relative to PM4.

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*GROWTH RESPONSES OF OPPOSITE SIGN AMONG DIFFERENT NEURON TYPES EXPOSED TO THYROID HORMONE**

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Read before the Academy, April 23, 1951

Introduction.—The metamorphic changes transforming the tissues of the tadpole into those of the mature frog have been shown to be under the "control" of the thyroid hormone. That is, they fail to occur, if the hormone is absent. However, it is becoming increasingly clear that "control" does not mean the determination of the specific character of the ensuing changes, but refers merely to the reactivation and further sustenance of different chains of morphogenetic events, temporarily arrested in the larval stage, then continuing in each tissue reacting according to its own characteristic properties. Hormone action does not initiate heterogeneity in homogeneous tissues; it merely leads to the realization, including visualization, of latent differences based on pre-existing heterogeneity. The mosaic of terminal hormone effects is anticipated by a corresponding latent mosaic of differential susceptibility and response among the various reacting tissues (Weiss, 1924). In brief, the morphogenetic action of a hormone is not too unlike the action of the photographic developer in

bringing out the latent picture on an exposed plate.

It is less clear whether tissues react essentially as units, perhaps as a result of regional vascular or metabolic changes, or whether each cell responds separately as an individual. This problem has broad significance particularly in the case of the nervous system. Many behavior patterns, e.g., mating behavior, are dependent on sex hormones (see Beach, 1950) much in the same way as amphibian metamorphosis depends on thyroid. The fact that metamorphosis is accompanied by profound behavioral changes makes the parallel even closer. Yet the neurological basis of these hormonal effects on behavior is still quite obscure. It is reasonable to

TABLE 1

NUCLEAR SIZE OF MAUTHNER'S CELLS AFTER UNILATERAL TRANSECTION OF SPINAL CORD

CASE	AGE AT OPERATION	STAGE	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS OF M-CELLS IN UNITS[a] EXPERIMENTAL	CONTROL
SA7^{I}	33	V	5	9 × 7	9 × 6
SA7^{II}	31	IV	5	10 × 7	11 × 6
SA8	31	IV	7	12 × 10	12 × 7
SA1	22	II	7	11 × 6	9 × 7
SA5	33	V	8	12 × 8	13 × 9
SA3^{I}	43	VII	9	11 × 7	11 × 7
SA3^{III}	43	VII	9	11 × 6	12 × 6
SA4	43	VII	9	12 × 7	10 × 7
SA6^{III}	31	IV	11	11 × 6	10 × 9
SA5^{I}	33	V	12	13 × 7	11 × 9
SA11^{I}	33	V	28	11 × 8	15 × 8
SA15^{II}	31	IV	28	14 × 7	12 × 7
SA12^{II}	31	IV	29	10 × 6	10 × 8
			Av.	11.3 × 7.1	11.1 × 7.4
				80.2	81.4

[a] 1 unit equals 1.75 μ. The final averages are given as products of the average largest and smallest diameters.

assume that it consists of the selective and differential response to hormone of certain neuron systems, in contradistinction to others, based on innate differences among the respective neurons. The following account presents a crucial demonstration of such critical differences among neuron types in their response to the metamorphosing hormone.

The Test Object: Mauthner's Cell (M-Cell).—The hind brain of the tadpole contains, at the level of the entrance of the vestibular nerve, a single pair of giant cell bodies, so-called Mauthner's cells (hereafter referred to as "M-cells") with large descending axons generally considered to be concerned with swimming movements of tail and trunk. For further details

about these cells, we may refer to two recent reviews (Stefanelli 1950, 1951). During the larval period, the M-cells can easily be told from all other neurons of the surroundings by their extreme size. After metamorphosis, evidently correlated with the loss of undulatory swimming,

TABLE 2

MITOTIC ACTIVITY IN NORMAL HIND BRAIN

a	b	c	f	g	h
CASE	AGE AT FIXATION	STAGE	MITOTIC COUNT HIND BRAIN	MITOTIC COUNT SPINAL CORD	MITOTIC INDEX, HIND BRAIN
NP2[6]	33	V	18	20	11.3
NP2[5]	33	V	12	5	7.5
NP2[9]	43	VII	20	27	11.1
NP2[10]	43	VII	17	15	9.4
NP3	64	XIII	19	..	9.0
				Av.	9.7
NP4[III]	91	XXII	2	..	0.8
NP4[IV]	93	XXIII	0	..	0.0

they undergo regression,[1] losing size until they can no longer be distinguished. Our experiments were designed to analyze the causes of this regression.

According to recent studies (summarized in Young, 1950 and Weiss,

TABLE 3

MITOTIC COUNT IN HIND BRAINS WITH GRAFTS OF SUBMAXILLARY GLAND

a	b	c	d	e	f	h
CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	MITOTIC COUNT	MITOTIC INDEX HIND BRAIN
			FRESH SUBMAXILLARY			
FS1	36	V	V	3	27	16.4
FS2	36	V	V	3	10	5.9
FS3	39	VI	VI	3	10	5.8
FS4	39	VI	VI	3	10	5.8
FS5	39	VI	VI	3	14	8.2
FS6	39	VI	VI	3	12	7.0
					Av.	8.2
			BOILED SUBMAXILLARY			
BS1	34–36	IV–V	V	3	22	13.8
BS2	34–36	IV–V	IV–V	3	13	8.1
BS3	34–36	IV–V	IV–V	3	37	25.2
BS5	34–36	IV–V	IV–V	3	14	8.7
BS6	34–36	IV–V	IV–V	3	26	16.2
					Av.	14.8
					Grand Av.	11.2

