MORPHOLOGIC AND FUNCTIONAL CHANGES ASSOCIATED WITH TRANSPLANTABLE ACTH-PRODUCING PITUITARY TUMORS OF MICE *

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Recently, Furth and associates[1] reported the occurrence of transplantable pituitary adenomas in mice exposed to ionizing radiation. This study concerns one of the two types of pituitary tumors induced by radiation which caused marked hypertrophy of the adrenal glands of the primary hosts.[2] The following data are on the morphology and function of such primary and transplanted tumors and the ultimate effects of tumors upon the hosts. It will be shown by a variety of endocrinologic techniques that these tumors synthesized adrenocorticotropic (ACTH) hormone. Prolonged uncontrolled secretory activity of the neoplasms resulted in hypertrophy and hyperfunction of the adrenal cortices of the hosts. The ultimate clinical manifestations of these changes were obesity, lymphopenia, cessation of estrual cycle, a diabetes insipidus-like syndrome, and a fatal, generalized, pyogenic infection. These features constitute a syndrome, characteristic in mice of hyperadrenocorticalism.

MATERIALS AND METHODS

A total of approximately 300 LAF_1 male and female mice were used in this study. They were maintained in individual metabolic cages or in a colony and all received a standard diet consisting of Purina Chow and tap water ad libitum, supplemented weekly with fresh greens.

Details relating to the occurrence of the primary pituitary tumors have been reported by Upton and Furth.[3] The three primary hosts upon which this study is based were female LAF_1 mice which survived exposure to ionizing radiation from an experimental atomic detonation composed principally of gamma rays with a small admixture of neutrons, totaling 200 to 700 rep. All three mice were sacrificed within 24 to 30 months following exposure to radiation at a time when each appeared to be moribund.

In order to obtain additional data concerning the functional nature

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of the primary tumors, the bulk of the tumors was minced in saline solution and injected into the thigh muscles of male and female mice. The following descriptions are limited to the effects of the first 5 generations of transplants which produced the greatest degrees of adrenal hypertrophy and the most marked functional alterations. The tumors are still functional after 4 years during which time over ten successful passages were made.

All mice were necropsied. Since the major changes recorded were observed consistently, they will be described without stating the numbers of specimens observed except when the observations were based on small groups of animals. Most tissues saved for paraffin sections were fixed in Zenker's or Bouin's fluid. All staining procedures, unless specifically stated, are those described by Lillie.[4] Numerous animals have been preserved *in toto* for future studies.

Results

Primary Tumors and Hosts

Shortly following exposure to radiation, the three primary hosts showed moderate graying of the hair over the entire body. The mice were 24 to 36 months old when sacrificed. Grossly, each of the three pituitary glands measured about 5 mm. in average diameter. Histologically, the primary tumors, which henceforth shall be referred to as AtT* 1, 2, and 6, were similar and composed of uniform polyhedral cells. Mitotic figures were present. No specific chromophilic cytoplasmic granules could be identified by hematoxylin and eosin or by the Gude-Martins-Mallory stains.[5] Tumors 1 and 2 were confined to the sella turcica while tumor 6 extended into the floor of the sella and infiltrated into the tissues about the intracranial segment of the internal carotid artery.

The adrenal glands of the primary hosts were estimated to be twice normal size. Histologically, they showed marked hypertrophy of the fascicular and reticular zones. The cells of the zona glomerulosa were only slightly enlarged. The x-zone, usually present in the juxtamedullary portion of the adrenal cortex of female mice, was absent. The cells of the adrenal medulla were large and contained prominent chromaffin granules (Figs. 2, 4, 6, 8, 9, and 10).

Functional Effects of Transplanted Tumors

Within 2 to 6 months following transplantation, at a time when the grafted tumors were barely palpable, a typical clinical syndrome de-

* Adrenotropic tumor.

veloped in both male and female mice. The changes consisted of obesity, polydipsia, polyuria, and lymphopenia. In female mice there was cessation of the estrual cycle. The magnitude of these changes during the 7-day period of maximum urine volume is recorded in Table I. Hyperglycemia and glycosuria occurred only in a few mice bearing transplants of the first generation of tumor strains 1 and 2. In all other tumor-bearing mice neither glycosuria nor hyperglycemia was observed. The results of studies of glucose tolerance, of sodium, potassium, and nitrogen balances, and of urinary steroid excretion are reported separately.[6-8]

Immediate Cause of Death

Some mice died with no gross morphologic or histologic lesions, except hypertrophy of the adrenal cortex, to account for death. In these mice it was assumed that the electrolyte disturbances attending hyperadrenal cortical activity were fatal. Death in most tumor-bearing mice was preceded by a generalized pyogenic infection during which abscesses formed in the lungs (Fig. 13), kidneys, liver, myocardium (Fig. 11), endocardium (Fig. 12), pancreas, and adrenal glands. Microscopically, these lesions were composed of ne-

