

The Intracellular Localization of Hormonal Activity in Transplantable Thyrotropin-Secreting Pituitary Tumors in Mice*

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ABSTRACT

Mouse pituitary tumors secreting almost exclusively thyroid stimulating hormone have been characterized electron microscopically.

Tumors of known thyrotropin content were separated into nuclear, mitochondrial, microsomal, and soluble fractions by differential centrifugation. The hormonal activity of these fractions was correlated with that of the total homogenates and with their nitrogen and phosphorus content. Essentially all the thyrotropin of the homogenate was recovered in a particulate fraction sedimenting between 20,000 and 40,000 g. This fraction contained the RNA granules and membranous components typical of microsomal pellets, but also showed the presence of small dense bodies surrounded by smooth membranes. These bodies were also visible within the endoplasmic reticulum of intact cells, and it is postulated that these bodies may represent the sites of intracellular elaboration and/or storage of TSH.

Thyrotropin is tightly associated with microsomal particles but can be brought into solution by treatment with alkaline media, deoxycholate, and certain organic solvents.

INTRODUCTION

The nature of the sites of hormone synthesis and storage within the cells of adenohypophysis is a subject of considerable importance. It has recently been demonstrated by Purves and Griesbach (21, 22), Halmi (14), and Barrnett *et al.* (1) that in the rat TSH is produced by the basophilic cells of the anterior pituitary. On the other hand, Brown and Hess (4) employing the technique of cell-fractionation by differential centrifugation found TSH in both the acidophilic and basophilic granules of beef pituitary, whereas Herlant (16) claimed to have found it mostly in the basophilic granules and the supernatant.

There was hitherto no information on the intracellular localization of hormonal activity in

the TSH-secreting transplantable pituitary tumors that have been developed recently in mice. The mechanism of induction of these tumors, their monomorphous nature, and histologic identification as chromophobes or faint basophils, their high TSH content and ability to secrete almost exclusively TSH over several transplant generations have been described (2, 3, 11, 12). The present study is oriented toward the identification of the sites of TSH-synthesis and storage as well as an understanding of the mechanisms which control the rate of formation and release of hormone. This report deals with the isolation of hormone-bearing subcellular particles and their subsequent characterization.

Materials and Methods

The material used in this study came from eight tumor grafts in two successive generations of the same dependent tumor strain carried in radiothyroidecto-

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mized female LAF₁ mice. Tumors were used about five to seven months after graft or two to three months after they became palpable.

Homogenization and Cell-Fractionation.—Tumors were removed from the animals under ether anaesthesia and placed in a petri dish containing ice cold 0.25 M sucrose. They were dissected free of connective tissue and necrotic areas, blotted, weighed, and homogenized in precooled (0°C.) 0.25 M sucrose. A motor driven Potter-Elvehjem homogenizer fitted with a teflon pestle was used to make a 10 per cent (weight/volume) homogenate. In initial experiments, the homogenates were fractionated exactly according to Schneider and Hogeboom (23). Once it was demonstrated that the mitochondria contained little or no TSH activity, the following procedure was adopted:

1. Nuclei, unbroken cells, and debris were removed at 700 g in 10 minutes.
2. Mitochondria and large granules were sedimented at 20,000 g in 10 minutes.
3. Small granules were sedimented by centrifuging the 20,000 g supernatant at 40,000 g for 60 minutes (MS 40,000 g) or at 105,000 g for 60 minutes (MS 105,000 g). All centrifugations were done between 0° and 3°C. In all the experiments, each particulate fraction except (MS 40,000 g) was washed at least once and the washings were combined with the successive supernatant during the course of serial centrifugation. All fractions were finally resuspended to a known volume in 0.25 M sucrose. Aliquots of whole homogenate and fractions were frozen until assayed.

To determine the effect of environment on the TSH content of the microsomes, granules obtained by centrifugation at 40,000 g in 0.25 M sucrose were incubated under the conditions stated in Table II and thereafter resedimented at 40,000 g for 60 minutes. The sediment was resuspended in a known volume of 0.25 M sucrose and assayed. Aliquots of the supernatant were also assayed.

Total N was determined by direct nesslerization after digestion of aliquots in sealed tubes at 400°C.

Total P was determined by the method of Lowry *et al.* (19).

Spectroscopic determinations of protein and RNA were carried out in the Beckman model DU-spectrophotometer at 280 and 258 m μ respectively.

Biological Assay of Thyrotropic Activity.—Thyrotropic activity of each preparation was determined by assay using a method employing day-old white leghorn chicks¹ in which the five hour uptake of I¹³¹ was measured 24 hours following the last of three daily subcutaneous injections of control and test solutions. This is a method derived from earlier observations of others [17, 24].

The cockerels received on the day of hatching were

injected immediately subcutaneously in the neck with 1 milliliter amounts of 0.15 M NaCl solutions of thyrotropic standards of unknown fractions. Five to eight animals were used per fraction and constituted a group. Each injected chick was labeled, placed in a cage with others of his group, and subsequently kept at brooder temperatures of 85–95°F. A second and third TSH injection was given at daily intervals. Tap water was available throughout the assay and a low iodine starter mash² was used from the evening of the second to the evening of the third assay day. A control group receiving 0.15 M NaCl and four groups injected with u.s.p. Thyrotropin Reference Standard at dosages from 1.56 to 50 International milliunits per milliliter were included in each assay of unknown fractions.

