EXPERIMENTAL OBSERVATION OF RAT NEUROSECRETION UNDER DERANGED STEROID HORMONE MILIEU

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INTRODUCTION

A mass of evidence has been accumulated steadily since the original reports of *Oliver* and *Schafer* (1895, Vasopressin), *Magnus* and *Schafer* (1901, Adiuretin) and *Dale* (1906, 1909, Oxytocin) to show that three well defined hormonal activity can be ascribed to the posterior pituitary.

Hormonal activity of the posterior pituitary has remained a physiological and pharmacological concept lacking detailed morphological foundation, although many progresses have been made in physiology, chemistry and pharmacology of three hormones. It seemed unlikely that the endocrine function of posterior pituitary is due to pituicyte devoid of any secretory characteristics.

Many problems remained unsolved concerning the identification of glandular cell responsible for hormone formation. These problems were readily solved by the development of concept of neurosecretion. *Scharrer* (1928) proposed a concept of neurosecretion, based on cytological observation of hypothalamus of Phoxinus. In the reptile, bird and mammal, there are two nuclei, Nucleus supraopticus and Nucleus paraventricularis. On the other hand, in fish and amphibia, there is one nucleus, N. praeopticus. Thus the universality in all vertebrates of neurosecretory phenomenon was recognized. A mass of neurosecretory cells can be distinguished from other aggregation of cells in central nervous system not only by their cytological characteristics but also by their rich blood supply. Neurosecretory cells are neurones with Nissl-bodies, dendrites, axons and neurofibrils which also resemble gland cell in that they show a cytological characteristics of secretory activity. They produce granules and droplets of substance which can be stained by a variety of methods of which chrome alum hematoxylin phloxine, first described by *Gomori* (1941) for Langerhans' Islet, has been shown by *Bargmann* (1949) to be particularly suitable. When the hypothalamo-hypophyseal neurosecretory pathway was interrupted operatively, the supraopticohypophyseal tract showed accumulation of neurosecretory material proximal to the site of operation, indicating that the neurosecretory products pass down the tract into the posterior pituitary, where they are stored and excreted on demand (*Drager* 1950, *Hild* 1951, *Scharrer* 1952, *Scharrer* and *Wittenstein* 1952). Parallelism was ascertained between neurosecretory content and pharmacological activity of three hormones (*Hild*

1952, *Hild* und *Zetler* 1952). Neurosecretory granules are interpreted as vehicle or carrier substance transporting three hormones from hypothalamic center into posterior pituitary. Accordingly most of the works on neurosecretion are that dealing with water and salt metabolism. The intimate relationship between hypothalamus and anterior pituitary function became generally accepted as the concept of hypophyseal portal vessel was advanced. The view seemed probable that the neurosecretion may play significant role by neurohumoral mechanism in the hypothalamic control over anterior pituitary. Based on the study of influence of endocrinoneurotic milieu on the development of neoplasm, theory of endocrinoneurotic milieu was evolved by Professor Dr. *Mori* (1935) with regards to disposition. Not only the development of neoplasm but also every biological phenomenon including differentiation, growth and sexual function are modulated by endocrinoneurotic milieu according to the theory. After the establishment of this theory, the implication of the theory has been gradually advanced on basis of experimental data on the development of tumor, arteriosclerosis, tuberculosis and avitaminosis under deranged hormone milieu.

Hypothalamus, regarded as the center of autonomic nervous system, has been shown to produce neurosecretory substance which is supposed capable of regulating anterior pituitary function. It can be supposed in the light of above mentioned endocrinoneurotic theory of *Mori* that hypothalamic center is of great significance as a single regulator of both endorine and nervous milieu.

The present work on rats was undertaken in an attempt to clarify the morphological influence of various steroid hormones upon neurosecretory activity.

Normal Picture of Hypothalamic Neurosecretion (Fig. 1 & 2)

Nuclei of neurosecretory cell are generally large and vesicular, frequently located eccentrically in the cytoplasma, containing a single nucleolus with a marked affinity to phloxine. Sometimes there are slight indentations, irregularity or a crease in the nuclear membrane. Nissl substance is arranged in a few clumps in the periphery of cytoplasma leaving perinuclear zone free of Nissl and stains reddish blue. The remaining cytoplasma of the nerve cell is more or less granulated evenly with neurosecretory material in the form of extremely fine granule. The neurosecretory granules measure 0.2–0.3μ in diameter, smallest being barely visible with light microscope. The amount of neurosecretory material varies considerably from neuron to neuron, depending upon the phase of secretory activity. The dendrites of the neurosecretory cell are irregularly enlarged along their course and contain dense accumulation of C.H.P. positive granules. Along the course of the axon, there are many fusiform enlargements which were shown to be the accumulations of neurosecretory material on way of transport into posterior pituitary.

Neurosecretory cells are densely packed and constitute conspicuous, well demarcated cell group, interspersed by rich blood supply, readily distinguished from the adjacent tissue by virtue of foregoing specific features.

Supraoptic nucleus is a small cell group located just laterally to chiasma opticum, associated rarely with nucleus supraopticus accessorius.

In frontal section taken through the center of supraoptic and paraventricular nucleus, the nucleus paraventricularis constitutes a triangular configuration. Dorsal extremity of this mass is expanded laterally, longest side of triangle lies parallel to the wall of III-ventricle. Continuing posteriorly, the ventral portion of the nucleus is blended by small light staining cell of the periventricular gray matter. Anterior posterior length of the nucleus mass was reported to be quite constant (0.65–0.70mm) (*Frykman* 1942). The paraventricular nucleus is generally considered to be composed of two portions, a dorsolateral part called the magnocellular portion containing large dark staining cell and a ventromedial part or paravocellular portion, containing medium-sized dark cell.

Hypophysectomy (Fig. 3)

Supraoptic nucleus underwent alomst complete disintegration, lacking even one of normal neurosecretory cell.

The paraventricular nucleus in the hypophysectomized rat retained its triangular configuration on frontal section but the nuclear mass stood out less conspicuous than normal. The boundary of the nucleus became less distinct than normal, even difficult to point out.

Due to rterograde degeneration, reduction in the number of typical cell with peripherally located Nissl and eccentrically situated nucleus was shown in the dorsolateral or magnocellular portion of the paraventricular nucleus. Even a trace of neurosecretory material was not found throughout the section.

The majority of the paraventricular cell underwent following severe degenerative process. Namely, cytoplasma shrunk away from the nuclear membrane, leaving the nucleus completely surrounded by a clear space and became finely vacuolated with increased affinity to phloxine. Loss of Nissl and *Gomori* substance was also recognized. Nuclear pyknosis and loss of affinity for phloxine of nucleolus were encountered very often in degenerating neurosecretory cells.

In the heavily affected cell, cytoplasmic vacuolation proceeded to the point where nothing but fine interwoven shreds of cytoplasma is left. Nucleus underwent pyknosis and shrinkage through which it ultimately disappeared. Some neurosecretory cells were converted into large vacuoles with only very thin rings of cytoplasma surrounding them with the disappearance of nucleus. Some cells have completely disappeared leaving a free space corresponding their original shape in cerebral substance.