1950a), neuronal size depends on trophic interactions with the peripheral tissues, perhaps through functional reinforcement (Hamberger and Hydén, 1945; Edds, 1950). These effects seem to operate, however, upon a background of intrinsically different growth potentials among different kinds of

neurons, emphasizing the multifactorial character of size determination. M-cells, specifically, have been shown to acquire their excessive growth capacity early in development (Rossetti, 1947; Stefanelli, 1951), though remaining subject to size variation in accordance with the extent of their dendritic fields. Nothing was known, however, about the factors causing their metamorphic reduction. Considering that neurons deprived of peripheral connections atrophy (Weiss, Edds and Cavanaugh, 1945; Sanders and Young, 1946; Cavanaugh, 1951), the M-cell regression could have been ascribed to the shrinkage of their peripheral field in the course of tail resorption. Failing this explanation, the next plausible assumption would be that the M-cell body itself reacts adversely to the metamorphosing

TABLE 4

MITOTIC COUNT IN HIND BRAIN AND CORD WITH THYROID GRAFTS

CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	MITOTIC COUNT HIND BRAIN	MITOTIC COUNT SPINAL CORD	MITOTIC INDEX, HIND BRAIN
T35[2]	34	IV	V	3	107	134	67.0
T34[2]	34	IV	V	3	78	35	49.0
T33[2]	34	IV	V	3	106	104	66.1
T32[2]	34	IV	V	3	74	149	46.1
T36[2]	35	IV	V	4	91	117	56.9
T18[I]	37	V	X	4	71	58	44.0
T39	41	V	VI	3	110	44	65.0
T18[III]	43	VI	XX	5	42	40	23.5
T27[I]	44	VI	XXII	6	45	44	25.0
T27[II]	44	VI	XXI	6	36	37	20.0
T27[III]	44	VI	XXI	6	55	...	30.5
T5[II]	45	VI	XXI	7	55	69	30.0
T5[III]	45	VI	XXI	7	52	50	29.0
T41[2]	46	VII	VIII	3	133	145	76.0
T26[I]	49	VII	XXI	6	17	14	9.0
T3[III]	58	VII	XXI	15	13	15	6.3
T28[II]	60	XI	XXI	5	42	20	19.9
T25[II]	62	XI	XX	7	25	8	11.8
						Av.	37.5

hormone. Experiments were therefore undertaken in which the effects on the M-cell of loss of tail innervation and of thyroid hormone could be tested separately, and both prior to, hence uncomplicated by, the normal metamorphosis of the test animal.

Materials and Methods.—Tadpoles of Rana pipiens, Xenopus laevis and Pseudacris versicolor were used. The former were staged according to Taylor and Kollros.

To test the loss of tail innervation, either tailless larvae were produced by removing the tail bud in the embryo, or the tail was later amputated and kept from regenerating by periodic reamputations, or, lastly, the spinal cord was hemisected in the second trunk segment, the anterior stump be-

ing turned upward to prevent reconnection of the longitudinal tracts containing the M-axon. The success of the operation was later checked histologically.

The effect of thyroid hormone on the nerve cells was tested by the technique developed in this laboratory by Kollros (1943), implanting a hormone source in the vicinity of the hind brain. We used either fragments of rat thyroid gland or flakes of agar ca. 0.5 mm. across, soaked for 20 minutes in a 1:1000 solution of thyroxin (synthetic "Roche Organon"). In control experiments, fragments of parotid gland of rats were used. The grafts were placed either into the fourth ventricle itself or on top of the choroid plexus.

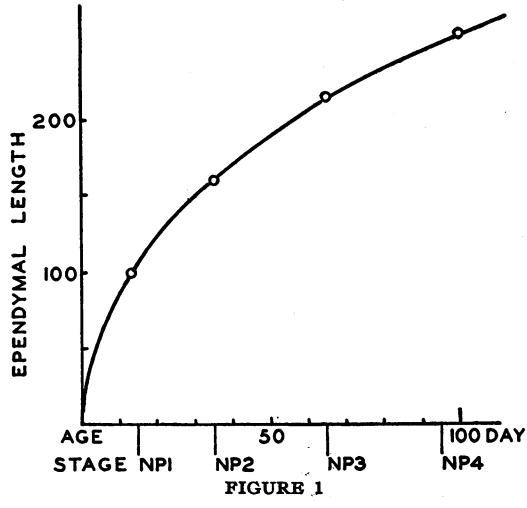

FIGURE 1

Growth curve of 4th ventricle at level of M-cells. Length of the ependymal lining in cross-sections of hind brain (in arbitrary units) plotted over larval age.

Most animals were sacrificed within the first week after the operation. They were fixed and stained either by Bodian's protargol technique or, for mitotic counts, by Heidenhain's iron hematoxylin. Brain cells were measured by projecting the slides on paper under high magnification, selecting in each preparation 100 cells at random from the vicinity of the M-cells and recording their nuclear diameters in equidistant size classes, as described previously for nerve fibers (Weiss, Edds and Cavanaugh, 1945; Cavanaugh, 1951). In M-cells, the longest and shortest nuclear diameters were measured, and in addition, the area of the largest cross-section of the whole M-cell body was determined by planimeter.

M-Cell Bodies after Amputation of M-Fibers.—Both tail amputation and high spinal cord severance deprived the M-fibers of a major portion of their

length and all the corresponding innervation area. Contrary to expectations, this trenchant reduction of peripheral load left the size of the cell bodies essentially unaffected.