Twenty-four hours after the third TSH injection, 0.2 to 0.3 μ c. of carrier-free I¹³¹ was given subcutaneously to all animals. Five hours later, the chicks were sacrificed by chloroform inhalation, thyroids excised, and the per cent uptake of the injected I¹³¹ calculated from measurements of the radioactivity determined by a well-type scintillation detector. The data obtained were statistically evaluated according to the method described by Gaddum (13), unknown fractions containing thyrotropic activity being tested at two dose levels. Probabilities indicating the significance of differences between mean values of tested fractions were determined by analysis of variance (6, 10).

Previous experience with this assay (5) has shown a sensitivity (minimal detectable dose) of 1.5 to 3.0 International milliunits per injected dose with a log dose response over a range covering approximately a 20-fold increase in dose. The mean index of precision (λ) for thirty assays was 0.26 ± 0.01 , a value which is not significantly different from that of the assays reported here.

Electron Microscopy.—Small portions of the tumor from which the microsomal fraction MS 40,000 g was obtained were fixed in cold, (0°–5°C.) buffered 1 per cent osmium tetroxide containing 0.25 M sucrose for 1 to 1½ hours. They were dehydrated rapidly in ascending concentrations of methanol and embedded in *n*-butyl methacrylate which had been partially polymerized at 60°C. for 30 minutes. Polymerization was completed by incubation of the methacrylate-containing gelatin capsules at 60°C. overnight. Sections were cut at 40 to 60 m μ with a Porter-Blum microtome and picked up on carbon-coated and celloidin-covered copper grids and examined in an RCA EMU-2E microscope. Electron micrographs were taken at original magnifications ranging from 3600 to 13,000.

An aliquot of the microsome suspension was sedimented as indicated above, the supernatant decanted, and the cold, buffered 1 per cent osmium tetroxide

¹Hall Brothers Hatchery, Inc., Wallingford, Connecticut.

²Wirthmore Feeds, Boston, courtesy of Dr. W. A. Glista.

containing 0.25 M sucrose was added to the pellet in the plastic tube. The pellet was dislodged intact with a thin spatula from the wall of the plastic tube and fixation proceeded for 1 to 2 hours at the same temperature. The round, biconvex pellet was divided into a number of wedge-shaped blocks so that the full thickness of the pellet could be oriented in the embedding capsule to coincide with the facing surface of the block at microtomy. Dehydration embedding and sectioning were carried out as mentioned above.

RESULTS

1. *Distribution of Hormonal Activity, Total N, and Total P in Subcellular Fractions of TSH-*

Secreting Tumors.—Initial experiments with six different tumors showed that the nuclei and mitochondria contained little of the hormonal activity of the tumor homogenate and that most of the TSH could be recovered in a particulate fraction, sedimenting between 20,000 and 105,000 g (Table I). Subsequent studies showed that over 90 per cent of the TSH could be recovered in the granules sedimenting between 20,000 and 40,000 g with no additional recovery at higher centrifugation speeds. Table I also shows the distribution of total N and total P in tumor cell fractions in two typical experiments. Most of the nitrogen and

TABLE I
Distribution of Thyrotropic Activity, Total N, and Total P in Tumor Homogenate and Fractions (Mouse Pituitary Tumor Transplant)

Preparation	Experiment No.	Units* of TSH/ gm.‡ ± s.e.	Activity recovered	Total N mg./gm.‡	N re- covered	mμ TSH per mg. N in fraction	Total-P μg./gm.‡	P re- covered
			<i>per cent</i>		<i>per cent</i>			<i>per cent</i>
Whole homogenate	1	10.65 ± 0.95	100	14.90	100	714	2273	100
	2	10.80 ± 1.25	100	14.75	100	732	2273	100
Nuclear fraction	1	1.25 ± 0.27	11.8	5.00	33.6	250	706	31
	2	1.66 ± 0.16	15	4.84	32.8	342	687	30.2
Mitochondria and large granules	1	0.75 ± 0.05	7	1.47	9.9	510	298	13.1
	2	—§	<9	1.60	10.8	<629	253	11.1
MS 40,000 g fraction	1	11.85 ± 1.45	112	1.12	7.5	10580	344	15.1
	2	10.12 ± 1.24	100	1.60	10.8	6325	407	17.9
Supernatant 40,000 g	1	0.83 ± 0.1	7.8	7.26	48.7	115	918	40.4
	2	0.6	5.5	6.63	45.0	90	978	43.0
MS 105,000 g fraction	1	10.5 ± 2.1	92	3.34	22.4	3143	592	26.0
	2	10.8 ± 1.3	100	3.19	21.6	3384	522	23.0
Supernatant 105,000 g	1	1.48 ± 0.16	13	4.96	33.3	298	744	32.7
	2	0.75 ± 0.1	7	5.02	34	149	835	36.7
Total additive recovery	1		123–138		99–99.5			99–102
			129–131		94–97			100–102

* The mean index of precision (λ) of these assays was 0.289 ± 0.062 .

‡ Per total preparation derived from 1 gram wet weight of fresh tissue.