Table 1. Hormone used and manner of treatment

Hormone used	normal group				hypophysectomized group			
	daily dosis	days of administration	no. of rat		daily dosis	days of administration	no. of rat	
			male	female			male	female
Estradiol	30 γ	30	2	2	30 γ	30	2	1
	100 γ	30	3	3				
		60	4	4				
Estradiol pellet	2 mg	60	2	1				
	10 mg	60	2	1				
Hexesterol	50 γ	30	2	2	50 γ	30	2	2
Acrylonitrile	1 mg	30	2	2	1 mg	30	2	2
Testosterone propionate	1 mg	20	1	1	2.5mg	30	2	2
	2 mg	10	1	1				
		30	1	1				
	3 mg	30	1	1				
		60	1	1				
Testosterone depot	20 mg	60	1	1				
	40 mg	60	1	1				
	60 mg	60	1	1				
Methylandrostenediol	2.5mg	30	2	2	2.5mg	30	2	2
		60	1	1				
Methyltestosterone	2.5mg	30	1	1	2.5mg	30	1	1
Progesterone	1 mg	30	1	1	1 mg	30	2	2
	5 mg	30	2	2	5 mg	30	1	
	25 mg	60	1					
Cortisone acetate	10 mg	30	3	2	10 mg	30	5	2
DOCA	2.5mg	30	1	1	2.5mg	30	1	1

Morphological Observations of Effect of Steroid Hormone Upon Neurosecretion

Material and Method

Adult albino rats (Japanese Gifu strain), weighing 100–150g were employed

in the experiment. The animals were divided into two groups. One is normal group and the other is hypophysectomized group. Both groups were kept in the same environmental and dietary condition. A variety of steroid homone were administered intramuscularly daily, with the exception of estradiol pellet and testosterone depot in the manner as shown in table 1. The animals were sacrificed at the interval ranging from 10 days to 60 days. After heart puncture, brain and hypophysis were carefully dissected out through the skull and immersed in Zenkel-formol overnight, then dehydrated and embedded in paraffin according to routine methods. Serial sections (5μ) were stained by *Gomori* chrome alum hematoxylin for the observation of neurosecretory granule. Diameter of nucleus was measured by micrometer under oil immersion as the average of 200 neurosecretory cells taken at random.

Result

1. *Morphological picture of neurosecretion on steroid hormone treatment.*
"Supraoptic and paraventricular nucleus"

Non Hypophysectomized Group

(Female sex hormone)

Estradiol (Fig. 4,5,6, and 7): Majority of the neurosecretory cell was swollen with enlarged nucleus. It was noted that peripherally located Nissl substance and chromatin content markedly decreased in most of neurosecretory cells. The cells seemed to be heavily packed due to swelling of cytopalsma. The fact that blood vessel, interspersed intercellularly, appeared dilated would be consistent with the presumption of increased activity due to estradiol administration. Neurosecretory granules increased markedly. Very fine granules were distributed evenly throughout the cytoplasma. Extracellular neurosecretory material, taking form of fusiform accumulation along the pathway also increased in size and number both in supraoptic and paraventricular region. It might seem that fusiform aggregates of neurosecretory material are distributed mainly near the upper extremity of paraventricular nucleus and distributed principally in narrow marginal zone beneath supraptic nucleus in frontal section. Increased neurosecretory granule was shown to be very fine and evenly distributed in all cells of short term experiment ($30\gamma \times 30$ days) while to be somehow coarse and to vary considerably in amount from cell to cell in long term experiment ($100\gamma \times 60$ days). In pellet implantation, neurosecretory material became very fine as compared to daily injection.

Hexesterol (Fig. 8): It was observed that the neurosecretory content increased moderately and blood vessel became dilated. It was noteworthy that 2 types of cells were recognized in all cases. One of them was swollen neurosecretory cell with enlarged nucleolus, containing poor chromatin content and was

depleted of *Gomori* substance, being stained light. The other of them was shrunken cell with pyknotic nucleus and was filled with neurosecretory material. The cytoplasma became strongly phloxinophilic.

Acrylonitrile: No morphological change was noted in acrylonitrile treated group. Blood vessel supplying neurosecretory center seemed somehow dilated. Neurosecretory content and morphology of neurosecretory cell remained unchanged.

Progesterone: Neurosecretory content increased considerably in progesterone treated rat. Dense aggregate of neurosecretion was seen to obscure the nucleus in some cells. Increase in size of cell body was recognized as well as that of nucleolus. Very minute granules were scattered mainly around the nucleus and became sparse peripherally. No difference of effect was noted among 1, 5 and 25 mg of progesterone.

(Male sex hormone)

Testosterone propionate (Fig. 9): Enlargement of nucleus accompanied by a depletion of chromatin substance could be recognized in all experimental animals. Swollen cell body was completely depleted of Nissl substance. Reduction of chromatin content in nucleus and loss of affinity for phloxine of nucleolus were also noted, which made nucleus and nucleolus less conspicuous than were those of normal controls respectively. Blood vessels in close association with the neurosecretory cell were seen to be highly dilated as compared to degree elicited by other hormone treatment. Neurosecretory materials were very fine in size and increased considerably in amount both intracellularly and extracellularly. Any difference of morphological effect was not observed among 1, 2 and 3mg of testosterone used. Neurosecretory material became increased markedly in 10 days after treatment. In 20 days, much more increase in amount of granule was seen. In 60 days the content of neurosecretory material returned to the same degree as brought by 20 days treatment. It was shown that the effect of testosterone depot to increase the neurosecretory content was maximum in 40 mg among dosis of 20, 40 and 60mg.

MAD (Fig. 10): MAD was also shown to increase the size of nucleus and the amount of neurosecretory material. Extra fine granule was distributed evenly throughout the cytoplasma. Fusiform accumulations along the nerve tract were observed in abundance intercellularly both in supraoptic and paraventricular nucleus.

Methyltestosterone: No conspicuous alteration was noted but that the injection of blood vessel supplying the nuclei takes place in female.

(Adrenocortical hormone)

Cortisone (Fig. 11): The rats were in poor condition at autopsy because of high dosis of the hormone given. In short, the histological alterations were of

character comparable to that induced by hypophysectomy. In the neurosecretory cell of supraoptic and paraventricular nucleus, cortisone elicited the complete loss of Nissl and *Gomori* substance. In some cells, nucleus was shrunken to be pyknotic, was completely isolated from cytoplasma by a clear empty zone. Some nuclei were flattened and pushed to one side of cytoplasma. Some nucleus had but a trace of nucleolus left with diminished affinity for phloxine. In most of cells, cytoplasma showed more or less vacuolation and shrunk away from the nuclear membrane. Vacuoles proceeded to enlarge to the extent where fine thread-like cytoplasma was interwoven. Majority of cells seemed to have completely disappeared until nothing was left but a free space corresponding to the original shape of neurosecretory cell. It was observed in the experiment that cortisone induced more widespread degeneration of thalamic and hypothalamic nerve cell as shown by *Castor* et al (1951).

DOCA (Fig. 12): Morphological picture induced by DOCA was of the same nature as that induced by administration of sodium chloride (*Leveque*, 1953). It was characteristically observed that the neurosecretory cells with signs of degeneration and the cells with signs of heightened function were simultaneously present mixedly. In half of neurosecretory cells, there were nuclear and nucleolar enlargement, depletion of Nissl and *Gomori* substance in association with loss of affinity for phloxine and vacuolation of nucleolus. These signs were interpreted as an indication of increased synthesis of neurosecretory material (*Leveque*, 1953). In other half of cells, nuclear pyknosis, disappearance of nucleus and cytoplasmic vacuolation were present. Cytoplasma became strongly phloxinophilic and replete with a large quantity of neurosecretory material to the point where dense accumulation of *Gomori* substance covered completely the surface of entire cell body. These changes may be one of the steps in the irreversible degeneration. A large quantity of *Gomori* substance in cell under the degenerative process was considered to be due to the inability of the cell to discharge them. The former with a sign of elevated function was converted ultimately to the latter with a sign of degeneration because the stimulation was continued beyond the capacity of cell.

HYPOPHYSECTOMIZED GROUP

(Female sex hormone)

Estradiol (Fig. 13): Minority of neurosecretory cells underwent degenerative process and disappeared in estradiol treated hypophysectomized rat. It was noted that the cell seemed to be distributed sparsely by virtue of disintegration. Remaining cells were swollen. Nucleolus was blurred due to the loss of affinity for ploxine in some cells. Blood vessels were moderately dilated. The effect of estradiol to activate neurosecretion was recognized also in hypophysectomized rat, judged from the content of neurosecretory material. The

observation that neurosecretory material was increased with nuclear enlargement even in estradiol treated hypophysectomized rat, was considered suggestive of the presence of direct action of estradiol upon the neurosecretory cell without any mediation by pituitary.