Tail Amputation: Tails (15–25 mm. in length) were amputated in 6 tadpoles (28–36 mm. total length). The 12 M-cell nuclei of these animals (1, 3 and 6 days postoperative) measured 82.7 square units on an average, while 12 M-cells of normal control animals of comparable age and size (tail length 20–23 mm., total length 30–35 mm.) averaged 81.6 square units. Thyroid treatment, as will be shown later, produces its most marked effects on cell size during the first week. The fact that tail amputation had no effect whatsoever during the corresponding period is therefore significant.

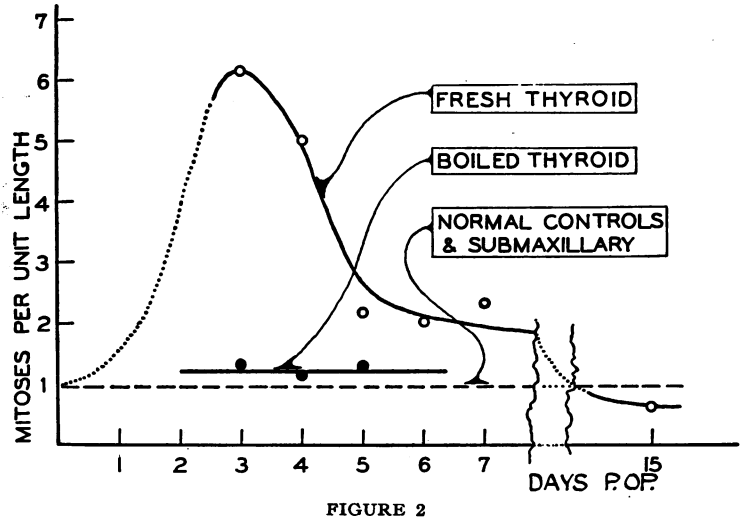

FIGURE 2

Mitotic index in hind brains of controls (broken line) and experimental cases (solid lines).

Spinal Cord Hemisection: Table 1 summarizes the measurements of M-cells in 13 animals preserved at various intervals after unilateral cord section. Of the two M-cells of each animal, one ("control") has an intact fiber with normal peripheral distribution, while the other ("experimental") has only a fiber stump. Yet both cells have remained about equal in size up to 29 days after the operation, averaging 81.4 and 80.2 square units, respectively, which is nearly the same value as in the preceding series.

In conclusion, amputation of a major portion of an M-fiber by either method has failed to cause regression of the cell body within the period of investigation. The Nissl pattern of the cell bodies was likewise found to

have remained unaltered. These facts seem to prove that, contrary to Bodian's (1947) assumption, the mere loss of axonal substance does not of itself cause neuronal atrophy. Such atrophy, where it occurs, is rather the result of the loss of functional connections (Weiss, 1950). In the present case this would lead one to suspect that the residual collateral connections of the M-fiber stump or newly formed connections at the level of transection may have been sufficient to preserve the integrity of the neuron.

At any rate, these results rule out the idea that M-cells regress after metamorphosis because the resorption of the tail has deprived them of innervation territory. We therefore turned to the second alternative, that of a direct response of the M-cell body to the metamorphosing hormone of the thyroid.

TABLE 5

MITOTIC COUNT IN HIND BRAIN WITH BOILED THYROID GRAFTS

a	b	c	d	e	f	g	h
					MITOTIC COUNT		
CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	HIND BRAIN	SPINAL CORD	MITOTIC INDEX, HIND BRAIN
TC42	52	IX	IX	4	30	20	19.0
TC43	55	X	XII	4	9	..	4.4
TC48	48	VIII	XI	3	36	22	18.0
TC46	48	VIII	VIII	3	7	..	3.5
TC49	58	XI	XIII	3	48	34	23.0
TC35	60	XI	XV	5	45	19	21.5
TC50	58	XI	XII	3	18	..	8.6
TC38	60	XI	XIII	5	27	32	12.8
TC32	60	XI	XV	5	15	12	7.2
TC40	60	XII	XIII	5	8	..	3.8
TC31	60	XI	XIII	5	34	10	16.2
TC30	60	XI	XIII	5	36	19	17.1
						Av.	13.0

Effect of Thyroid Grafts on Local Brain Growth.—A. Mitotic Effect: During metamorphosis, brain growth takes a marked spurt accompanied by intensified mitotic activity. Mitoses are confined to the ependymal layer lining the brain ventricles, and must therefore be referred to the number of nuclei in that layer rather than to the total number of brain cells. Being densely packed, the ependymal cells cannot be counted with the desired accuracy. But since the packing is uniform, the number of cells per unit length of ependymal lining is rather constant, hence mitotic rate can be expressed, for comparative purposes, as the number of mitoses per unit length of the ventricular contour. Thyroid grafts accelerate the local growth of the adjacent brain parts. In comparing their mitotic rates with those of normal tadpole brains, this expansion must be taken into account. For this purpose, the normal growth curve of the inner contour of the fourth ventricle in cross-section (at the M-cell level) was secured from a

series of measurements in normal samples stages (Fig. 1). From this curve the ependymal length, i.e., the denominator for the mitotic index, could then be read directly for any given age.

The mitotic data are summarized in tables 2–5 and figure 2. In view of the precipitate metamorphosis of their head region, animals with thyroid grafts were staged (column d) according to head criteria. In each case all mitoses in 10 alternate sections through the hind brain (column f) and 10 alternate sections through the spinal cord about 500 μ farther caudally (column g) were recorded. The total count amounts to 2335 mitoses.