§ This signifies no measurable activity.

|| This represents figures obtained by adding per cent recovery values for nuclear fraction, mitochondria and large granules, MS 40,000 g (or MS 105,000 g) and supernatant 40,000 g (or supernatant 105,000 g).

Details are given in methods and materials section. In Experiment 1, the homogenate and fractions were assayed at comparable doses. In Experiment 2, the nuclear fraction, mitochondrial, and large granular fraction and the final supernatant fraction were assayed at concentrations five times greater than those of the whole homogenate and the microsomal fractions.

TABLE II

The Effect of Environmental Variables on the Thyrotropic Activity of the Microsomal Fraction (MS 105,000 g)

Conditions of treatment	Units* of TSH in fraction			Significance of difference between means (P)‡	
	Pellet before treatment	Treated pellet	Supernatant	Original vs. treated	Original pellet vs. supernatant
A. Frozen and thawed 3 times in 0.25 M sucrose	4.5	3.0	—§	0.3-0.4	<0.01
B. Incubated at 0°C. for 30 min. in					
I. 0.25 M sucrose	4.5	4.5	—§	0.6	<0.01
II. distilled water	4.5	3.0	—§	0.4	<0.01
III. sodium chloride					
0.05 M	4.2	5.5	—	0.6-0.7	<0.01
0.15 M	4.2	5.0	—	0.5-0.6	<0.01
IV. calcium chloride					
0.05 M	4.2	5.5	—	0.8-0.9	<0.01
0.15 M	4.2	4.5	—	0.5-0.6	<0.01
V. magnesium acetate					
0.05 M	4.2	6.0	—	0.7-0.8	<0.01
0.15 M	4.2	6.0	—	0.9	<0.01
VI. disodium EDTA					
0.05 M	4.2	6.0	—	0.9	<0.01
0.15 M	4.2	6.0	—	0.9	<0.01
VII. acetate buffer					
pH (3.5) 0.15 M	4.2	1.2	1.2	0.2-0.3	0.2-0.3
VIII. tris (trihydroxymethyl amino ethane) buffer					
pH (8.6) 0.15 M	4.8	0.5	4.2	0.1-0.2	0.6-0.7
C. Incubated at 30°C. for 30 min. in					
I. 0.25 M sucrose (pH 7.2)	7.5	4.5	0.15	0.4	0.1
II. 0.25 M sucrose containing 0.02 per cent RNase	7.5	5.0	0.75	0.4	0.1
D. Incubated at 0°C. in 0.25 M sucrose containing 0.3 per cent deoxycholate. (pH 7.45) for					
I. 3 min.	15	7.5	7.5	0.2-0.3	0.2-0.3
II. 30 min.	7.5	—§	7.5	<0.01	>0.95

* The mean index of precision (λ) of these assays was 0.301 ± 0.05 .

‡ From "t" table, Fisher, 1946.

§ This signifies no measurable activity at comparable or higher doses.

|| Supernatant fractions in these cases were brought to neutral pH and frozen until assay.

¶ Crystalline ribonuclease of Nutritional Biochemicals Corporation, Cleveland, Ohio, was used.

Details are given in methods and materials section. Each preparation was assayed twice. Mean values of potency are reported for each preparation.

phosphorus is found in the final supernatant and nuclear fraction. It is of interest to note that in Experiment 2, in which the least active fractions were tested at five times the concentration of the homogenate and the active pellets, the mitochondria, and the final supernatant contained less than 10 per cent of the original activity. The small amount of activity found in the nuclear fraction in both the experiments is probably due to unbroken cells which sediment with the nuclei. The MS

40,000 g pellet is an incompletely sedimented microsomal fraction but is more representative of the hormone-bearing granules than that obtained at higher centrifugation speeds (MS 105,000 g). Thus, MS 40,000 g pellet has a hormonal content of 6 to 10 units/mg. N compared with 3 to 3.5 units/mg. N for MS 105,000 g pellet and 0.7 to 0.75 units/mg. N for the total homogenate. The over-all additive recovery of TSH in subcellular components which is about 130 per cent of the

total homogenate is within the limits of error of the assay, and does not represent a significant increase in the activity.

2. *Effect of Environmental Variables.*—Data listed in Table II demonstrate that TSH is very tightly bound to the active particles. Freezing and thawing as well as a wide range of environmental conditions of ionic strength and ionic composition are not effective in producing release of the hormonal activity into solution. Furthermore, treatment with ribonuclease did not solubilize significant activity, whereas exposure to alkaline pH solubilized most of the pellet activity. Treatment with deoxycholate readily brought into solution significant amounts of TSH. The slight increase of thyrotropic activity in the case of pellets treated with salt is not significant. In acid pH, however, there is some loss of activity probably due to inactivation. The validity of these statements was tested by analysis of variance between groups representing untreated pellets and treated fraction with computation of P values (10).

3. *Extraction of TSH from Active Particles.*—Upon extraction of microsomes at -5°C . with 10 volumes of methylal:methanol (4:1) the thyrotrophin remained in the insoluble precipitate. After treatment of this residue with 70 per cent ethanol at -5°C ., more than 70 per cent of the hormonal activity could be recovered from the precipitate by extraction with 0.005 M NaCl, yielding material with a potency of 2 units/mg. dry weight. Similar treatment of whole tumors gave material with a potency of 0.25 to 0.3 units/mg.