TABLE I

Ranges of Functional Effects of Transplanted Tumors in Intact Mice

Tumor strain	No. mice	Food per day		Water per day		Urine per day		Urine specific gravity		Total leukocytes $\times 10^3$		Lymphocytes	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min	Max.
		gm.	gm.	ml.	ml.	ml.	ml.					%	%
Normal	51	2.3	7.1	2.0	10.4	0.2	2.9	1,060	1,070	9.7	12.1	83	96
1	16	2.1	8.0	9.5	71.0	5.0	59.0	1,014	1,060	0.3	4.8	0	16
2	3	5.4	6.5	18.0	39.0	4.1	34.0	1,014	1,030	1.6	4.4	0	5
2	17	3.0	8.7	5.0	27.0	2.0	17.0					4	28
6	3					2.0	27.0			2.0	3.4	0	24

crotic amorphous débris and masses of Gram positive, periodic acid-Schiff (PAS) positive bacilli (Fig. 14). The leukocytic infiltration of tissues surrounding abscesses was not extensive and there was no evidence of delimiting fibroblastic activity. A diphtheroid organism (*Corynebacterium Kutcheri*) was repeatedly isolated from such lesions. Bilateral total adrenalectomy was the only effective means of controlling manifest infection. Slight infection could be controlled with terramycin in drinking water. This proved to be an excellent prophylactic measure.

Morphology of Transplanted Tumors

The transplanted tumors were small and at death usually weighed less than 500 mg. The tumors did not metastasize to regional lymph nodes or to other organs but infiltrated muscles at the site of implantation (Fig. 16). Histologically, the tumors were composed of relatively small cells which usually were arranged in cords about foci of necrosis. Necrosis was common and extensive in normal hosts, scant in adrenalectomized hosts. The nuclei were hyperchromatic and mitotic figures were present. The cytoplasm showed moderate diffuse basophilia when stained with azure-eosin mixture (pH 4.5), but contained no specific chromophilic granules when stained with the Gude-Martins-Mallory,[5] chromalum hematoxylin-phloxine, phosphotungstic acid-hematoxylin, periodic acid-Schiff, aldehyde fuchsin, and hematoxylin and eosin stains. By the usual criteria, the tumors can thus be considered to be chromophobic adenomas (Fig. 15).

Prolonged serial transplantation of tumor 2 (Table II) resulted in increased pleomorphism of neoplastic cells accompanied by marked increase in the maximum weight of the tumor and decrease in ACTH production as judged by adrenal weights and water intake of tumor-bearing mice.

Morphologic Effects of Transplanted Tumors

The weights of organs of intact mice bearing tumors are recorded in Table II. Most tumor-bearing mice were very obese. The maximum body weight of some mice reached more than 50 gm. as compared to a maximum of 35 gm. in control mice of the same age and sex. The body contour of tumor-bearing mice was rotund. There was a marked increase in the thickness of subcutaneous fat depots. The peritoneal fat was also increased as demonstrated by the observations that the mean weight of the testicular fat pad of 23 male tumor-bearing mice was 1.2 gm. as compared to a mean of 0.6 gm. in ten normal males of the same age.

The adrenal glands of all mice bearing transplanted tumors were

TABLE II

Mean Organ Weights of Intact Mice Bearing Transplanted Tumors

Tumor strain	Sex	No. mice	Final body weight	Testicular fat body	Adrenal glands	Seminal vesicles	Testes	Uterus	Ovaries	Tumor
			gm.	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Normal	F	13	23.4		6.4			111	10.0	0
	M	10	34.8	0.6	4.6	325	212			0
1 (G 1-3)	F	55	30.0		H			N	N	200
	M	36	30.0		H	N	N			200
2 (G 1-3)	F	30	30.0		H			N	N	200
	M	30	30.0		H	N	N			200
2 (G 4-5)	F	11	30.0		16.6			86	5.6	1,300
	M	23	35.3	1.2	13.8	316	166			344
2 (G 7)	F	22	35.1		7.3			N	N	6,400
6 (G 1)	F	20	30.0		H				N	200
	M	20	30.0		H	N	N	N		200

G = generation, H = hypertrophy, N = normal.

enlarged to approximately three times normal size. The cells of the zonae fasciculata and reticularis were greatly enlarged and almost completely depleted of lipid and stainable carbonyl groups as demonstrated by the direct Schiff and Ashbel-Seligman techniques. The cells of the zona glomerulosa were only slightly enlarged and, unlike the other zones, retained their lipid content (Figs. 3 and 19).