Table 2 lists mitotic activity in normal controls for the age group used in the experiments. Omitting the last two stages (XXII and XXIII), during which mitotic activity has practically ceased as a result of maturation, an average mitotic index (stages V through XIII) of 9.7 is calculated. Table 3 gives the mitotic counts in animals of comparable ages which had received implants of fresh or boiled rat parotid glands in or near the fourth ventricle, 3 days after the operation, which is the time when the mitotic effect of thyroid grafts (see below) reaches its peak. The table shows that neither the operation as such nor the presence and discharges of a foreign gland of similar consistency as thryoid have any marked effect on mitotic activity, the average index (11.2) being not much above that of unoperated controls. This average of the pooled data has been used as control base in figure 2 (dotted line).

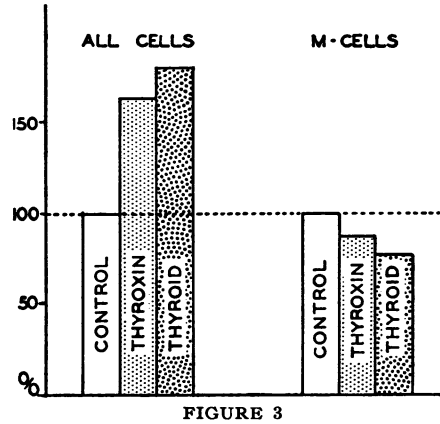

FIGURE 3

Relative volumes of non-M-cells (left) and M-cells (right), with controls set at 100%. Each non-M bar represents 1000 cells.

By contrast, table 4, summarizing the cases with thyroid grafts, shows a marked elevation of the mitotic index, particularly during the early days following the operation. On the third day postoperative, the mitotic index reaches an average (6 cases) of 61.5, which is about 600% of the normal and control indices. From this peak the index gradually declines (Fig. 2), until by day 15 it has returned to a normal level. Comparison of columns c and d shows that external features of the animals have advanced by several stages, indicating a regional acceleration of metamorphic events by the thyroid discharge from the grafts. Thus by the sixth day postoperative the various heads showed characters of regular stage XXI–XXII larvae. In view of the fact that the mitotic index at such late stages would normally

already be very low (less than 1; see table 2), the average of 22.2 for the seven animals counted on days 6 and 7 postoperative is still extremely high. It is difficult, therefore, to decide how much of the decline of the curve in figure 2 is due to the dissipation of the hormone source as a result of the disintegration and phagocytosis of the graft, and how much to the precipitate maturation, hence depletion of germinal cells, of the ependymal layer. But the fact that hormone delivery from the graft is of limited duration will be further supported below.

In the brains with thyroid grafts, an occasional extra-ependymal mitosis was observed, but these seem to have been true germinal cells which had simply been crowded out from their ventricular sites during division.

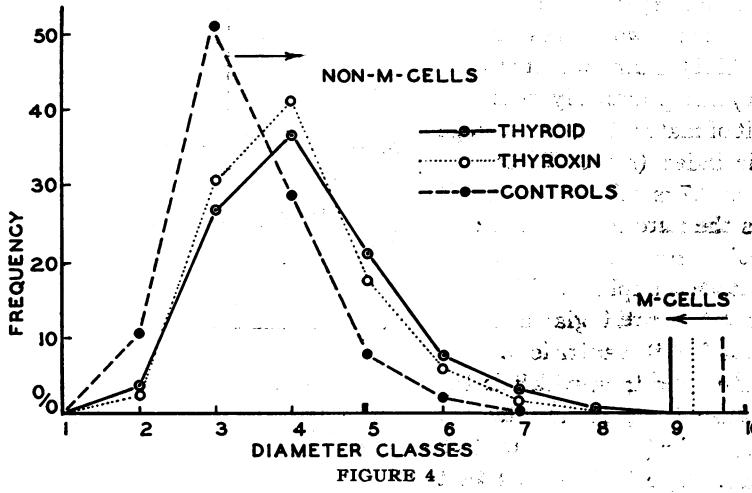

FIGURE 4

Histograms of nuclear diameters in 1000 non-M-cells each of control, thyroid and thyroxin cases. Mean diameters of M-cells for corresponding series are also indicated.

Table 5 gives the mitotic counts in the vicinity of grafts of boiled rat thyroid. The average index of 13.0 (Fig. 2) is only slightly higher than that of non-thyroid controls (tables 2 and 3). Even if the five ostensibly negative cases (TC32, 40, 43, 46 and 50) are omitted, the remaining average of 18.2 is still considerably lower than the average of 51.4 calculated from table 4 for cases with live thyroid grafts of comparable postoperative ages. Boiled thyroid thus seems to have had a perceptible, but weak, mitotic effect. Since it is generally recognized that thyroid hormone as such does not lose potency in boiling, the different effectiveness of fresh and boiled glands in our experiments must presumably be attributed to differences in the mode of release. Either the cells of the fresh gland continue to produce hormone even after grafting, or, more likely, the boiled gland has been so altered in

the process of boiling and coagulation that hormone will not be as readily released, especially since the boiled cells, in contrast to live ones, will not undergo autolysis.