Hexesterol and acrylonitrile: The neurosecretory nuclear regions were constituted of the cells with a variety of signs of degeneration, morphological picture being identical to that induced by hypophysectomy. No *Gomori* substance was present. Hexesterol and acrylonitrile were recongized to have no effect on neurosecretion in hypophysectomized rat.

Progesterone (Fig. 14): There was no cell with any signs of degeneration. The cells were distributed somewhat sparsely and depleted of *Gomori* substance but remained normal in form, size and other morphological features. These observations were indicative of that progesterone acts upon the neurosecretory cell directly to prevent retrograde degeneration inevitable after hypophysectomy.

(Male sex hormone)

Testosterone (Fig. 15): It was observed as in normal rat treated with DOCA that two kinds of neurosecretory cell, showing distinct morphological pictures, were evenly distributed. One of them was shown to be in a degenerative process. Shrunken cytoplasma was completely depleted of Nissl and *Gomori* substance. Vacuolation of cytoplasma and nucleus proceeded to the point where nothing was left but two thin rings of nuclear and cellular membrane. The other of neurosecretory cell was interpreted to be exempted by testosterone given from degeneration due to hypophysectomy.

They showed marked increase in amount of *Gomori* substance and in size of nucleus. Testosterone was considered to activate the neurosecretory activity even in hypophysectomized rat through direct action upon the neurosecretory center.

MAD: No degenerative process was observed. Only a trace of *Gomori* substance was present in some cells.

Methyltestosterone: The picture observed was nearly identical to that of hypophysectomy and so no effect on neurosecretion under hypophysectomy was recognized.

(Adrenocortical hormone)

Cortisone (Fig. 16): Cortisone was described to affect the nerve cell extensively in normal rat. In hypophysectomized rat, the destructive effect was shown to be present also. In the neurosecretory center, complete destruction was encountered possibly both due to retrograde degeneration and due to original destructive action of cortisone, therefore neurosecretory center seemed at a glance spongiform because of the empty space left after degenerating cell.

DOCA: The finding obtained was shown nearly identical to that elicited by hypophysectomy. No peculiar effect was noted of DOCA on hypophysectomized rat.

"Posterior pituitary"

Posterior pituitary is considered to be constructed of pituicyte and ending of Tractus supraopticohypophyseus, terminating on the wall of blood vessel. Fine neurosecretory granule is densely and evenly distributed over the entire gland, obscuring fine structure of posterior pituitary.

(Male and female sex hormones)

No striking alterations were noted except that the neurosecretory granule became coarse and uniform in size, though these hormones were shown to exert prominent effects upon neurosecretory cell.

(Adrenocortical hormones)

Complete loss of neurosecretory material was seen in cortisone and DOCA treated rat. With the loss of neurosecretory granule details of stroma, obscured previously, became obvious. The loss of neurosecretory material induced by cortisone is interpreted due to cessation of production of material in degenerated center while the loss induced by DOCA due to exhaustion of neurosecretory center which was brought about by continued heavy load beyond the capacity.

2. Brain weight (Table 2)

Little difference was recognized among both sexes as to brain weight per body weight 100g. In hypophysectomized rat, pronounced increase of brain weight was noted as compared with that of normal rat, presumably because of loss of body weight inevitable after hypophysectomy. Estradiol had no effect upon brain weight in normal group but was shown to have the action to increase brain weight in hypophysectomized group. On the contrary, marked increase in brain weight was shown by synthetic estrogen hexesterol and acrylonitrile in normal group, which was abolished completely in hypophysectomized group. Cortisone had the action to increase brain weight in both normal and hypophysectomized group, possibly due to loss of body weight by its toxicity. No effect was noted upon brain weight in male sex hormone, progesterone and DOCA treatment for both normal and hypophysectomized groups.

3. Microscopic measurement of diamter of nucleus. (Fig. 1 and Table 2)

It is of great importance for estimation of secretory activity what sort of criterion represents its functional state more strictly. An attempt to estimate secretory activity on basis of contents of neurosecretory material has been made by *Leveque* (1953) with unsuccessful results as he observed that the neurosecretory cell with signs of degeneration may still contain a large quantity of granule. After *Leveque's* statement, the extent to which Nissl substance disappears and the size of necleolus

Table 2. Body weight, brain weight and nuclear diameter of paraventricular & supraoptic neurosecretory cell

	normal group										hypophysectomized group									
	male					female					male					female				
	body weight (g)	brain weight(g)		nuclear diameter		body weight (g)	brain weight(g)		nuclear diameter		body weight (g)	brain weight(g)		nuclear diameter		boby weight (g)	brain weight(g)		nuclear diameter	
			body weight	P.V.	S.O.			body weight	P.V.	S.O.			body weight	P.V.	S.O.			body weight	P.V.	S.O.
control	150	1.7	1.13	10	11	138	1.52	1.1	10	11	111	1.75	1.57	8.9	8.9	100	1.55	1.55	8.4	8.4
castration	182	1.9	1.04	10.5	13.5	150	1.8	1.2	12.5	13.5	116	1.8	1.55	10.5	12	88	1.8	2.04	10.5	11.5
Estradiol	170	1.95	1.15	12	13.1	163	1.8	1.1	10	11	100	1.9	1.9	12	13.7	108	1.95	1.8	10	11
Hexesterol	158	2.0	1.26	12	13	126	1.85	1.47	10	12	130	1.9	1.46	9.6	9.6	110	1.65	1.50	9.5	9.5
Acrylonitrile	130	1·9	1.46	10.5	11.8	119	1.6	1.34	10.2	11.4	100	1.48	1.48	7.9	8.3	108	1.65	1.53	8.8	10
Testosterone	188	1.95	1.04	12.5	13.5	170	1.85	1.09	13	13.8	120	2.0	1.66	10	11.1	115	1.8	1.56	10.1	12.5
MAD	168	1.9	1.13	12.5	12.9	141	1.6	1.13	12.9	13.1	104	1.5	1.44	10.1	11.9	98	1.55	1.58	10.1	12.1
Methyltestosterone	190	2.0	1.05	9.0	9.8	140	1.5	1.07	12.4	13.1	135	1.8	1.33	9.2	9.2	136	1.7	1.25	9.1	9.1
Progesterone	140	1.65	1.18	11	13.5	150	1.65	1.10	11	13.5	132	1.9	1.44	10	13.4	109	1.7	1.56	10.5	13.2
Cortisone	104	1.65	1.59	8.4	8.9	114	1.75	1.53	9.0	9.5	80	1.85	2.31	8.5	9.4	88	2.0	2.27	8.2	8.3
DOCA	210	2.3	1.09	8.0	9.6	190	2.1	1.1	8.5	9.0	110	1.7	1.54	8.4	8.9	122	1.85	1.51	9.0	9.1

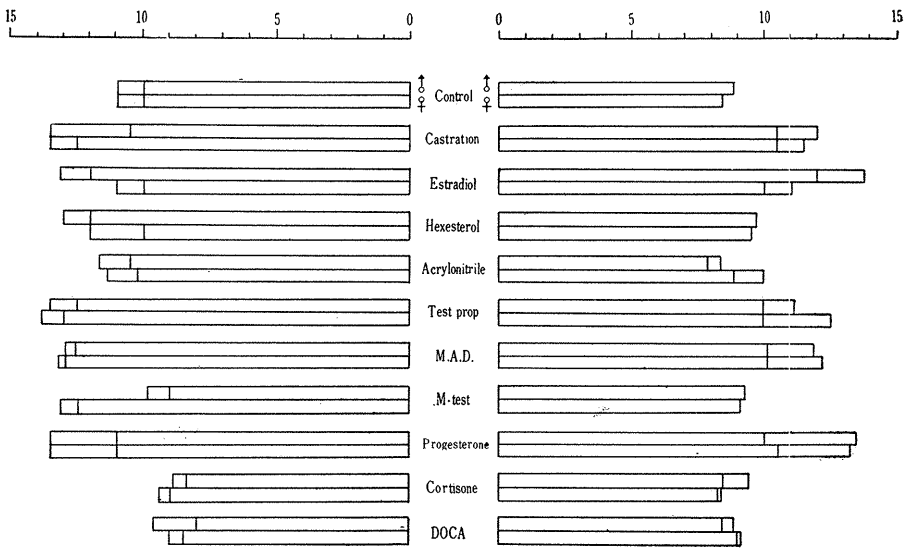

Fig. 1. Nuclear size (μ) of supraoptic and paraventricular cell

increases may be used as a more reliable criterion for the estimation of neurosecretory activity than granular contents.