The ovary was moderately atrophic and contained only a few small and medium-sized follicles. Atretic follicles were plentiful. There were no large follicles or corpora lutea. Even the interstitial débris of old corpora lutea was lacking. The nuclei of the interstitial cells were pyknotic (Fig. 18). The uteri were normal grossly. Histologically, the tall columnar uterine epithelium often exhibited characteristic clear basal vacuoles. The associated endometrial stroma was usually edematous.

The vaginal mucosa never was completely cornified. The epithelium consisted of an inner zone composed of a few layers of cells of cuboidal basal type and an outer zone of mucus-containing cells (Fig. 17). Polymorphonuclear leukocytes could be identified in both epithelial zones and within the lumen of the vagina. In some female mice the breast tissue was hypertrophic. Both ductal and alveolar elements were at times enlarged and contained vacuolated eosinophilic secretions.

The testes, seminal vesicles, prostate, and epididymides were essentially normal histologically.

The structural changes in the kidneys were not as marked as the observed functional defects. There was no evidence of glomerular or vascular lesions of either nephrosclerosis or periarteritis nodosa. In mice with greatest urine volume there was moderate to marked dilatation of the distal convoluted and the collecting tubules. The kidneys of most mice which died spontaneously showed foci of acute pyelonephritis and abscesses related to the previously described terminal septicemia.

Histochemical staining procedures were carried out on renal tissue from a few tumor-bearing mice. The distribution and intensity of staining caused by acid and alkaline phosphatase, succinic dehydrogenase, and β-glucuronidase did not differ from controls. The normal pattern of PAS staining of the brush border of the proximal convoluted tubules was maintained. There was no evidence of androgen-induced changes in the epithelium of Bowman's capsule of female mice.

The skin revealed a consistent increase in the thickness of the subcutaneous panniculus. The hair cycle was in the inactive phase and no changes were found in the pigmentation. Since melanocytes were not readily found in most areas of skin, evaluation of cutaneous melanino-

genesis was difficult. One exception was in the corium of the perineum where large hypertrophic melanocytes were easily seen in both normal and tumor-bearing mice. Examination of the iris, choroid, and the pigmented layer of the retina revealed no consistent changes in tumor-bearing mice.

There was marked atrophy of the thymic cortex and the splenic follicles. The erythroid and myeloid components of the bone marrow were prominent and the spleen often contained prominent megakaryocytes (Fig. 20).

Synthesis of Pituitary Hormones by Tumors

The biologic properties of the hormones synthesized by the tumors were first established by observations of the effects of the tumor upon the hypophysectomized host. Seventeen male and female tumor-bearing mice survived complete hypophysectomy for more than 3 weeks. During this interval there was no skeletal growth. At necropsy each animal showed atrophy of the gonads, seminal vesicles or uterus, and the thyroid gland, while the adrenal cortex was hypertrophied (Fig. 5; Table III). Histologic examination of

TABLE III

Mean Organ Weights of Hypophysectomized Mice Bearing Transplanted Tumors

Tumor strain	No.	Sex	Body weight	Adrenal glands	Testes	Seminal vesicles	Ovaries	Uterus	Thyroid gland
			gm.	mg.	mg.	mg.	mg.	mg.	mg.
Control	4	F	24.3	3.3			3.0	20.3	A
Control	1	M	18.0	2.4	19.8	24			A
1	1	F	18.4	H			A	A	A
2	4	F	17.5	H			A	A	A
2	5	M	25.0	H	A	A			A
2	1	F	22.0	19.0			3.0	25.0	A
2	2	M	23.5	16.7	92.0	44			A
6	2	F	20.0	H			A	A	A
6	2	M	25.0	H	A	A			A

H = hypertrophy, A = atrophy.

the tibial epiphyses revealed no cartilaginous thickening. This evidence indicates that the tumor synthesized and secreted ACTH but did not secrete gonadotropins, thyrotropin, or growth hormone.

Extracts of lyophilized tumor tissue were also assayed* for prolactin, gonadotropins, thyrotropin (TSH), adrenocorticotropin (ACTH), and melanocyte expanding hormone (MSH). There was no evidence of gonadotropins, prolactin, or TSH in 10 mg. of tumor powder. However, the concentration of ACTH and MSH activity in tumor extracts was about one tenth to one thousandth that of beef anterior lobe powder.

Discussion

All the primary and transplanted tumors described in this paper are morphologically similar. They are composed of relatively smaller cells than the thyrotropin secreting tumors previously described.[9] The absence of specific intracellular granulation prohibits any conclusions concerning the cytologic origin of the tumor. The term chromophobe adenoma is obviously unsatisfactory for it merely indicates the absence of any positive histologic diagnostic criteria.