The mitotic effect of thyroid grafts placed near the fourth ventricle spreads far down into the spinal cord. Table 4 reveals mitotic counts similar to those of the hind brain (column f) at a cord level 500 μ behind (column

TABLE 6

DISTRIBUTION OF NUCLEAR SIZES IN HIND BRAIN SAMPLES OF RANA PIPIENS

CASE	AGE OR STAGE (DAYS)	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS IN UNIT CLASSES 1	2	3	4	5	6	7	8	MEAN NUCLEAR DIAMETER OF M-CELLS, $d_m = \sqrt{d_{max} \cdot d_{min}}$	
						Controls						
NP3[II]	64		1	6	48	25	13	7	0	0	8.8	9.8
NP2[9]	43		0	11	41	39	6	2	1	0	..	..
NP2[10]	43		0	9	52	28	9	2	0	0	9.5	9.8
NP2[5]	33		0	1	45	38	12	3	1	0	9.9	9.4
NP2[II]	33		0	2	56	32	6	4	0	0	9.4	10.0
NP2[6]	33		1	8	54	27	9	0	1	0	7.9	8.8
NP2[III]	33		0	22	55	17	6	0	0	0	..	..
NP2[IV]	33		0	11	44	36	6	3	0	0	11.4	10.8
NP2[2]	31		1	22	53	19	5	0	0	0	10.0	..
NP2[1]	31		0	14	53	27	5	1	0	0	9.9	9.2
Av.	38		0.3	10.6	50.1	28.8	7.7	2.2	0.3	0	Av. = (see text)	9.7
Total relative volume 4822 cu. units.												
						Thyroid Grafts						
T39	38	3	0	1	14	35	33	12	4	1	7.3	9.4
T41	43	3	0	1	17	43	22	8	9	0	9.8	9.2
T6[I]	38	13	0	6	31	44	10	8	1	0	9.5	9.2
T8[I]	33	8	0	6	34	42	13	5	0	0	8.9	8.8
T6[II]	38	13	1	8	55	25	7	4	0	0	8.5	9.9
T7[II]	33	10	0	4	26	31	21	14	4	0	8.8	12.2
T3[II]	43	9	0	2	24	42	24	5	3	0	8.1	8.4
T18[I]	33	4	0	2	17	35	26	11	5	4	8.8	8.8
T5[II]	38	7	0	3	26	32	28	6	4	1	9.4	8.5
T36	31	4	0	1	25	40	28	5	1	0	8.0	7.8
Av.	37		0.1	3.4	26.9	36.9	21.2	7.8	3.1	0.6	Av. =	9.0
Total relative nuclear volume 8821 cu. units.												
						Thyroxin-Agar Grafts						
C5[II]	II–IV[a]	6	0	4	35	41	13	4	3	0	9.5	9.4
C5[I]	II–IV	6	0	1	22	42	24	10	1	0	8.8	9.4
C13[III]	VI–IX	7	0	4	32	35	16	9	4	0	8.1	11.4
C13[IV]	VI–IX	7	0	2	26	38	22	9	3	0	9.4	8.8
C1	V	4	0	2	24	53	18	3	0	0	8.5	7.9
C7	V	6	0	3	30	35	18	9	5	0	8.8	8.9
C12	VIII–IX	8	0	2	33	41	17	6	1	0	8.8	9.0
C3	V–VIII	6	0	3	33	42	14	6	1	1	10.5	10.5
C11[2]	V	4	0	3	36	43	16	2	0	0	10.2	7.9
C13	V–IX	4	0	3	38	40	15	4	0	0	9.9	8.9
Av.			0.0	2.7	30.9	41.0	17.3	6.2	1.8	0.1	Av. =	9.3
Total relative nuclear volume 7650 cu. units.												

[a] Indicates head stages at beginning and end of experiments.

g). To obtain a more complete picture of the spread, two normal larvae (33 days old; stage V) and two larvae with thyroid grafts (36 days, stage V, and 46 days, stage VII), the latter 3 days after implantation, were sectioned frontally, and all mitoses in alternate sections of the spinal cord were counted. The counts were, for the normal animals, 462 and 662 mitoses (average, 562), and for the ones with grafts, 1840 and 1180 (average, 1510)

mitoses, revealing a two- to threefold increase above normal. Since according to table 4, a higher increase was found at a far anterior cord level, it is evident that the effect grades off anteroposteriorly within the cord. Presumably it spreads by diffusion from the ventricle through the central canal.

B. Effect on Cell Size: While the ventricular cells near thyroid grafts grew and divided, the cells of the mantle, which had lost their mitotic capacity, just grew in size. Table 6 lists 10 cases with thyroid grafts, 10 cases with thyroxin-agar implants and 10 normal controls of comparable average ages; in each case, a random sample of 100 nuclei from the vicinity of the M-cells was taken and their distribution according to diameter classes recorded (the nuclei being nearly spherical). From these figures, the total relative volume of the 100 nuclei was calculated according to the formula:

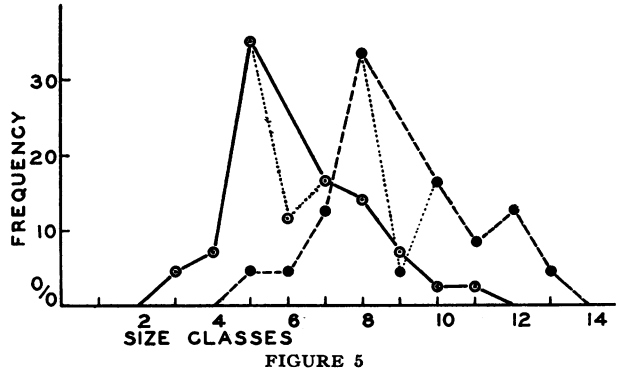

FIGURE 5

Histograms of M-cells (largest cross-sectional areas) in controls (- - ● - -) and thyroid- or thyroxin-grafted cases (—⊙—) of Rana pipiens.