MORPHOLOGY

1. *Tumor.*—Sections of this functional TSH-secreting tumor which were examined by light and electron microscopy showed a well organized cellular parenchyma, many mitotic figures, and evidence of good vascularization (Figs. 1 and 2). The nuclei appear polymorphic, and in thin sections it was common to find circumscribed regions of cytoplasm seemingly isolated within the karyoplasm. In such instances a nuclear envelope could be seen separating the islands of cytoplasm from the karyoplasm (Fig. 3, lower nucleus). The limits of the plasma membrane can be defined only with difficulty even in a suitably oriented thin section (Figs. 2 and 3).

The presence of large vacuolar spaces (*erv*) of varying size within the cytoplasm of the tumor cells as well as at the cell margins is characteristic

of this tissue (Figs. 1 to 3) (7). These vacuoles are similar to the colloid-filled vesicles appearing in the thyrotrophic basophils of the rat adenohypophysis after thyroidectomy (15, 8) but lack the colloid and occasional dense granulation contained in the vesicles.

Near the larger vacuolar spaces are small dense bodies (*sv*) approximately 30 to 80 μm in their largest diameter; their contents are rather homogeneous and are moderately electron-opaque (Figs. 2 to 5). These dense bodies, in favorable regions of section, are surrounded by smooth surfaced membranes. The similar electron-scattering properties of the dense bodies and the surrounding membranes make the identification of the two elements rather difficult in some areas of the sectioned material. It could very well be that some of the dense bodies lack a membrane, or that some of them just contain a larger space between the granule and the enclosing membrane. The membranes that surround the granules appear to be similar to the membranous components of the endoplasmic reticulum and are presumably derived from the region of the Golgi complex (Fig. 5). The appearance of vacuolar spaces in thyrotrophs in response to thyroidectomy and in functional TSH-secreting tumor material may represent a physiological transformation of the membranous elements of the endoplasmic reticulum and Golgi complex, into a discontinuous system of dilated vacuoles which form in the cytoplasm in varying amounts depending upon the nature and extent of the stimulus which induces such a cellular response.

The dense bodies are smaller than the granules found in thyrotrophs of normal rat pituitaries (8). They are so often near vacuoles or near the plasma membrane as to suggest that they may represent a stored form of the hormone, whose active principle may be released *via* this vacuolar system to the outside upon some appropriate stimulus.

Mitochondria appear to be normal morphologically; they are disposed randomly throughout the cytoplasm and have no preferential orientation to other cell constituents.

2. *Pellet.*—Electron micrographs of microsomal pellet MS 40,000 g, show numerous elements of the endoplasmic reticulum (*er*) as well as the small dense bodies (*sv*) (Figs. 6 and 7). The largest number of endoplasmic reticulum fragments and dense bodies is found at the lowermost part of the

pellet. There are few RNA particles at this depth; they are most numerous in the upper regions of the pellet. In Fig. 6, a portion of the central part of the pellet was enlarged to show the similarity in size and electron opacity of the isolated dense bodies (*sv*) to those found *in situ* (Fig. 5).

Particles having the identifying features of molecular ferritin (18) are frequently seen in the pellet material (Fig. 6 *f*). Similar ferritin particles were not seen within the cell cytoplasm. The significance of this observation is obscure.

Fig. 7 and 8 are two comparable regions of control and deoxycholate-treated microsomal pellets MS 40,000 and MS 105,000 g, respectively. The former is an untreated pellet and shows a large population of dense bodies (*sv*), membranous and canalicular elements of endoplasmic reticulum (*er*), and a few RNA-rich particles in the background. Untreated pellets spun at 105,000 g were not examined by electron microscopy but on the basis of their TSH content, these would be expected to show a similar concentration of dense bodies. In Fig. 8 the deoxycholate-treated pellet MS 105,000 g shows a striking reduction in the number of dense bodies (*sv*), the presence of some remaining elements of the endoplasmic reticulum, and the fragmentation of much of the membranous material. RNA-rich particles were present in sections taken at higher levels of this pellet, and there appeared to be no difference in their morphology as a result of treatment. The thyrotropic activity of this fraction was reduced by about 50 per cent and the residual hormonal activity is presumably attributable to the dense bodies and other remaining membranous components.

DISCUSSION

The ultrastructure of the dependent transplantable TSH-secreting tumor which we have described and illustrated briefly confirms the recent findings of Farquhar and Furth (7).

We have been able to show biochemically that the thyrotropic activity is associated with the membranous portion of the tumor microsomes. In addition we have been able to localize, within the tumor cells, as well as within the microsomal pellets, a small electron-opaque microgranule, approximately 30 to 89 μ in diameter, which in favorable regions appears to be surrounded by smooth surfaced membranes presumably derived from the endoplasmic reticulum. In addition, the relationship of these dense bodies to the Golgi

complex suggests that the microgranule formation may occur at or near this cytoplasmic site. In normal cells of the anterior pituitary, the Golgi complex has been implicated as the principal locus of secretory granule formation (9, 20).