Eichner (1953) considered the enlargement of nucleus as being the best indicator for the determination of neurosecretory activity from the experiment where dogs were thirsted by withdrawal of drinking water.

1. *In normal rat*

Diameter of nucleus of supraoptic and paraventricular neurosecretory cell measured 11μ and 10μ respectively without any difference among both sexes.

(Female sex hormone)

Treatment with estradiol and hexesterol was recognized to increase nuclear size markedly in male while acrylonitrile was shown ineffective as to the nuclear size in both sexes. Progesterone was also shown to have the action to enlarge nuclear size for both sexes, unlike estradiol and hexesterol.

(Male sex hormone)

Size of nucleus was found to increase in both sexes by Testosterone propionate and MAD, somewhat prominently in female than does in male. However, synthetic androgen, methyltestosterone did not increase the size of nucleus in male but did only in female.

(Adrenocortical hormone)

Conspicuous diminution of nuclear size was observed by treatment of cortisone and DOCA, corresponding to morphological pictures. The degree of nuclear shrinkage by cortisone and DOCA was comparable to that due to hypophysectomy.

2. *In hypophysectomized rat*

Diameter of nucleus of both supraoptic and paraventricular neurosecretory cell became shrunken to be 8.9μ in male and 8.4μ in female.

(Female sex hormone)

Estradiol was observed to have the same excitatory action for male to enlarge nuclear size in hypophysectomized rat as shown in non-hypophysectomized rat. Retrograde degeneration specific to hypophysectomy was nearly completely arrested by estradiol treatment as judged from nuclear size. It seemed likely to assume that estradiol acts directly upon the hypothalamic neurosecretory cell to activate it without intervention of pituitary because the excitatory action of estradiol on neurosecretory center was recognized similarly both in normal and hypophysectomized rat.

Hexesterol, effective in normal rat as to nuclear size and acrylonitrile were shown ineffective in hypophysectomized rat. Therefore the nuclear size after hexesterol and acrylonitrile treatment in hypophysectomized rat was nearly the same as that after hypophysectomy without any treatments.

Progesterone: The effect of progesterone to enlarge nuclear size was observed in hypophysectomized rat for both sexes, suggesting that progesterone acted

directly on hypothalamic neurosecretory center to activate it and to prevent retrograde degeneration following hypophysectomy.

(Male sex hormone)

Testosterone propionate and MAD increased nuclear size in hypophysectomized rat as in non-hypophysectomized rat.

Methyltestosterone was ineffective in hypophysectomized rat though effective in normal female.

(Adrenocortical hormone)

Cortisone and DOCA treatment had no effect upon nuclear size in hypophysectomized rat.

In short estradiol, progesterone, testosterone and MAD were found to have excitatory action on hypothalamic neurosecretion by direct way without mediation of pituitary, as judged from the fact that actions of above mentioned four hormones were observed still in hypophysectomized rat. The action seemed somewhat prominent for the opposite sex except that progesterone exerts its effect equally on both sexes. The effect to enlarge nuclear size of hexesterol and methyltestosterone was abolished by hypophysectomy because the effect may possibly be mediated by pituitary gland. Cortisone and DOCA reduced the nuclear size. These finding stands in good accordance with that of morphological alterations above mentioned.

Neurosecretion Under Low Steroid Hormone Milieu

Following observation on the morphological pictures of neurosecretion under high steroid hormone milieu, neurosecretion of rat under castration and adrenalectomy was studied morphologically with some results.

Material and Method

Sixteen adult albino rats, weighing 150–200g were used. Eight rats were castrated and sacrificed at intervals of 10, 30 and 60 days after operation. Eight rats were adrenalectomized, maintained on 1% NaCl and were sacrificed at the intervals of 5, 14, 18, 22 and 30 days after the exstirpation. Methods used for microscopy are the same as written previously.

Result

Castration (Fig. 17 and 18)

Paraventricular neurosecretory cells were swollen and replete with increased *Gomori* substance without any degenerative process. But in most cells of supraoptic nucleus, nucleus showed pyknotic change and cytoplasma became strongly phloxinophilic. The cells showing nuclear pyknosis were diminished in number as time lapsed. It was noteworthy that in one of castrated rats, cells with elevated activity and cells in the step of irreversible degeneration were encountered as in

DOCA treated rats. In posterior pituitary, no remarkable changes were noted.
Adrenalectomy (Fig. 19)

Some of supraoptic neurosecretory cells were vacuolated with pyknotic nucleus. Nucleolus became prominent as empty vesicle in pyknotic nucleus. Pyknosis of nucleus was encountered in case of 5 and 14 days after adrenalectomy but was absent in 18, 22 and 30 days after operation. Neurosecretory granule seemed to decrease moderately in 5 and 14 days and disappear in 18 days or more days. In paraventricular nucleus, reduction of *Gomori* substance was similarly observed as in supraoptic nucleus but no pyknotic change was present. Neurosecretory content of posterior pituitary became also reduced considerably in 14 days, thereafter neurosecretory material was not diminished. It was peculiar that in both adrenalectomy and castration nuclear pyknosis were richly encountered. The cellular degeneration such as pyknosis of nucleus was interpreted to be ascribable to stress incidental to the operation (*Pandalay* and *Leveque* 1953).

Morphological Observation on the Effect of ACTH and Synahorin on Neurosecretion

The neurosecretion may be considered to stand in close relation to the steroid hormone level from the experimental data described above under deranged condition of steroid hormones. The steroid hormone milieu is under control of pituitary trophic hormones. Therefore, it may be of interest to study the effect of pituitary trophic hormone on neurosecretion.

Material and Method

Two rabbit-units of synahorin (pituitary and chorionic gonadotrophin) and 5mg of ACTH (Armour) were injected daily for 30 days to adult albino rats, weighing an average of 100g. Sections of hypothalamus and posterior pituitary were prepared according to the same manner as written previously.

Result

ACTH: Following degenerative changes were encountered consistently in paraventricular nucleus as reported in part by *Castor* 1951. Basophilia of cytoplasma was reduced throughout the paraventricular nucleus because of marked decrease of Nissl substance. Reduction in size of nucleus and nucleolus was also recognized as well as reduction in size of cell body. Nucleus became for the most part pyknotic. Shrunken cytoplasma was seen to become more phloxinophilic. Half of them was still replete with *Gomori* substance, which was interpreted to accumulate because of the inability to discharge due to degeneration. Half of them was completely depleted of *Gomori* substance which was interpreted due to inability to produce neurosecretory material.

The changes elicited by ACTH treatment were considered of the same nature as that elicited by cortisone except that the supraoptic nucleus was not affected in ACTH treatment. Slight decrease in *Gomori* substance was seen in posterior pituitary, the decrease being most prominent in the center of posterior pituitary.