$V = M_1 + 2^3M_2 + 3^3M_3 + 4^3M_4 + \ldots + n^3M_n$; where M_1, M_2, M_3, etc., represent the numbers of nuclei recorded in size classes 1, 2, 3, etc. The mean diameter of each ellipsoid nucleus of the M-cells was determined as the square root of the product of the longest times the shortest diameters. The relative volumes of the M-nuclei are given as the cubes of the mean diameters. The resultant changes in nuclear volumes are summarized in the graph, figure 3, as percentages of the normal control values. The nuclei of non-M-cells have gained 59% with thyroxin-agar grafts, and 83% with thyroid grafts, while the nuclei of the M-cells of the same animals have lost 12% and 19%, respectively. It is uncertain whether the lower effectiveness of the synthetic, as compared to the natural, product is a matter of potency or merely of dosage. But it is evident that thyroid hormone in either form affects the sizes of M-cells and of non-M-cells in opposite directions.

This antagonistic response of the two neuron types suggests some fundamental difference in their constitution. On the other hand, it is equally conceivable that the growth stimulation by thyroid is merely an inverse function of cell size, reversing its sign at a critical size level above that of the largest non-M-cells, but below that of the M-cells. This possibility, however, is ruled out by the fact that all size classes of non-M-cells are about equally enhanced. Figure 4 shows the histogram of nuclear diameters for the non-M-cells in the three groups of animals listed in table 6. It can be seen that the thyroid treatment has moved the whole nuclear population up by approximately one size class; the larger size classes are, if anything, favored. The graph also gives for comparison the mean diameter values of the M-cell nuclei, showing the displacement in the reverse direction from that of the histograms of non-M-nuclei.

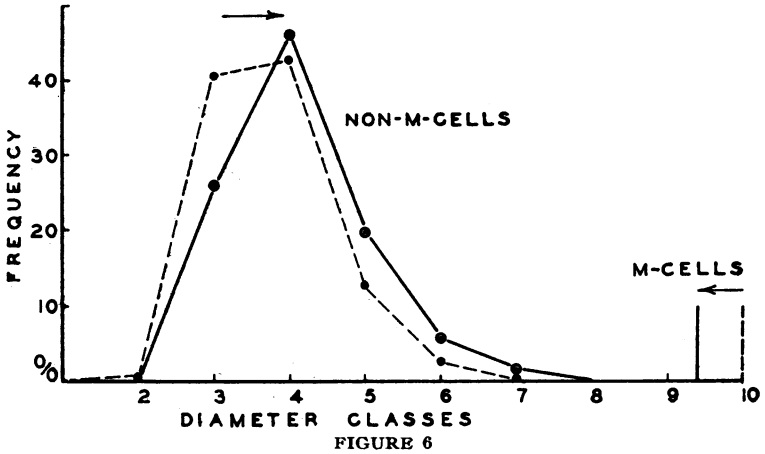

FIGURE 6

Histograms of non-M-nuclei in normal (- - ● - -) and thyroid-grafted cases (—⊙—) and record of the mean diameters of the M-nuclei (Xenopus laevis).

Histograms for the M-cells were made from planimetric measurements of the largest cross-sections of the cell bodies rather than from nuclear measurements; the greater accuracy of this method seemed indicated in view of the fact that the size of the sample of M-cells was only one-fiftieth of that of non-M-cells. Values for thyroid- and thyroxin-cases have been pooled. The resulting histograms for 24 normal (– – ● – –) and 43 experimental (— ⊙ —) cells are reproduced in figure 5; the size classes are on a different scale than those in figure 4. It is immediately evident from the graphs that thyroid treatment has affected all M-cells about equally, regardless of size, causing a shift of the whole population downward by about 3 size classes. These data prove clearly, particularly when taken in conjunction with those of figure 4, that the populations of M-cells and of non-M-cells

react as wholes in qualitatively different fashions, and that cell size as such is not a decisive factor.

The average volumes of M-cell bodies, computed from the planimetric data, were in the thyroid series approximately 60% of those of the control series. Comparing this value with the corresponding reduction of nuclear volumes to ca. 80% (Fig. 3), it appears that Bok's formula (1934), according to which the volumes of cell bodies of neurons vary as the squares of their nuclear volumes, holds roughly in the present case, too. It has likewise been found to apply to the atrophy of ganglion cells following peripheral disconnection (Cavanaugh, 1951).

All experiments described in the foregoing were done in Rana pipiens. An additional series with grafts of rat thyroid was carried out in the faster growing South African clawed toad, Xenopus laevis. The results, compiled