TSH is tightly bound to components of the microsomes, as shown by the fact that it cannot be solubilized by ribonuclease or exposure of microsomes to media of widely varying ionic strength, ionic composition, and osmolarity.

It is thought that the small dense bodies may represent a stored form of the hormone and mark the site of its formation and that hormone secretion may occur *via* the channels of the endoplasmic reticulum upon the proper stimulus.

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EXPLANATION OF PLATES

(The solid line next to each figure number represents 1 micron unless otherwise indicated.)

PLATE 111

FIG. 1. A portion of the transplantable TSH-secreting tumor, fixed in buffered osmium tetroxide, embedded in methacrylate, sectioned at approximately $2\ \mu$, and stained with periodic acid-Schiff (PAS) and hematoxylin. This field shows a number of cells having irregularly shaped oval or elongate nuclei within which are prominent hematoxylin-stained bodies. At the upper left margin is a mitotic figure in late prophase. The cytoplasm stains very weakly with PAS giving the impression of a slight pinkish wash. No PAS-positive granules are observable within the cytoplasm of the parenchymal cells. Cell boundaries are indistinct and only the basement membranes of the sinusoids (*bm*) stand out clearly. Occasionally, small lighter areas or vesicles can be distinguished and these are thought to be the dilated vesicular component of the endoplasmic reticulum (*erv*) as seen in Fig. 2. $\times 1800$.

FIG. 2. A survey field of the tumor showing the cytoplasm of six cells and the nuclei (*N*) of five of them. Immediately evident in this micrograph are the abundance of large intracellular vacuoles which are regarded as the dilated membranous components of the endoplasmic reticulum (*erv*). Other areas of the endoplasmic reticulum are organized into strands in a paranuclear position; it is at these sites that the small particulate RNP particles (*erp*) are found associated with the membrane. Occasionally, a plasma membrane (*pm*) can be identified. The difficulty in distinguishing the limits of the cell's cytoplasm appears to be due to the highly convoluted and involved intersusception of the apposed plasma membranes. At this relatively low magnification, small, moderately to high dense bodies (*sv*) are observed in favorable regions of the cytoplasm. Mitochondria (*M*). $\times 12,000$.