Synahorin (Fig. 20): It was distinguished in synahorin treatment that increase in amount of Nissl and *Gomori* substance and dilatation of blood vessel supplying neurosecretory cells were shown uniformly. The extent of increase in *Gomori* substance was shown comparable to that of testosterone or MAD. Varicose or fusiform aggregates of *Gomori* substance were abundantly encountered with the increased intracellular granule.

In the posterior pituitary, morphological picture remained nearly normal.

In short the effects of synahorin on neurosecretion were interpreted to resemble that of estradiol, progesterone, testosterone and MAD.

It was concluded from the experimental data that changes induced by ACTH or synahorin were comparable in their nature to that of corresponding hormones of the target organ respectively, namely cortisone and above mentioned four hormones.

Changes in Neurosecretion Induced by Some Stressful Stimuli

It was suggested recently by a series of experiments that the hypothalamic center may be involved in the mechanism responsible for the release of ACTH with subsequent liberation of adrenocortical hormone when exposed to stress. Some detailed reports on neurosecretion under stressful condition were presented (*Pandalay* and *Leveque* 1953, *Rothballer* 1953).

Material and Method

Twelve adult albino rats were sacrificed at the intervals ranging from 15 minutes to 24 hours after injection of 5% formalin 1.5cc. Another seven rats were subjected to painful stimuli by piercing the tail with needle in one minute and sacrificed similarly as in formalin treatment.

Result

Formalin (Fig. 21 and 22)

In hypothalamic center neurosecretory matrial was depleted completely and caliber of blood vessel was increased in 15 minutes. Cytoplasma became shrunken and more phloxinophilic in some of neurosecretory cells. Nucleus of the cell showed also pyknotic change, which is considered to be attributable to non-specific stressful stimuli (*Pandalay* and *Leveque* 1953). Degenerative cell with pyknotic nucleus was not present in 2 hours or more. Resumption of neurosecretion seem-

ed to begin in four hours as judged from appearance of perinuclearly arranged *Gomori* substance. Vasodilation was lessened simultaneously. Neurosecretory content seemed to exceed somewhat normal in 12 and 24 hours. As to changes of posterior pituitary induced by stress, Rothballer distinguished three stages. 1. resting stage, 2. stage of active discharge of neurosecretory material, 3. stage of restoration. A large quantity of neurosecretory material began to decrease already in 5 minutes and vanished almost completely with vasodilatation in 10 minutes. Neurosecretory granule seemed to reaccumulate in 12 hours from peripheral portion of posterior pituitary. Complete restoration to normal was seen in 24 hours when the posterior pituitary regained blue black colour due to affinity of the stored neurosecretory material to chrome hematoxyline. Restoration of neurosecrete in posterior pituitary after stress seemed to take place later than that of hypothalamic center.

Painful Stimuli of Pricks

The extent of vasodilatation and reduction of neurosecretion were shown to be less than that of formalin. Degenerative cell with pyknotic nucleus was also observed in both supraoptic and paraventricular nucleus. Changes in both hypothalamic center and posterior pituitary were demonstrated to be analogous to that elicited by formalin. From such morphological manifestations as nuclear pyknosis and reduction of *Gomori* substance, it may be supposed that the neurosecretory phenomenon can play some motive role in mobilization of pituitary adrenocortical response to stress.

Discussion

Sexual Function and Hypothalamus

Intimate relationship beween nervous system and sexual function is generally recognized. First Hohlweg and Junkmann postulated the sexual center in the hypothalamus which stimulated the production of gonadotrophic hormone. It may seem that the sexual center postulated in hypothalamus controls anterior pituitary by nervous or humoral pathway. Relation between hypothalamus and sexual function can be considered from the view point of relation between hypothalamus and anterior pituitary. A large quantity of reports has been accumulated as for the connection between hypothalamus and pituitary. It is definitely established that the neurohypophysis is controlled by hypothalamic nuclei. However there is no agreement that nerve fibre reaches the adenohypophysis from hypothalamic nuclei. The evidence that the axon of hypothalamic nuclei terminates amongst the glandular cell of the adenohypophysis is at present equivocal. The view is also accepted that those nerve fibres, whether sympathetic or parasympathetic, which enter anterior pituitary along its blood vessel are not secretomotor but vasomotor in function.

Based upon above mentioned reasons it seems unlikely that the pathway by which hypothalamus regulates the adenohypophyseal function is by nervous way.

Attention has been paid recently on the hypophyseal portal system of vessels as the controlling link between the hypothalamus and the pituitary, which drains blood from the median eminence and pours into the sinusoid of pars distalis. According to this alternative view, neurosecretory granules, produced in hypothalamic center, traverse down the hypothalamohypophyseal tract to the median eminence where the neurosecretory droplets are absorbed into the primary capillary loop of portal vessel.

Thereafter by the portal vessel stream, the neurosecretory granules reach the sinusoid of adenohypophysis to modulate the activity of secretion. The assumption that neurohumoral connection presumably by portal vessel plays an important role in the hypothalamic control of anterior hypophysis is devoid of direct evidence, but has been generally accepted by a variety of collateral observation (*Harris* 1936, *Taubenhaus* and *Soskin* 1941, *Markee*, *Sawyer* and *Hollinshead* 1946 and *Harris* 1950).

A number of workers has investigated the influence of stalk section on adenohypophyseal function to ascertain whether the pituitary stalk or portal vessel may play significant role for the integrity of adenohypophysis or not. But the results were equivocal. Contradictory results were reported with regard to derangement by severing stalk of endocrine function under hypophyseal control. It has been suggested that the pituitary portal vessels have regenerated in those cases where pituitary function is either restored or remains normal. According to Barnett and Greep, pituitary hypofunction resulting from stalk section is due to infarction of the adenohypophysis by the interruption of blood vessels. In this experiment, the rat neurosecretion was found to be activated by following four hormones in both normal and hypophysectomized group, estradiol, progesterone, testosterone and MAD. Cortisone was shown to cause degeneration in neurosecretory cell. DOCA was considered to load heavily ultimately to the point where half of neurosecretory cell displayed such signs of degeneration as nuclear pyknosis and cytoplasmic vacuolation.

Adrenalectomy, castration and stressful stimuli resulted in nuclear pyknosis. Changes obtained by treatment with ACTH and Synahorin were recognized to be identical to corresponding hormone of target organ, namely cortisone and sex hormones. It was with certainty concluded from present morphological data that the neurosecretion may respond actively to fluctuation in steroid hormone level.

Neurosecretion with Reference to Stress

Porter (1952) studied an electrical activity of brain in response to stress stimuli and reported a marked increase of electrical activity in posterior hypothalamus, indicating involvement of hypothalamus in pituitary adrenocortical response to stress. The localized electrolytic destruction of the posterior hypothalamus abolished the usual eosinopenic response to stress, due to presumed release of ACTH. Stimulation of the posterior hypothalamus was proved to induce stress condition

comparable to the state elicited by stressing agent, as judged by eosinopenic response, according to *Porter* (1953). Taking these reports in consideration, it seems likely that the hypothalamus plays a great role in stress. However the mechanism responsible for the involvement of hypothalamus in pituitary adrenocortical response to stress has to be left unsettled. *Hellerstein* (1952) detected leucopenia in infant rat by the injection of acetone extract of bovine hypothalamus. An extract of hypothalamus was shown by *Hume* (1949) capable of inducing eosinopenic response in normal animals and also in animals whose hypothalamic lesions render them incapable of responding to any stress.

An intact posterior hypothalamus appears to be essential for the release of ACTH in response to stress. A possible explanation has been proposed by Harris and Hume that neural impulse causes the production of hypothalamic neurohumor which passes down the portal vessel of the pituitary stalk into the anterior pituitary to provoke the secretion of ACTH. Some attempts were made to demonstrate the existence of this postulated neurohumor. Extracts of various areas of bovine brain were tested by *Slusher* and *Roberts* (1954) for ability to stimulate ACTH release. The results obtained revealed that the capacity to provoke the discharge of pituitary ACTH seemed to be present in protein and non-saponifiable lipide fractions of the posterior hypothalamus but not in comparable fraction prepared from other portions of the bovine brain.