Although devoid of special cytologic features, these tumors can easily be characterized by their effects on intact and hypophysectomized hosts and by direct bioassay. They differ from previously reported tumors in that they contain no TSH but do possess ACTH and MSH activities, and when transplanted cause a distinctive syndrome in the host. The tumors resemble the normal pituitary body in that they can synthesize biologically active substances, but whether these hormones are identical with the compounds secreted by the normal pituitary gland remains to be demonstrated. It is apparent that the tumor cells were not under the absolute control of the neurohumoral mechanisms which constantly regulate the pituitary-adrenal axis[10] of the normal animal, since fatal adrenal cortical hyperfunction occurred in the tumor-bearing mice.

The rôle of the adrenal gland in the causation of the clinical syndrome has been illustrated previously in studies of adrenalectomized hosts.[11] Following the removal of the adrenal glands of these tumor-bearing mice, the number of circulating lymphocytes increased; the urine volume, which had been increased, returned to normal; periodic vaginal cornification reappeared, and the generalized infections were controlled. When mice were adrenalectomized prior to the onset of

* Assay for gonadotropins, TSH, and prolactin by Dr. R. W. Bates, National Institutes of Health; assay for ACTH by Dr. S. L. Steelman, Armour Laboratories.

polyuria, the lymphopenic-polyuric syndrome was not observed, nor did the mice become obese. These findings are supported by the recovery of increased amounts of corticosteroids and ketosteroids from the blood of tumor-bearing mice.[8]

Although the features of this clinical syndrome differ somewhat from the typical Cushing's syndrome of man, they correspond to known effects of adrenal corticosteroids. The diabetes insipidus-like state characterized by marked polydypsia and polyuria, unaccompanied by profound glycosuria, has been produced in dogs and rats by desoxycorticosterone and may indicate excessive mineralocorticoid activity.[12] Lymphopenia, thymic atrophy, susceptibility to infection, and the occasional occurrence of hyperglycemia in the first generation of tumors can most likely be attributed to glucocorticoid activity.[13,14]

The occurrence of ovarian atrophy and vaginal mucification seems to indicate inhibition of pituitary gonadotropins possibly by adrenal ketosteroids.[15] It should be noted that in hypophysectomized tumor-bearing mice there was atrophy of the seminal vesicles in spite of adrenal hypertrophy, which indicates that the adrenal ketosteroids exhibited little true androgenic activity.

The genesis of obesity in tumor-bearing mice as well as in Cushing's syndrome of man is obscure. Heilman and Kendall[16] reported the occurrence of obesity in certain strains of mice implanted with pellets of either 11-dehydrocorticosterone or corticosterone. Implantation of pellets of cortisone or cortisone plus testosterone did not result in any increase in total body fat. Since obesity was most prominent in groups of tumor-bearing mice showing the most marked adrenal cortical hypertrophy and was prevented by adrenalectomy, it is likely that the adrenal cortex played an important etiologic rôle. In a recent study, Mayer and associates[17] have shown that the extrahepatic fat was increased in mice bearing the same ACTH-secreting tumor as those reported in this paper and this was accompanied by decreased spontaneous activity of the mice. Moreover, fat was synthesized in such mice from labeled precursors even when the animals were starved. (It may be that some adrenal corticoids secreted by the stimulated adrenal glands are capable of causing obesity by direct metabolic effects.)

Summary and Conclusions

Three primary pituitary adenomas of mice have been reported. Each tumor arose in the hypophysis of the primary host 24 to 36 months following exposure to total body radiation of 200 to 700 rep. The adrenal cortex of each mouse showed (1) hypertrophy of the zonae

fasciculata and reticularis, (2) little or no alteration in the zona glomerulosa, (3) atrophy of the x-zone.

Each primary tumor was grafted in series into the thighs of other mice of the same strain. In intact hosts the tumors caused a typical fatal syndrome characterized by adrenal hypertrophy, ovarian atrophy, thymic atrophy and lymphopenia, obesity, polydipsia and polyuria, cessation of estrual cycle, and susceptibility to infections. In hypopysectomized hosts, the transplanted tumors were shown to secrete ACTH.