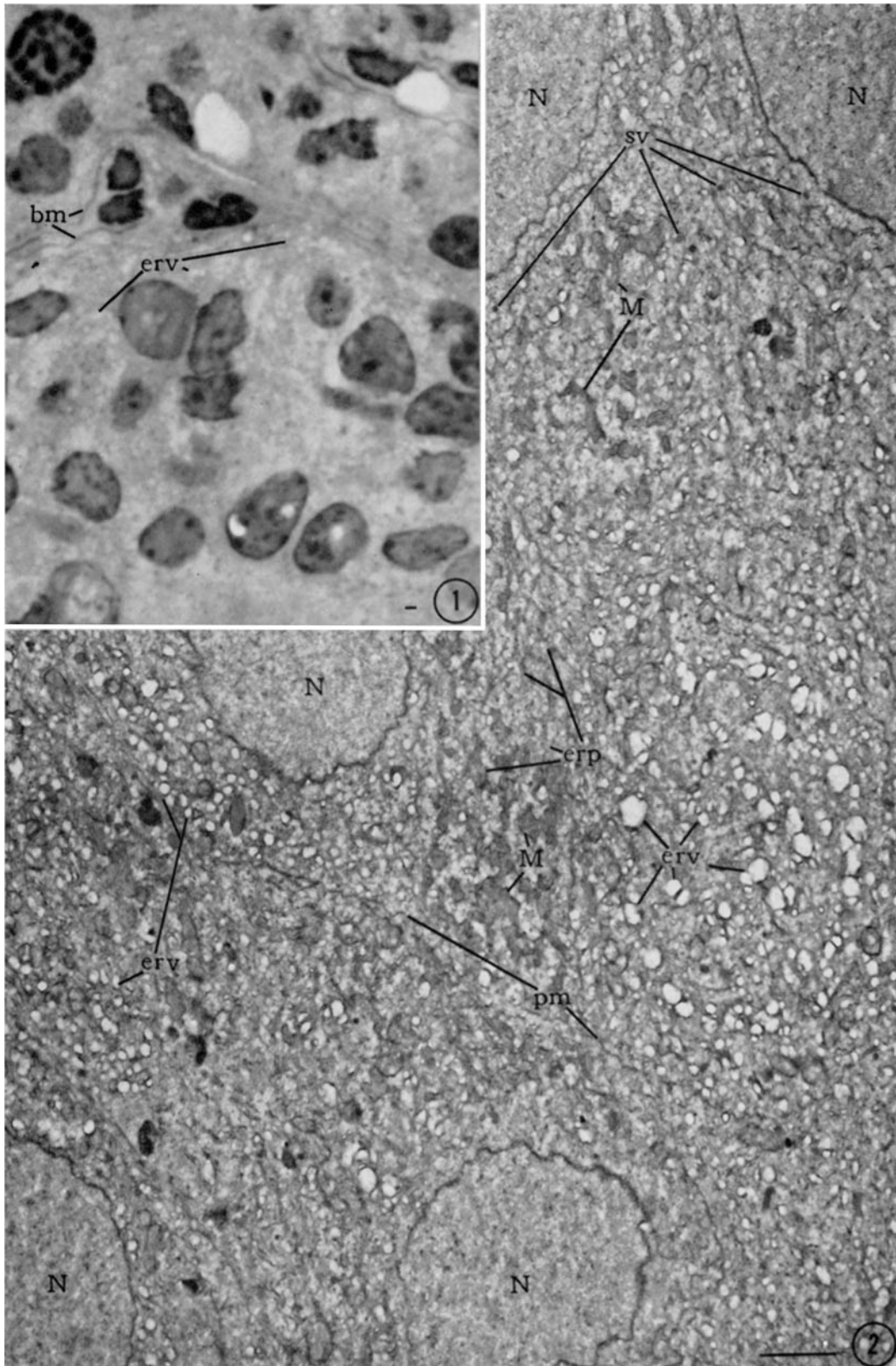


PLATE 112

FIG. 3. An area of the cytoplasm of three cells showing the extremely irregular course of the plasma membranes. The cell at the upper left appears to have a projection of cytoplasm about which the plasma membrane (*pm*) can be identified. Shown to good advantage are the small dense bodies (*sv*) within the cytoplasm. These have a definitive membrane about them; in certain areas (indicated by stars) these opaque bodies are seen in close proximity to the plasma membrane or to the larger vacuolar system of the endoplasmic reticulum within the cytoplasm (not labeled here, but equivalent to *erv* of Fig. 2; also see Fig. 4). $\times 22,500$.

FIG. 4. A higher magnification of a portion of the cytoplasm of tumor cells showing the relation of some of the small dense bodies (*sv*) to the larger vacuolar regions. The close proximity of these dense forms to the dilated membranous component of the endoplasmic reticulum may provide an avenue by which the material contained within the densities could be conducted out of the cell and released into the circulation. $\times 33,000$.

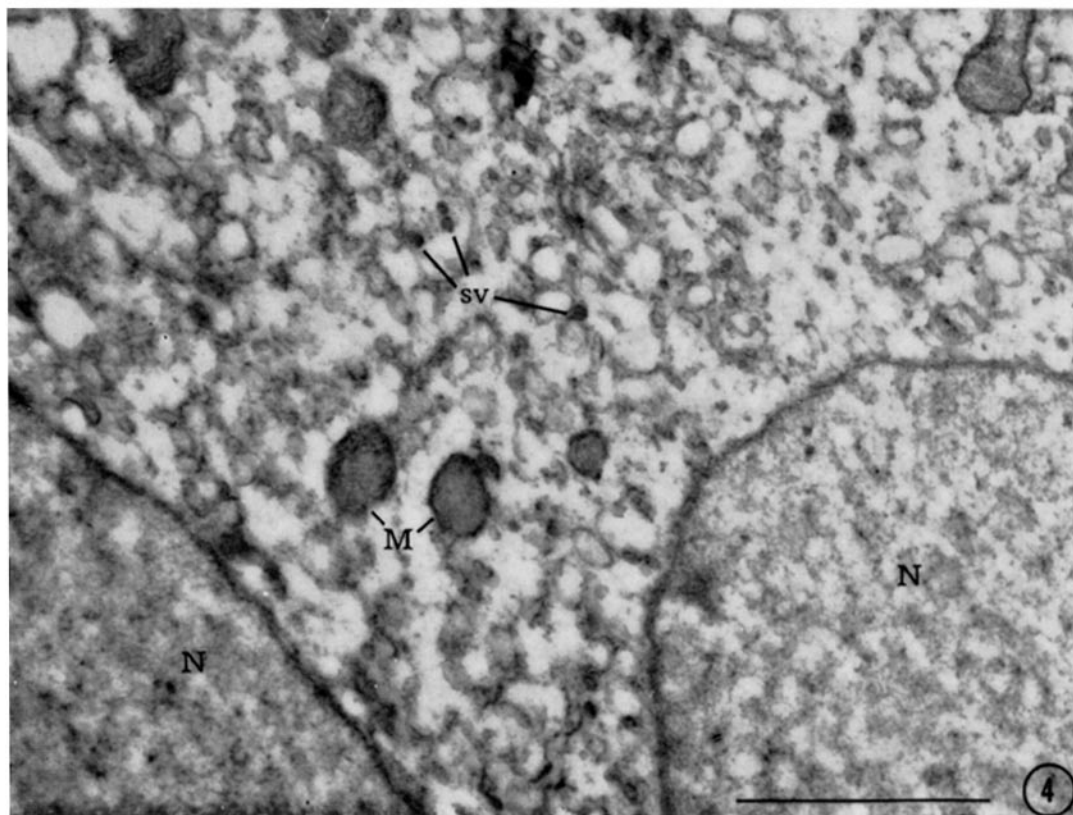
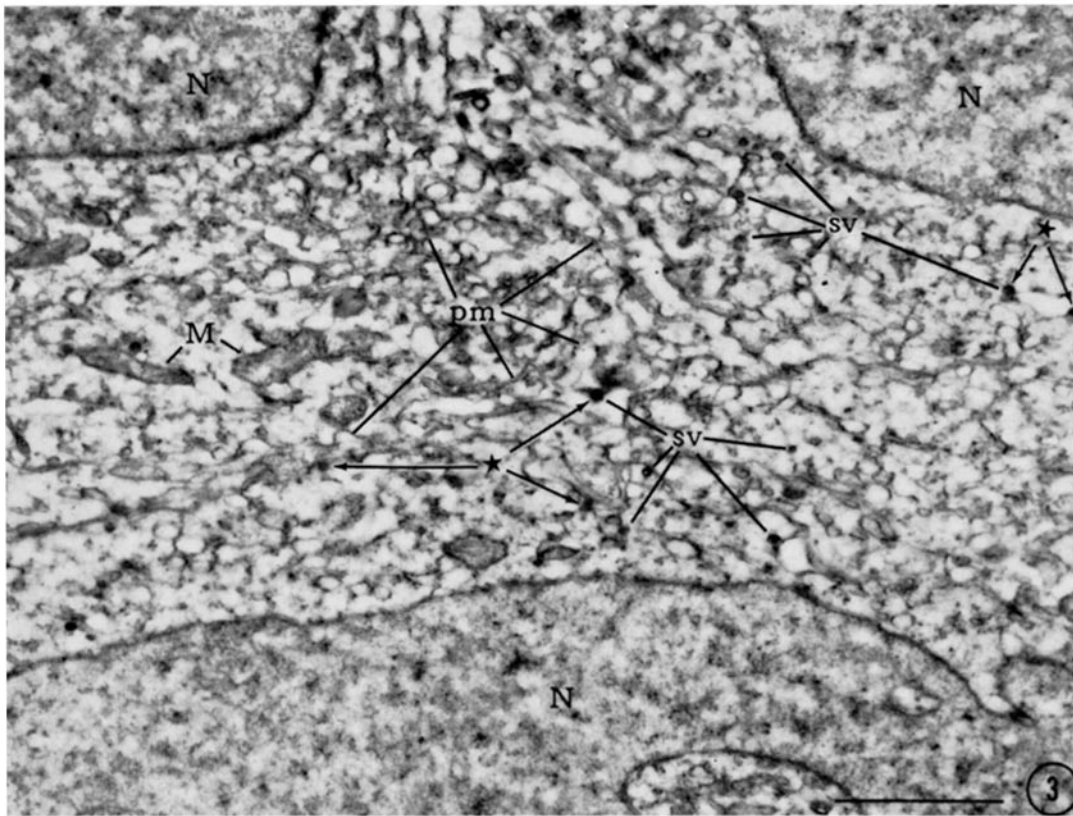