A study with combined tissue culture by *Guillemin* (1955) has shown that hypothalamic hypophysiotropic mediator, contained in posterior hypothalamus and median eminence, will stimulate anterior pituitary to release ACTH. Its mediator has been proved distinct from histamine, acetylcholine, adrenaline, noradrenalin, serotonine, vasopressin or oxytocin.

Guillemin et al (1957) dealt with the isolation from the hypothalamus of a purified material which causes the release of corticotrophin in vitro. The results obtained have shown that the substance, isolated from hypothalamus and posterior pituitary, capable of releasing ACTH from rat pituitary in vitro, appears to be a small peptide. Hypophyseal portal vessel can be considered as possible vascular pathway for the direct transport of such a blood borne substance from the hypothalamus to the anterior pituitary.

Porter and *Rumsfeld* (1956) dealt with the effect of the lyophilized plasma and plasma fraction from hypophyseal portal vessel blood as well as pitressin on adrenal ascorbic acid content and indicated that portal vessel plasma as well as pitressin contains a substance which promotes the release of ACTH and that the substance is either a large protein molecule or is bound to a large protein and is not identical with vasopressin.

Saffran et al (1955) elucidated that the vasopressin prepared by the method of *Stehle* and *Fraser* contains corticotrophin releasing factor as an impurity and corticotrophin releasing factor can be separated from *Stehle*'s vasopressin by paper

chromatography. Based on these observations, *Saffran* et al (1955) presumed that the posterior lobe of pituitary plays an important role in ACTH release and that corticotrophin releasing factor is elaborated within neurosecretory cells in the hypothalamus and is stored in the posterior pituitary to be excreted on demand, as is postulated for neurosecretion of vasopressin and oxytocin.

The identity of the suspected neurohumor is not known at present, but a plenty of observation were reported in favour of the possibility that the antidiuretic hormone or a substance closely associated with it in the neurohypophysis may be responsible for the release of ACTH in stressful condition.

In contrast to the view that the corticotrophin releasing factor is contained as contamination to vasopressin proposed by *Saffran* et al (1955), it has been proposed by a number of workers that the release of ACTH is under control of antidiuretic hormone per se liberated from the neurohypophysis (*McCann* and *Brobeck* 1954, *Mirsky*, *Stein* and *Paulisch* 1954, *McCann* 1957).

Thus a great deal of evidence indicates the existence of a neurohumoral mediator of hypothalamic origin, involved in ACTH release in response to stress. A neurohumoral substance responsible for the ACTH release is assumed to be antidiuretic hormone elaborated by neurosecretion.

The postulate of neurohumor is based upon a series of experiment where pituitary adrenocortical function was tested after electrical stimulation, stereotactic destruction of various portion of hypothalamus or after injection of portal vessel blood plasma and hypothalamic extract but is lacking the morphological evidences.

In the present experiment, cytoplasmic shrinkages with nuclear pyknosis were encountered in association with vasodilatation and reduction of *Gomori* substance in response to both formalin and painful stress.

These experimental results can be interpreted to offer morphological evidence for the postulated involvement of neurosecretion in stress mechanism. It may be assumed from these results obtained that pituitary adrenocortical response to stress will be provoked by neurosecretory phenomenon.

Summary

The neurosecretory phenomenon of rat was studied morphologically under some experimental conditions of high and low steroid hormone milieu.

1. Estradiol, progesterone, testosterone and MAD exert direct excitatory action on neurosecretion without any mediation of hypophysis. These four hormones prevent the neurosecretory cell from retrograde degeneration following hypophysectomy.

2. Cortisone affects extensively thalamic and hypothalamic area while DOCA loads neurosecretion heavily until some of cell undergoes degeneration due to ex-

haustion.

3. Effect of ACTH and synahorin on neurosecretion can be interpreted as that of hormones of corresponding target organs.

4. Castration results in nuclear pyknosis of some supraoptic cells. Slight increase in *Gomori* substance and in nuclear size were present in most of neurosecretory cells while adrenalectomy results in nuclear pyknosis of some supraoptic cells and in loss of neurosecretory material.

5. Nuclear pyknosis in some cells and disappearance of *Gomori* substance are induced by such stress as formalin and needle pricks on tail.

These results obtained suggest that presumed neurohumoral mechanism through which hypothalamus exerts its control over hypophysis may be neurosecretion itself.

The results of the experiments may offer morphological foundation in favor of accepted supposition that neurosecretion provokes the pituitary-adrenocortcial response to stress.

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EXPLANATION OF PLATES

Fig. 1. Normal picture of paraventricular and supraoptic nucleus of rat (H.E. × 40)

Fig. 2. Normal picture of paraventricular cells characterized by eccentrically located vesicular nucleus with a prominent nucleolus, peripherally situated Nissl substance, rich blood supply and by presence of some cells with fine, evenly distributed *Gomori* granula (C. H. P. × 600).

Fig. 3. paraventricular cells showing nuclear pyknosis and cytoplasmic vacuolation 30 days after hypophysectomy. Some of the cells disappeared completely, leaving empty space corresponding the shape of cell (C. H. P. × 600).

Fig. 4. Paraventricular cells of female rat treated with estradiol (30γ × 30 days). Fusiform accumulations of *Gomori* substance were increased markedly (C. H. P. × 700)

Fig. 5. Paraventricular cells of male rat treated with estradiol (100γ × 60 days). Cellular and nuclear enlargement with conspicuous increase of fusiform aggregate of *Gomori* substance (C. H. P. × 700).

Fig. 6. Supraoptic nucleus of male rat treated with estradiol (100γ × 60 days). Markedly increased *Gomori* substance were packed densely just beneath the supraoptic nuclear region. (C. H. P. × 700).

Fig. 7. Supraoptic nucleus of female rat after treatment with estradiol pellet (2mg) for 60 days. Moderate increases in amount of *Gomori* substance were encountered also beneath the supraoptic cell group (C. H. P. × 700).

Fig. 8. Paraventricular nucleus of male rat treated with hexesterol (50γ × 30 days) showing presence of two distinct types of neurosecretory cell, namely cells with pyknotic nucleus and shrunken cytoplasma and cells with enlarged vesicular nucleus (C. H. P. × 700).

Fig. 9. Paraventricular nucleus of female rat treated with testosterone (3mg × 30 days). Cells are densely granulated with fine *Gomori* substance. (C. H. P. × 700)

Fig. 10. Paraventricular neurosecretory cell with prominent increase of *Gomori* substance after treatment with MAD 2.5mg for 60 days. (C. H. P. × 700).

Fig. 11. Severely affected paraventricular cell of male rat treated with cortisone (10mg × 30days). Neurosecretory paraventricular region becomes spongiform because of the complete disintegration of cells. (C. H. P. × 700).

Fig. 12. Paraventricular nucleus of male rat treated with DOCA (2.5mg × 30days). Some cells are full of *Gomori* substance and some are depleted of *Gomori* substance. (C.H.P. × 700).

Fig. 13. Paraventricular nucleus of female hypophysectomized rat treated with estradiol (30γ × 30days). Neurosecretory materials are slightly increased. (C. H. P. × 700).

Fig. 14. Paraventricular nucleus of male hypophysectomized rat treated with progesterone (1mg × 30days). The cells are distributed somewhat sparsely and depleted of *Gomori* substance. (C. H. P. × 700).

Fig. 15. Paraventricular nucleus of female hypophysectomized rat treated with testosterone (2.5 mg × 30days). Cells filled with *Gomori* substance and cells showing serious degenerative signs are recognized. (D. H. P. × 700).