REFERENCES

1. Furth, J.; Upton, A. C.; Christenberry, K. W.; Benedict, W. H., and Moshman, J. Some late effects in mice of ionizing radiation from an experimental nuclear detonation. *Radiology*, 1954, **63**, 562–570.
2. Furth, J.; Gadsden, E. L., and Upton, A. C. ACTH secreting transplantable pituitary tumors. *Proc. Soc. Exper. Biol. & Med.*, 1953, **84**, 253–254.
3. Upton, A. C., and Furth, J. Spontaneous and radiation-induced pituitary adenomas of mice. *J. Nat. Cancer Inst.*, 1955, **15**, 1005–1021.
4. Lillie, R. D. Histopathologic Technic and Practical Histochemistry. The Blakiston Co., Inc., New York, 1947, 501 pp.
5. Gude, W. D. Modified Martins-Mallory stain for mouse pituitary gland. *Stain Technol.*, 1953, **28**, 161–162.
6. Bahn, R.; Wilson, H.; Anderson, E., and Furth, J. The physiological effects of prolonged endogenous stimulation of the adrenal cortex of the female mouse. Proceedings of the 20th International Physiological Congress, Brussels, 1956, p. 54.
7. Bahn, R.; Anderson, E.; Wilson, H.; Kedda, L., and Furth, J. Metabolic changes associated with transplantable ACTH producing pituitary tumors of mice. *Canad. J. Biochem. & Physiol.* (In press.)
8. Wilson, H.; Borris, J. J., and Bahn, R. C. Steroids in the blood and urine of female mice bearing an ACTH-producing pituitary tumor. *Endocrinology*. (In press.)
9. Furth, J. Morphologic changes associated with thyrotrophin-secreting pituitary tumors. *Am. J. Path.*, 1954, **30**, 421–463.
10. Harris, G. W. Neural Control of the Pituitary Gland. Williams & Wilkins Co., Baltimore, 1955, pp. 103–133.
11. Furth, J. Experimental pituitary tumors. In: Recent Progress in Hormone Research, Pincus, G. (ed.). Academic Press, Inc., New York, 1955, **11**, 221–255.
12. Gaunt, R. The adrenal cortex in salt and water metabolism. Recent Progress in Hormone Research, Pincus, G. (ed.). Academic Press, Inc., New York, 1951, **6**, 247–276.
13. Baker, B. L. A comparison of the histological changes induced by experimental hyperadrenalcorticalism and inanition. Recent Progress in Hormone Research, Pincus, G. (ed.). Academic Press, Inc., New York, 1952, **7**, 331–373.

14. Dorfman, R. I. The bioassay of adrenocortical hormones. Recent Progress in Hormone Research, Pincus, G. (ed.). Academic Press, Inc., New York, 1953, **8**, 87–116.

15. Greep, R. O., and Jones, I. C. Steroid control of pituitary function. Recent Progress in Hormone Research, Pincus, G. (ed.). Academic Press, Inc., New York, 1950, **5**, 197–261.

16. Heilman, F. R., and Kendall, E. C. The influence of the hormones of the adrenal cortex, Compounds A, B and E, on the deposition of fat in the mouse. *Proc. Staff Meet., Mayo Clin.*, 1956, **31**, 454–459.

17. Mayer, J.; Zomzely, C., and Furth, J. Body composition and energetics in obesity induced in mice by adrenotropic tumors. *Science*, 1956, **123**, 184–185.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Adrenal gland of normal adult LAF_1 female mouse. Hematoxylin and eosin stain. × 105.

FIG. 2. Hypertrophic adrenal gland of the primary host which was the source of adrenocorticotropic tumor 1 (AtT-1). Hematoxylin and eosin stain. × 105.

FIG. 3. Hypertrophic adrenal gland of mouse bearing transplanted adrenocorticotropic tumor 1 (AtT-1). Hematoxylin and eosin stain. × 105.

FIG. 4. Hypertrophic adrenal gland of the primary host which was the source of adrenocorticotropic tumor 2 (AtT-2). Hematoxylin and eosin stain. × 105.

FIG. 5. Hypertrophic adrenal gland of hypophysectomized (60 days) mouse bearing transplanted adrenocorticotropic tumor 1 (AtT-1). The architecture of the adrenal cortex is maintained despite removal of the anterior pituitary gland. Hematoxylin and eosin stain. × 105.

FIG. 6. Hypertrophic adrenal gland of the primary host which was the source of adrenocorticotropic tumor 6 (AtT-6). Hematoxylin and eosin stain. × 105.