PLATE 113

FIG. 5. A highly magnified portion of a cell showing some of the cytoplasmic relations in the juxtannuclear region. An area of the expanded Golgi complex (*GC*) occupies the major part of the field. At the periphery of the Golgi complex, small dense bodies (*sv*) of the type seen in the previous micrographs are encountered. In some of these bodies a distinct membrane can be distinguished enclosing the opaque material, whereas in others, the plane of section through the vesicular structure does not permit such an observation. These dense bodies measure from 30 to 80 $m\mu$ at their widest diameter. Such micrographs as this suggest that the dense material may be formed in the Golgi region, be pinched off as small vesicular units, or granules, and then make its way quickly to the nearest point of cellular exit which, in these cells, is the ubiquitous vacuolar system making up a portion of the endoplasmic reticulum. $\times 62,000$.

FIG. 6. A portion of a pellet which was spun at 40,000 *g* and which was derived from the tumor tissue seen in Fig. 5. The dense vesicular bodies (*sv*) illustrated here have a measured diameter from 43 $m\mu$ to 70 $m\mu$ and these figures agree quite closely with the size of these units *in situ*. Some of these bodies possess a more opaque, peripheral membrane; other vesicular profiles identified as portions of the endoplasmic reticulum, do not contain as dense a center. Frequently, particles resembling those of molecular ferritin (*f*) are seen in the pellets, but are not found in the cells. $\times 59,000$.