TABLE 7

DISTRIBUTION OF NUCLEAR SIZES IN HIND BRAIN SAMPLES OF XENOPUS LAEVIS

CASE	AGE AT FIXATION	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS IN UNIT CLASSES 1	2	3	4	5	6	7	8	MEAN NUCLEAR DIAMETER OF M-CELLS, $d_m = \sqrt{d_{max.} \cdot d_{min.}}$	
						Controls						
X1C	27		0	1	49	35	12	2	1	0	10.5	9.6
X4C	27		0	1	42	46	7	3	1	0	9.8	9.9
X1C1	27		0	1	43	42	11	3	0	0	10.5	10.5
X4CI	27		0	0	40	46	12	2	0	0	9.4	9.0
CX5	31		0	1	31	48	19	1	0	0	10.5	10.4
CX5-6	31		0	0	37	41	15	5	2	0	10.0	10.2
Av.			0	0.66	40.3	43.0	12.6	2.6	6.6	0	Av. =	10.0
						Thyroid Grafts						
XII2	20	5	0	0	37	47	12	3	1	0	8.8	9.9
X4[3]	22	5	0	0	38	42	12	7	1	0	8.8	9.2
X5-7	24	8	0	0	29	41	20	7	3	0	9.4	9.9
X5-6	24	8	0	0	26	41	19	12	2	0	8.9	9.4
X1II	17	10	0	0	15	55	25	4	1	0	8.9	
X4[3]	22	5	0	0	21	43	28	7	1	0	9.9	10.4
X1I	17	10	0	0	22	54	21	1	2	0	8.8	9.2
Av.			0	0	26.9	46.1	19.6	5.9	1.6	0	Av. =	9.4

in table 7 and figure 6, are quite similar to the previous ones. The thyroid hormone produced an increase in size of the non-M-cells, but reduction in the size of the M-cells (loss of M-nuclear volume: 17%). The histograms of the M-cells bodies based on the largest cross-sectional areas are given in figure 7, showing the shift of the thyroid-treated M-cells toward the smaller size classes.

Residual Effects.—The observed acceleration of proliferation and cell growth naturally is reflected in dimensional changes of the whole cross-section of the hind brain. While the processes thus far discussed augment the gray matter, concomitant outgrowth and, presumably caliber increase of nerve fibers from the enlarged brain parts add to the thickness of the white matter. The resulting over-all changes are summarized in the graphs,

figures 8–10. Figure 8 represents the normal "growth curve" of the cross-section of the hind brain during the larval period, based on planimetric measurements of the total cross-section, as well as of the gray matter only, in 23 normal controls. Figure 9 shows the increase in cross-sectional area in the medullae oblongatae containing thyroid grafts. As one can readily see, growth is initially accelerated, falling off by the 6th day, in general agreement with the detailed cell events discussed above. In figure 10, finally, the growth curves of the thyroid cases and of normal controls of comparable ages (taken from Fig. 8) are combined. These curves show quite conclusively that the thyroid-induced growth acceleration has been only temporary. After the initial rise, the curves for the experimental animals run strictly parallel to those for the controls. This means that the former retain their erstwhile advance, but otherwise continue to grow at a normal rate once the thyroid source has been exhausted. The action thus

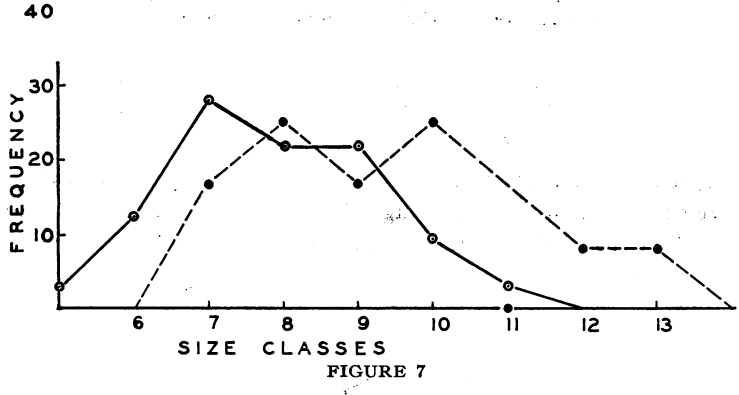

FIGURE 7

Histograms of the M-cell bodies in control (- - ● - -) and thyroid graft cases (Xenopus). Note opposite shift from that in figure 6.

is comparable to that of a single solid dose of hormone administered over a limited period, and the course of events clearly proved that the observed growth phenomena were direct, rather than secondary, consequences of the local hormone action.

Discussion.—The local hormone application in these experiments has but precipitated events which eventually would have taken place in the course of normal metamorphosis. In this regard, the results merely amplify earlier observations on circumscribed metamorphic changes in the vicinity of thyroid grafts (e.g., Hartwig, 1940; Kollros, 1943). In fact, Kollros, *et al.*, in a recent preliminary note (1950), report size increase in midbrain cells in response to thyroxin pellets implanted in to the cranial cavity, quite similar to the response of the non-M-cells of the hind brain in our own experiments.

The added significance of our results lies in the demonstration of the fact

that different neurons of the same locality, the M-cells and the non-M-cells, respond in wholly different ways, resulting in changes of opposite sign. Again, regression of M-cells amidst growing non-M-cells would have been

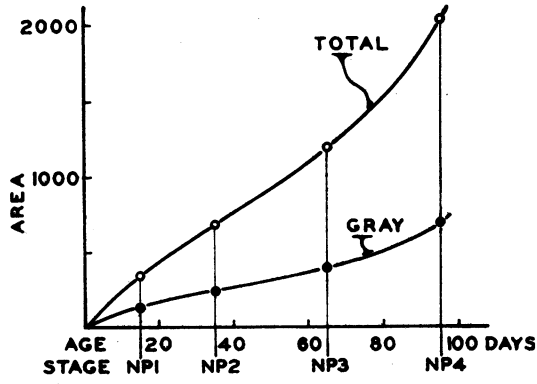

FIGURE 8

Cross-sectional increase (area in arbitrary units) of hind brain and its gray matter in normal larvae up to 100 days.

