from as limited observation as this. It appears unusual that each type of cell whether experimental or normal would arrest the majority of its mitotic cells at the metaphase regardless of the time between thyroidectomy and autopsy.

The two animals only partially thyroidectomized at 28 days show mitotic activity greater than that of the controls yet less than that of complete thyroidectomies. The presence of thyroid tissue was evidently primary in determining the extent of their division figures. In those animals thyroidectomized at 14 days which displayed only a few cells of thyroid tissue, the division count in one case was close to the normal while in the others the counts were consistent with those after thyroidectomy. This might indicate an inter-relationship of other factors upon thyroidectomy in accounting for mitotic changes in the pituitary.

Making valid conclusions or even pointing out indications of trends is difficult in a limited study. For example, in animals numbers 31 and 32 (table II) we find that the mitosis per square m.m. shows such a variation that to try to account for the difference between these two rats given the same treatment under the same known conditions, is difficult if not impossible. Other variations in the mitotic rate as between numbers 22, 24 and 26 (table II) or numbers 16 and 17 (table I) make correlation invalid between groups at five-day intervals after thyroidectomy. Because

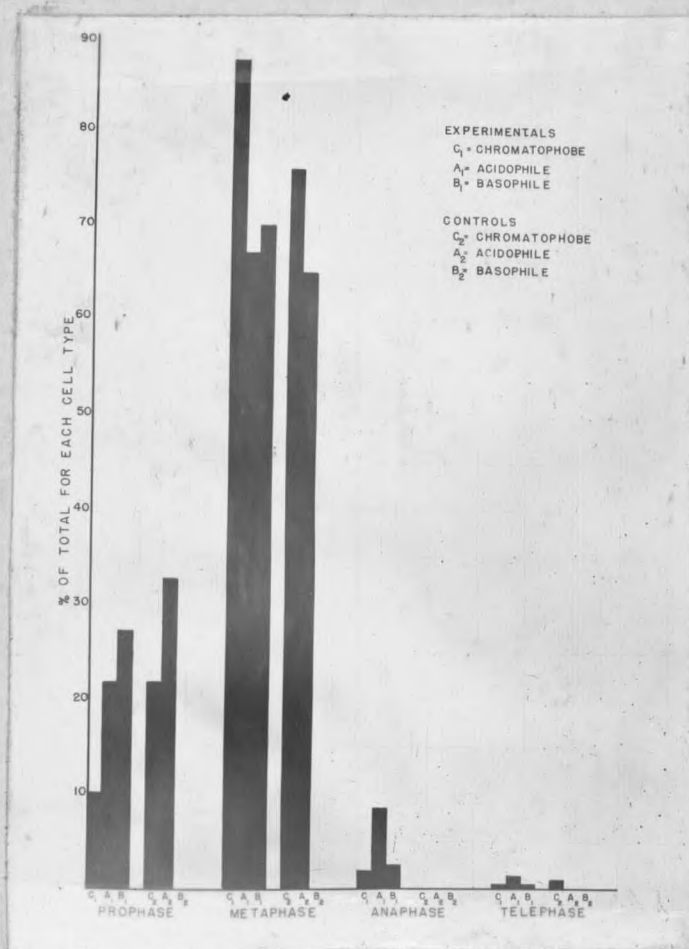


Figure 1. Total mitosis of the three cell types in 27 experimental animals and 12 controls. Six thousand dividing cells were found in the experimentals; about 340 were found in the controls.

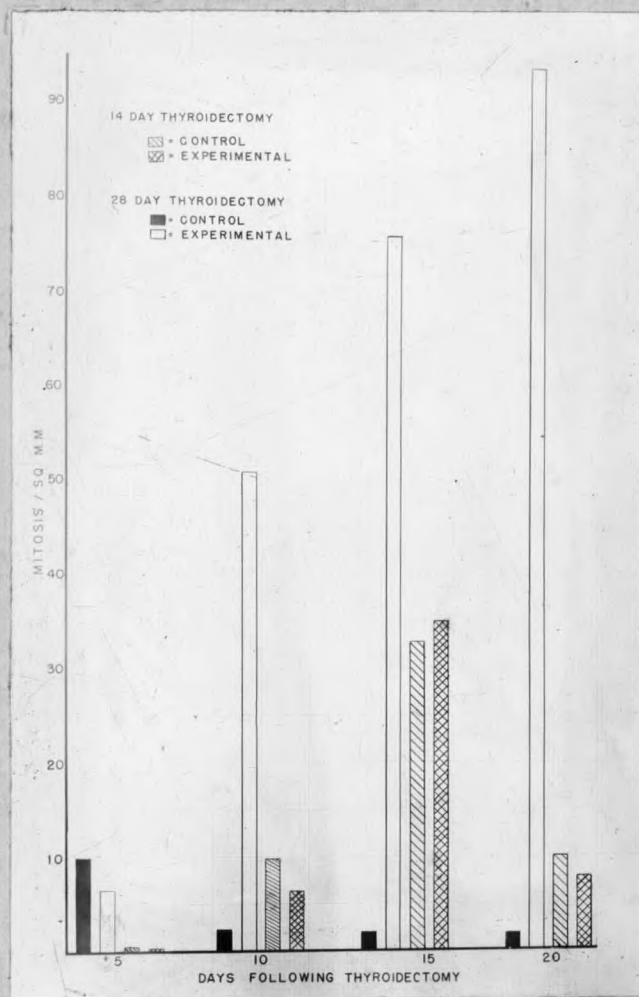


Figure 2. Mitosis per square m.m. for each cell type in two groups of animals thyroidectomized at 14 and 28 days and sacrificed at 5-day intervals up to 20 days.