Fig. 16. Paraventricular nucleus showing degeneration of female hypophysectomized rat treated with cortisone (10mg × 30days). (pyronine-methylgreen × 700).

Fig. 17. Paraventricular nucleus of male rat 30days after castration. Some cells are swollen and some shrunken with pyknotic nuclei. (C. H. P. × 700).

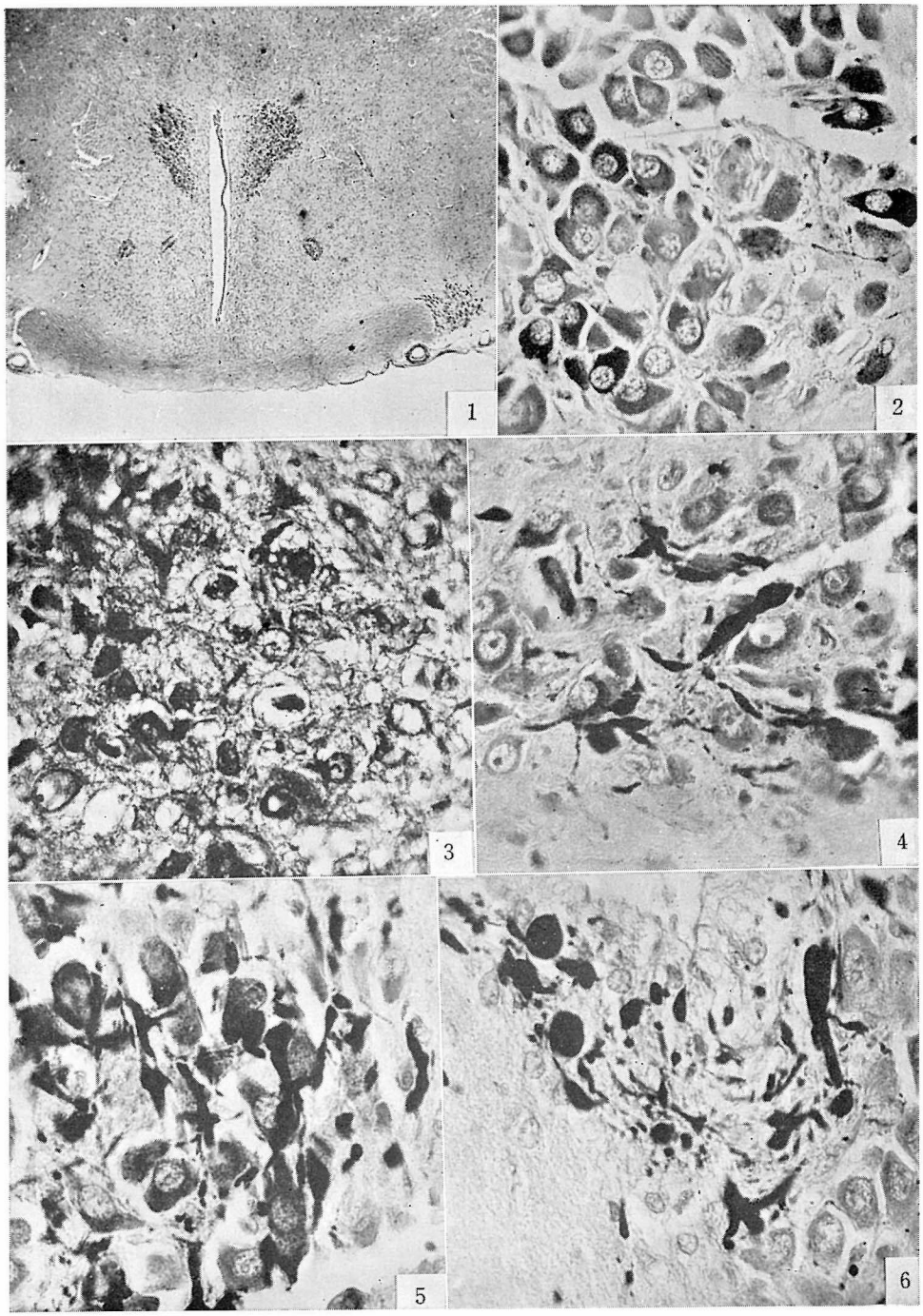
Fig. 18. Supraoptic nucleus of male rat 30days after castration, showing marked nuclear pyknosis. (C. H. P. × 800).

Fig. 19. Supraoptic nucleus of male rat 14 days after bilateral adrenalectomy, showing nuclear pyknosis with loss of affinity for phloxine of nucleolus. (C. H. P. × 700).

Fig. 20. Paraventricular nucleus of male rat treated with synahorin (2 rabbit-units × 30days). Marked increase of *Gomori* substance both intra-and extracellularly. (C. H. P. × 700).

Fig. 21. Normal picture of rat posterior pituitary with large quantity of *Gomori* substance. (C. H.P. × 65).