a normal feature of these brains during metamorphosis anyhow; but we now have proof that while the change as such is brought on by the thyroid hormone, the pattern of the change is predetermined in the responding cells,

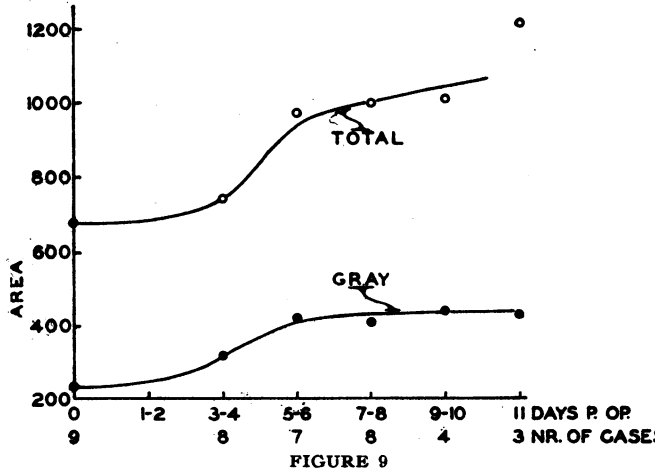

FIGURE 9

Growth of cross-section of hind brain following thyroid implantation at approximately stage NP2 of figure 8.

which evidently constitute a mosaic of quite diverse cell species. Thus, both the property of M-cells to respond to thyroid by involution, and the property of non-M-cells to respond to the same agent by growth, are but at-

tributes of the more distinctive constitutional differentials acquired by these various cell types previously in the course of embryonic differentiation. Having been dealt with comprehensively in some recent publications (Weiss, 1949, 1950), these problems need not be labored here further.

Two points, however, should be reemphasized. First, since the differently reacting cell types lie intimately intermingled, their differential response cannot be attributed to differential exposure to, and accessibility by, the hormonal pool. Second, since the non-M-cells of all sizes have been equally enhanced in their growth (figs. 4, 6), the degrowth of the M-cells

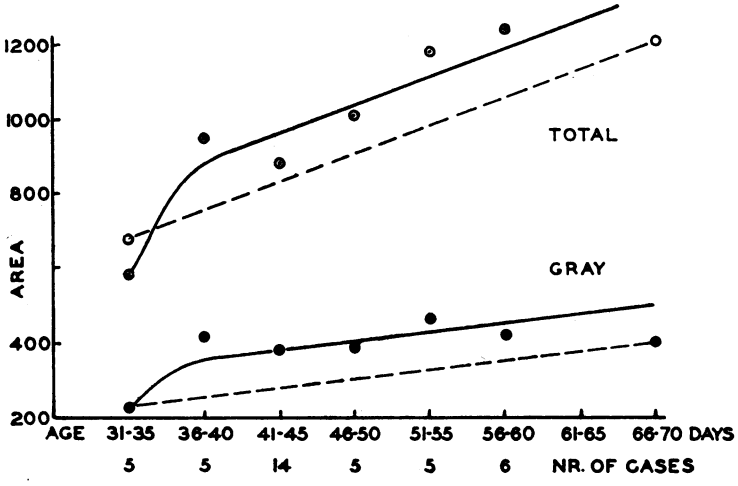

FIGURE 10

Growth curves of cross-section of hind brain in normal (broken lines) and thyroid graft cases (solid lines).

cannot be explained simply by their large size, that is, by mere quantitative extrapolation of the positive growth effect beyond a critical upper size level. The difference between the two cell types thus is not merely one of degree, but one of kind, and the large size differential between M-cells and non-M-cells is but one of the superficial signs of much more profound constitutional, i.e., physicochemical and metabolic distinctions. It is reasonable to assume that similar distinctions exist among the non-M-cells, except that either they do not become reflected in cell size or our techniques of measurement and group assay are too crude to detect them. Indirect evidence for the qualitative differentiation ("speciation") among neurons is accumulating rapidly; numerous examples have been cited in the recent symposium on Genetic Neurology (Weiss, 1950b). The evidence pertains both to the fact that specific qualitative differences among neurons exist and to the relevance of these differences to the establishment and maintenance of functional order in the nervous system. Their relevance to the insuring of de-

velopmental order, in counterplay with the humoral milieu, is demonstrated clearly by the results of this paper. Whether this also brings us any nearer to an understanding of the relation between hormone action and patterns of behavior remains to be ascertained.

Summary.—Grafts of rat thyroid or thyroxin-agar implants near the hind brain of tadpoles produce within a few days a burst of mitotic activity in the neural epithelium and marked growth of nerve cells with the exception of Mauthner's neurons, which atrophy. Evidence is presented to show that this antagonistic response, which occurs similarly in normal metamorphosis in response to the metamorphosing hormone of the thyroid, is based upon qualitative differences among the neuron types involved. The data confirm the view that hormones do not create differentials among equipotential tissue units, but merely help to realize preexisting latent differentials. The results stress anew how relevant qualitative diversity among neurons is to the development of neural order, and perhaps suggest a clue to the mechanism of hormonal "control" of behavior.

* This work has been aided by the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and by a research grant from the National Institutes of Health, Public Health Service.

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[1] A brief note that has come to our attention after this manuscript had gone to press (G. M. Baffoni and G. Catte, *Atti della Accad. Naz. dei Lincei*, 8th ser., vol. **9**, fasc. 5, pp. 282–287 (1950)) reports precipitate involution of Mauthner's cells in tadpoles kept in water with dilute thyroid extract.