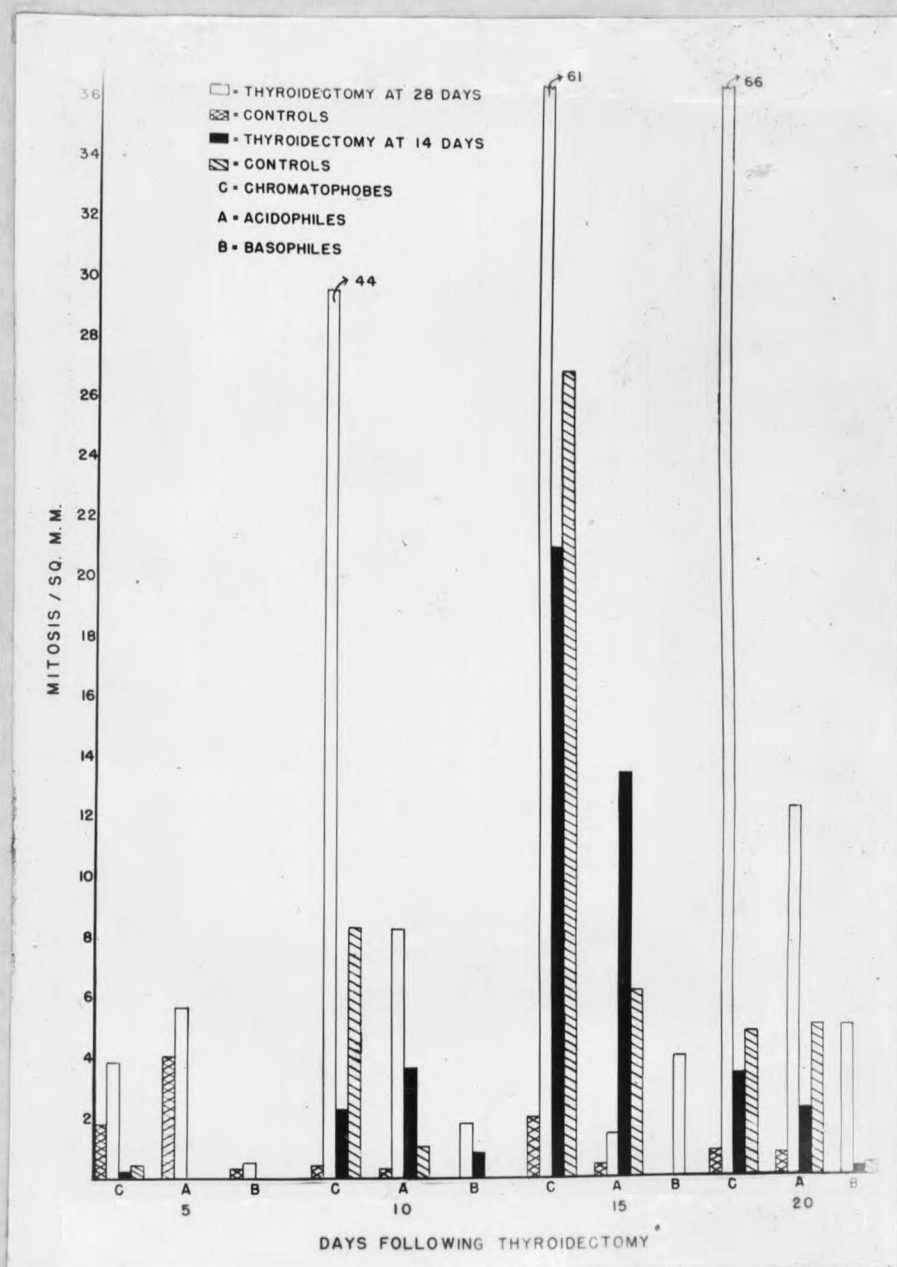


Figure 3. Total mitosis per square m.m. in rats thyroidectomized at 14 and 28 days as compared to their normals at 5-day intervals.

the numbers of animals in each group and the number of treatments are not extensive enough, it is impractical to give more than the possibility of certain trends or indications.

CONCLUSIONS

1. Thyroidectomy of rats at 28 days shows general increase (in terms of mitosis per square m.m.) in pituitary mitotic activity over the normal rate. The total percentage of chromatophobes in the operatives is increased over the normals while the mitotic acidophiles and basophiles are greatly decreased after thyroidectomy.
2. Thyroidectomy of rats at 14 days indicates little variation from normal activity of the anterior lobe of the pituitary up to 20 days after thyroidectomy. Both rise through the 15 days after thyroidectomy observation and drop again at the 20-day period.
3. Acidophiles reach a peak of mitotic activity 15 days after thyroidectomy at 14 days. Twenty-eight day operatives show their lowest acidophile mitosis in 15 days and rise to the peak again at the 20 days after thyroidectomy observation.
4. Data accumulated in this study indicates that the great majority of chromatophobes, basophiles and acidophiles in both experimentals and controls were arrested at the metaphase of the division cycle at autopsy.
5. A general inter-relationship between thyroid and pituitary is indicated. Since structure is a prerequisite of function the changes in pituitary structure in this study are indicative of a modification or variation of pituitary component functions in relation to thyroid deficiency.

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