Fig. 22. Posterior pituitary showing marked decrease of *Gomori* substance, 30 minutes after injection of 5% formalin 1.5cc intramuscularly. (C. H. P. × 80).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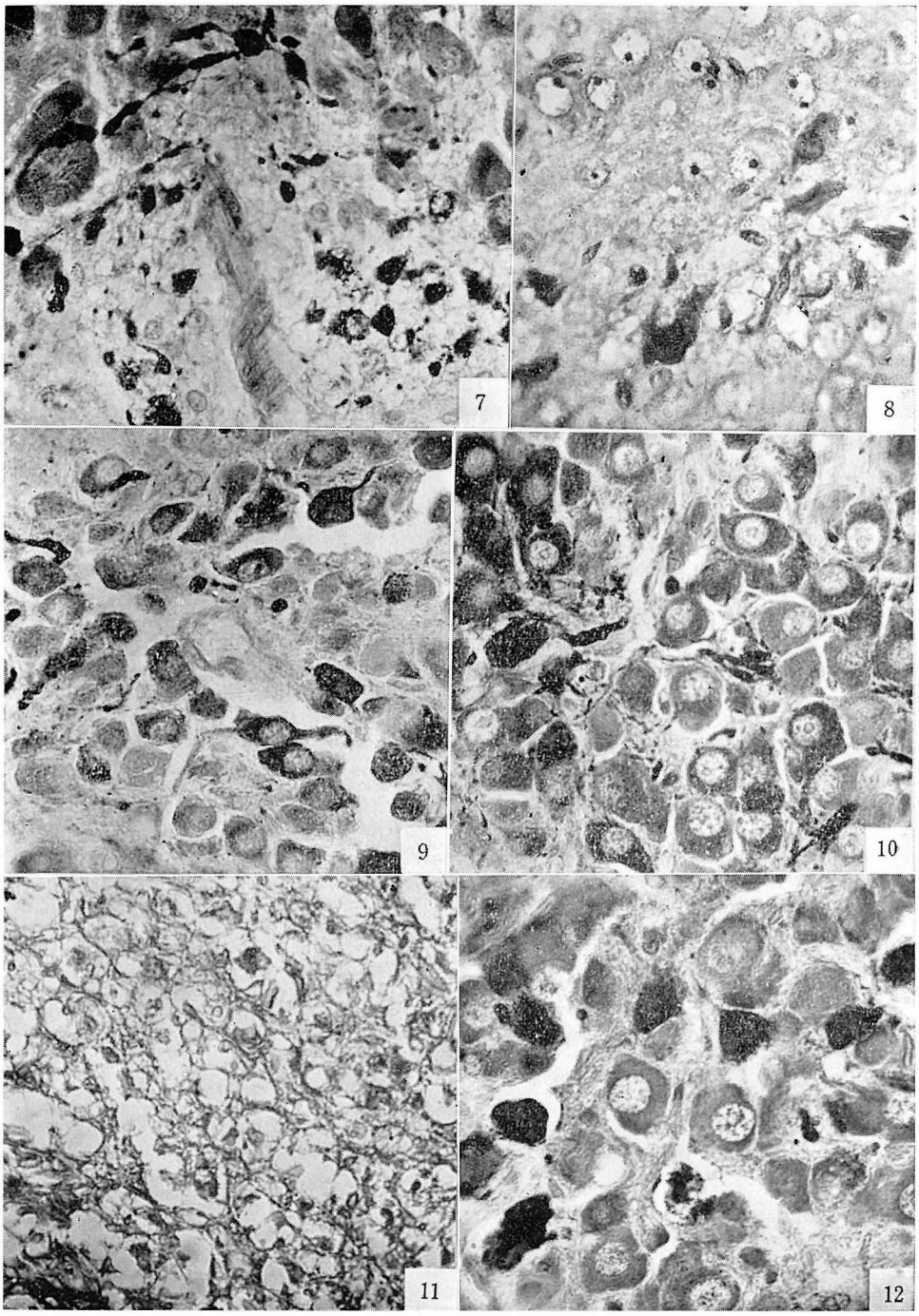
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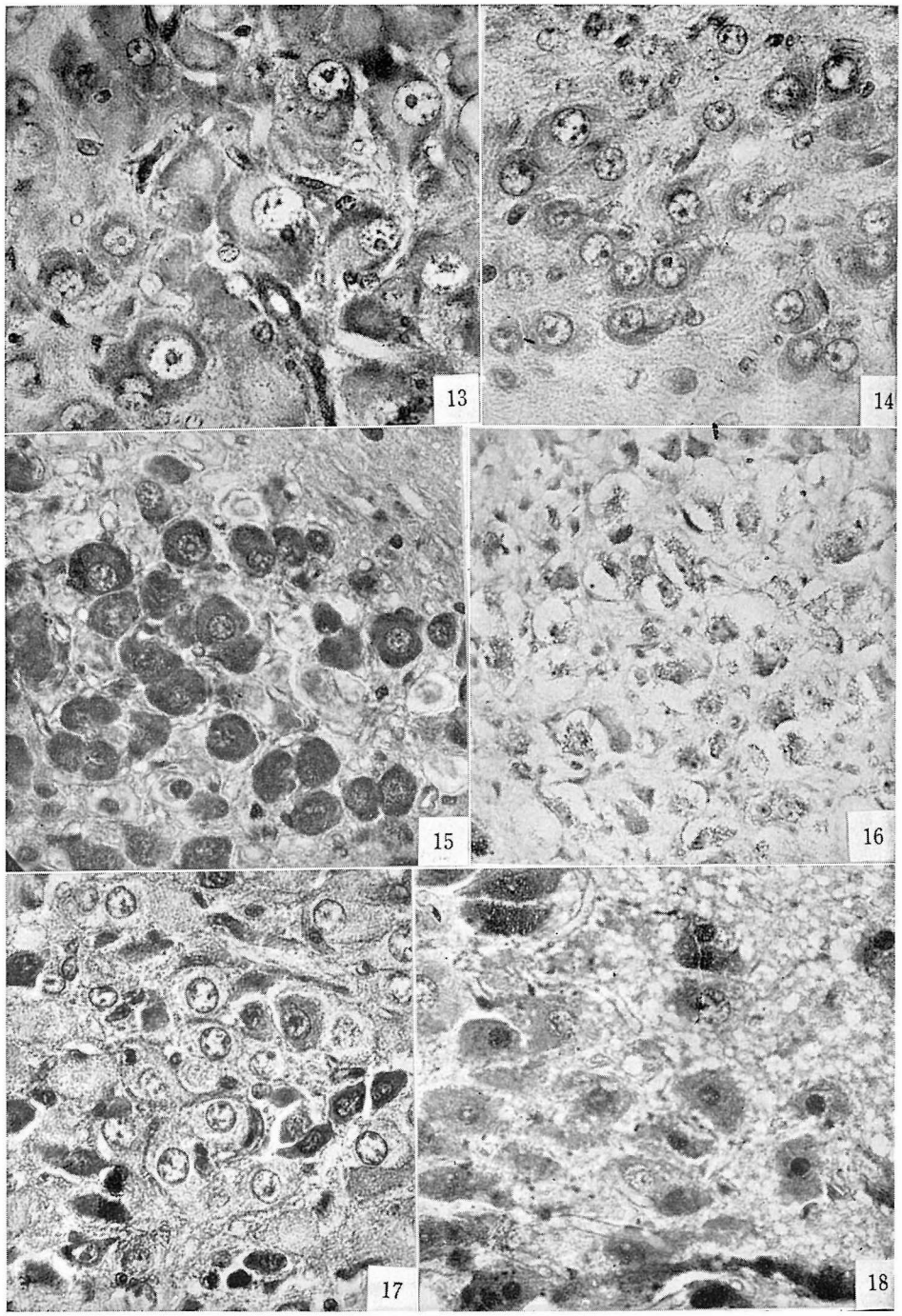
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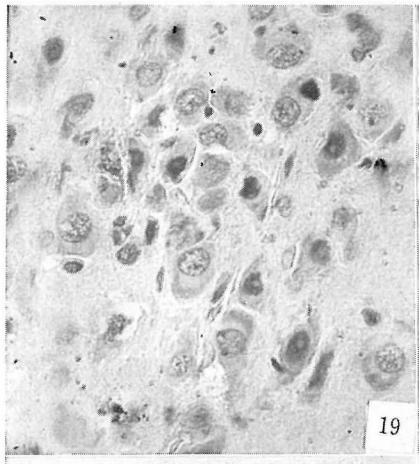
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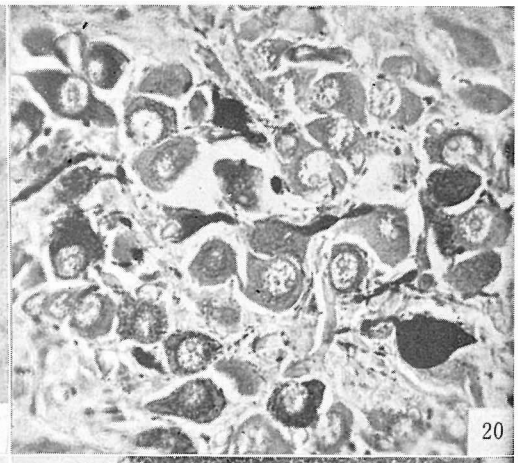
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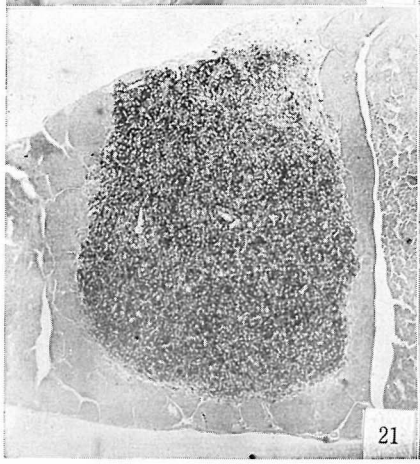
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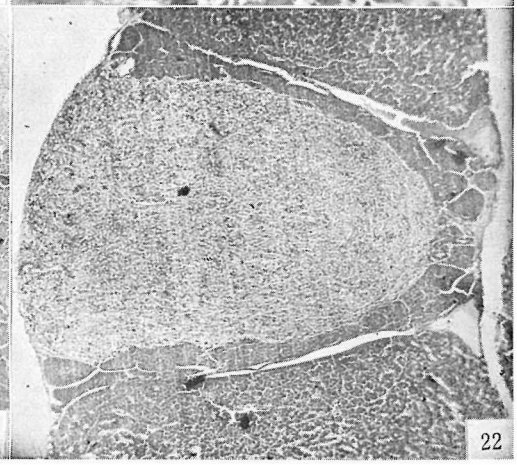
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