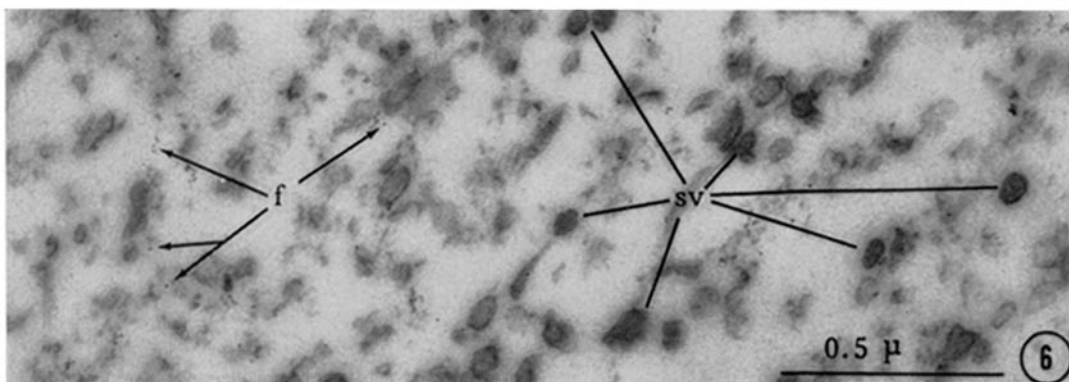
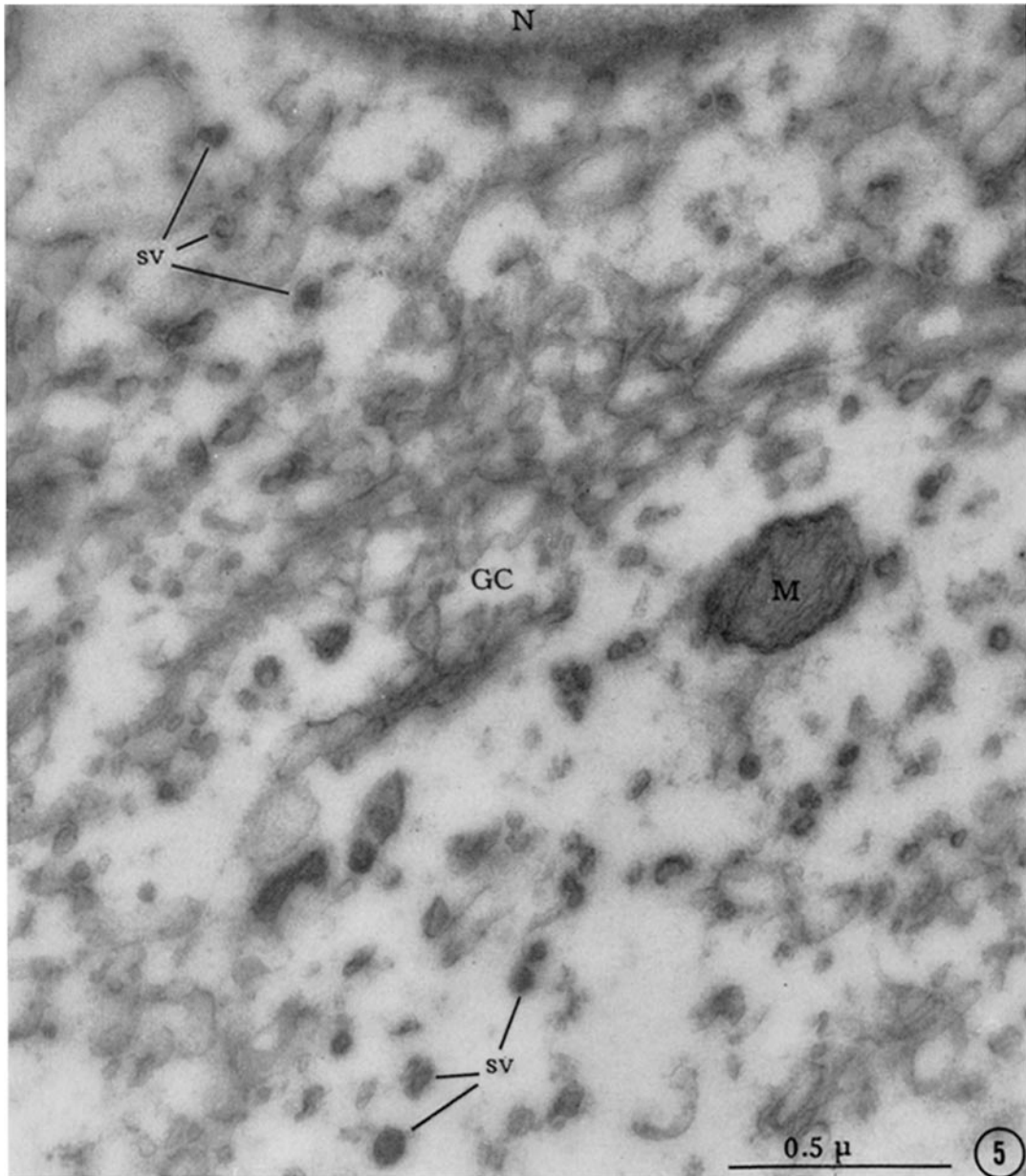


PLATE 114

FIG. 7. A portion of a pellet of the microsome fraction MS 40,000 *g* showing the concentration of many profiles of the dense vesicular bodies and of the smooth surfaced vesicular and canalicular elements of the endoplasmic reticulum. Some RNA-rich particles are diffusely spread in the areas between these two principal components of this fraction, but in this region of the pellet, they are not numerous. Aliquots of this pellet assayed for thyrotropic activity gave results comparable to those obtained with the original tissue homogenate. $\times 45,000$.

FIG. 8. A portion of a pellet of the microsome fraction MS 105,000 *g* which had been treated with deoxycholate and processed similarly to the pellet illustrated in Fig. 7. A region of this pellet comparable to that illustrated in Fig. 7 shows only a few small dense bodies (*sr*), and remnants of the various membranous components of the endoplasmic reticulum, and some seemingly unsolubilized vesicular profiles of typical vesicles of endoplasmic reticulum (*er*). The RNA-rich particles can be identified in other parts of the micrograph, but this region of the pellet does not contain the great bulk of the particles. The thyrotropic potency of aliquots of this microsomal fraction was reduced over 50 per cent; consistent with this loss in hormonal activity was the great reduction in the population of the small dense bodies and other smooth surfaced vesicular components of the endoplasmic reticulum. $\times 45,000$.