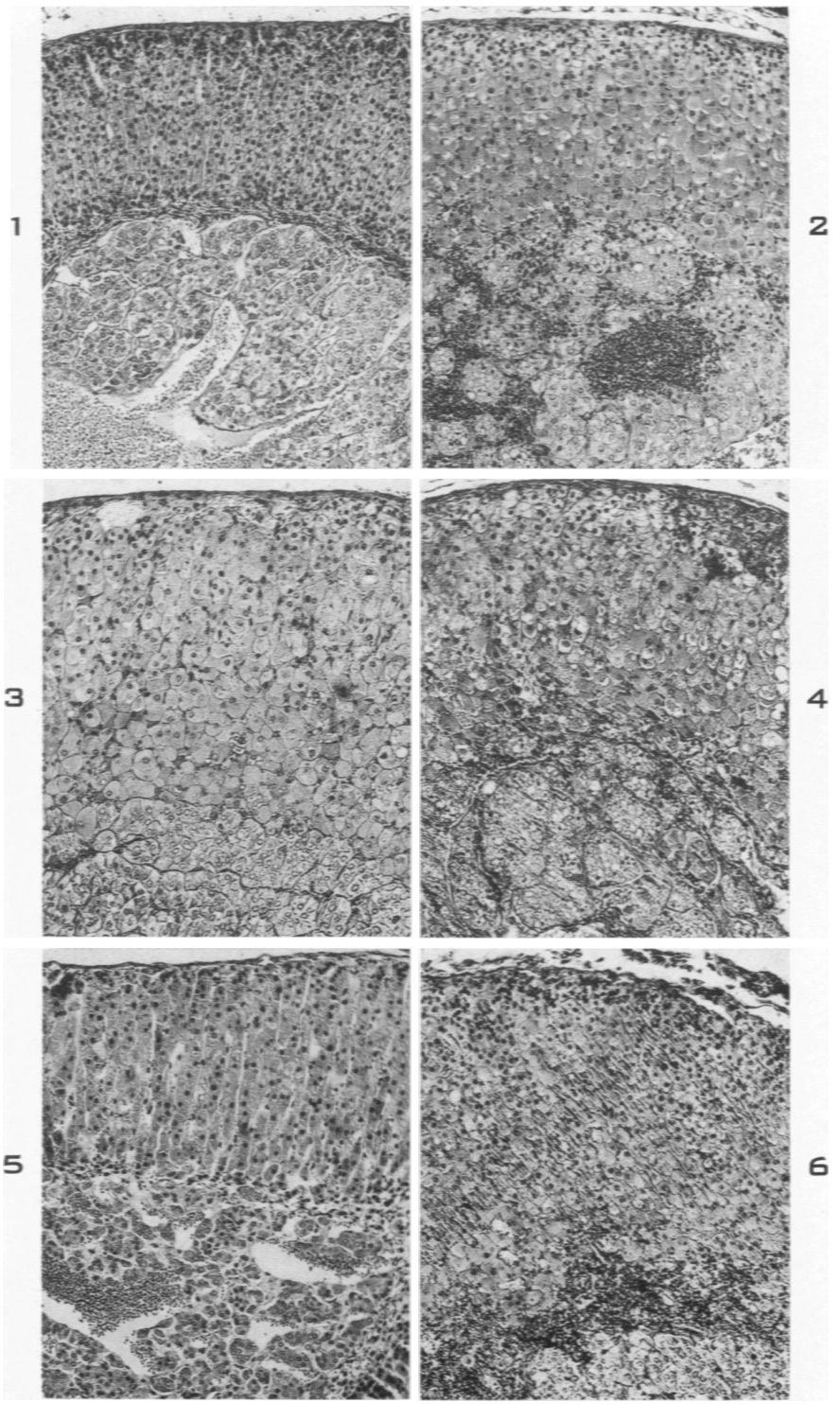
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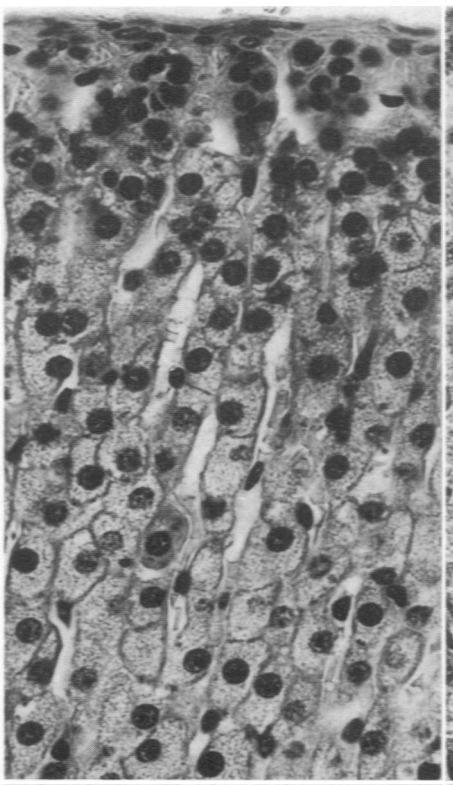
FIG. 7. Zonae glomerulosa and fasciculata of normal adult female LAF_1 mouse. Hematoxylin and eosin stain. × 500.

FIG. 8. Zonae glomerulosa and fasciculata of the primary host which was the source of AtT-1. Hypertrophy confined chiefly to the zona fasciculata. Hematoxylin and eosin stain. × 500.

FIG. 9. Zonae glomerulosa and fasciculata of the primary host which was the source of AtT-2. For comparison with Figures 7 and 8. Hematoxylin and eosin stain. × 500.

FIG. 10. Zonae glomerulosa and fasciculata of the primary host which was the source of AtT-6. For comparison with Figures 7 and 8. Hematoxylin and eosin stain. × 500.



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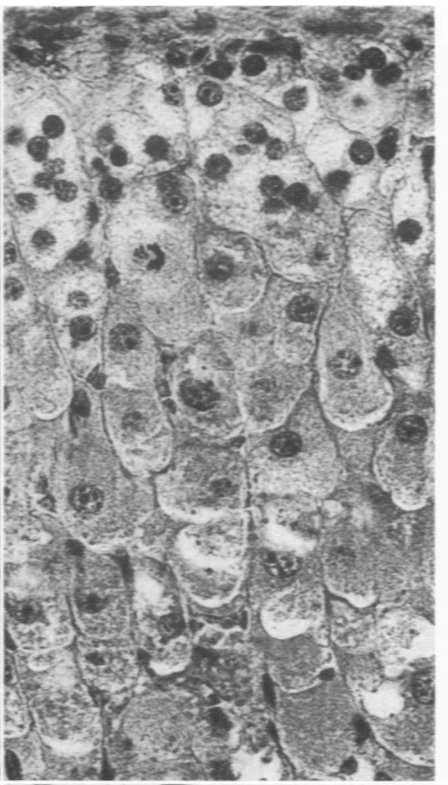

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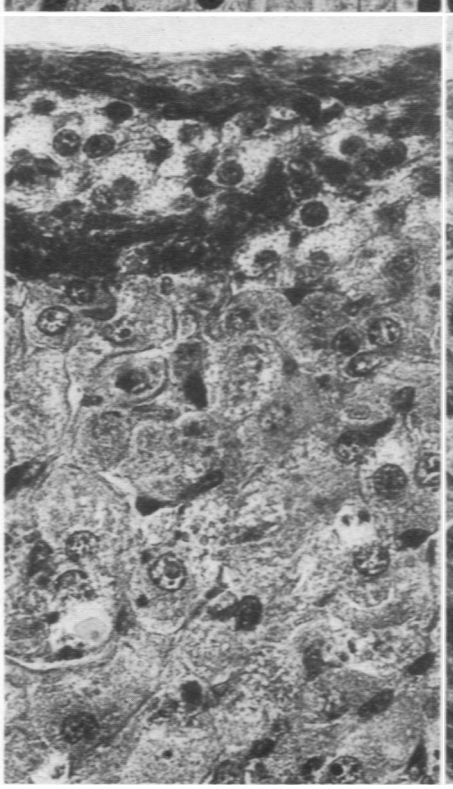

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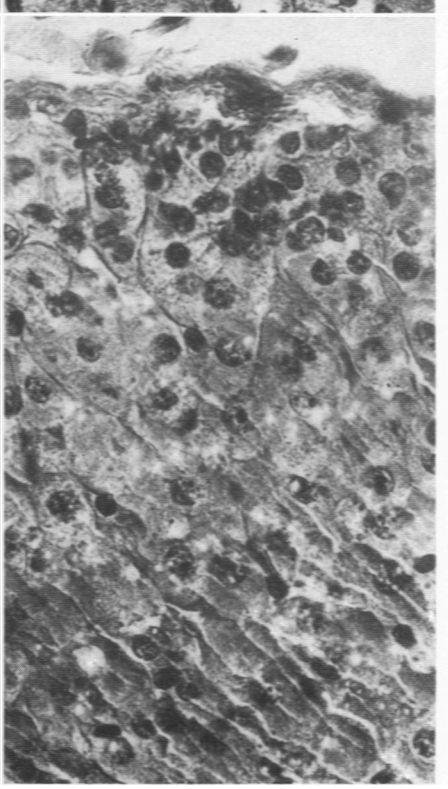

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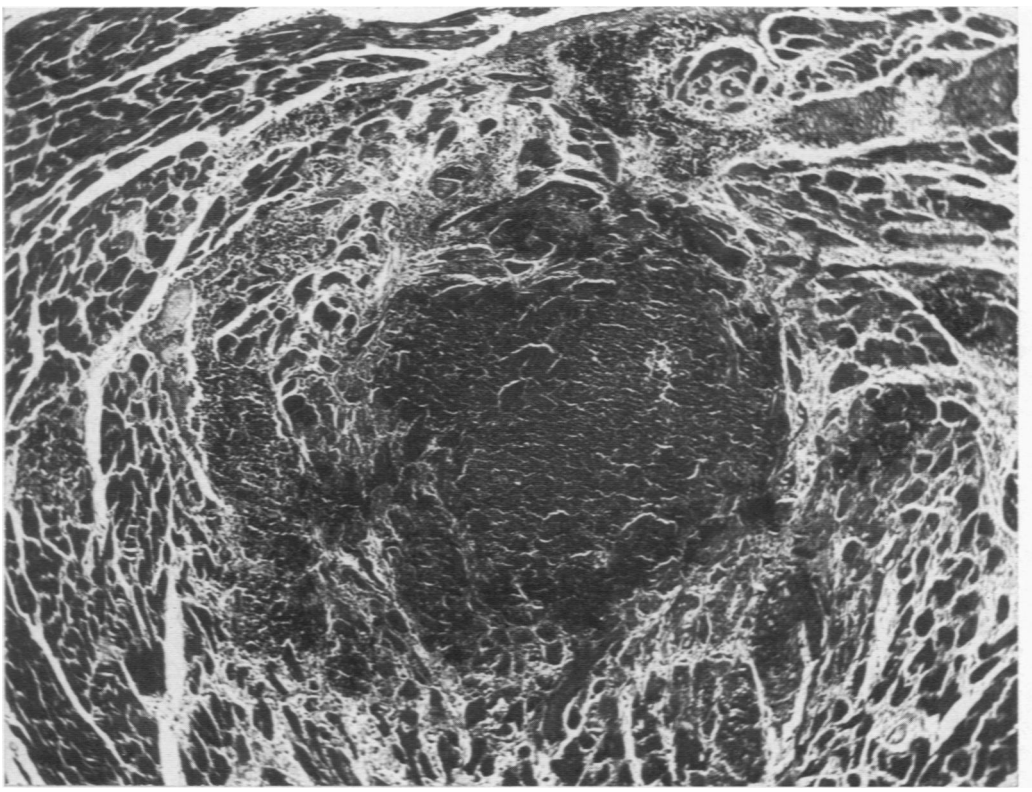
FIG. 11. Myocardial abscess in heart of mouse bearing transplanted AtT-1. Gram's stain. × 100.

FIG. 12. Acute mitral bacterial endocarditis in heart of mouse bearing transplanted AtT-6. Hematoxylin and eosin stain. × 105.



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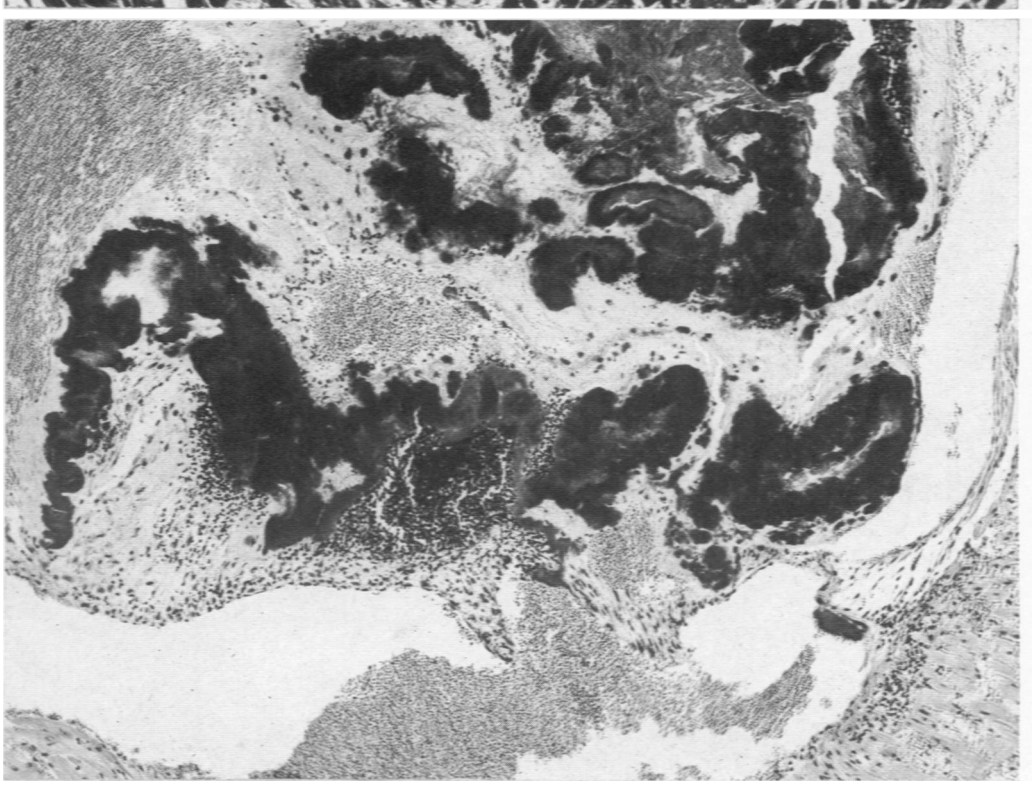

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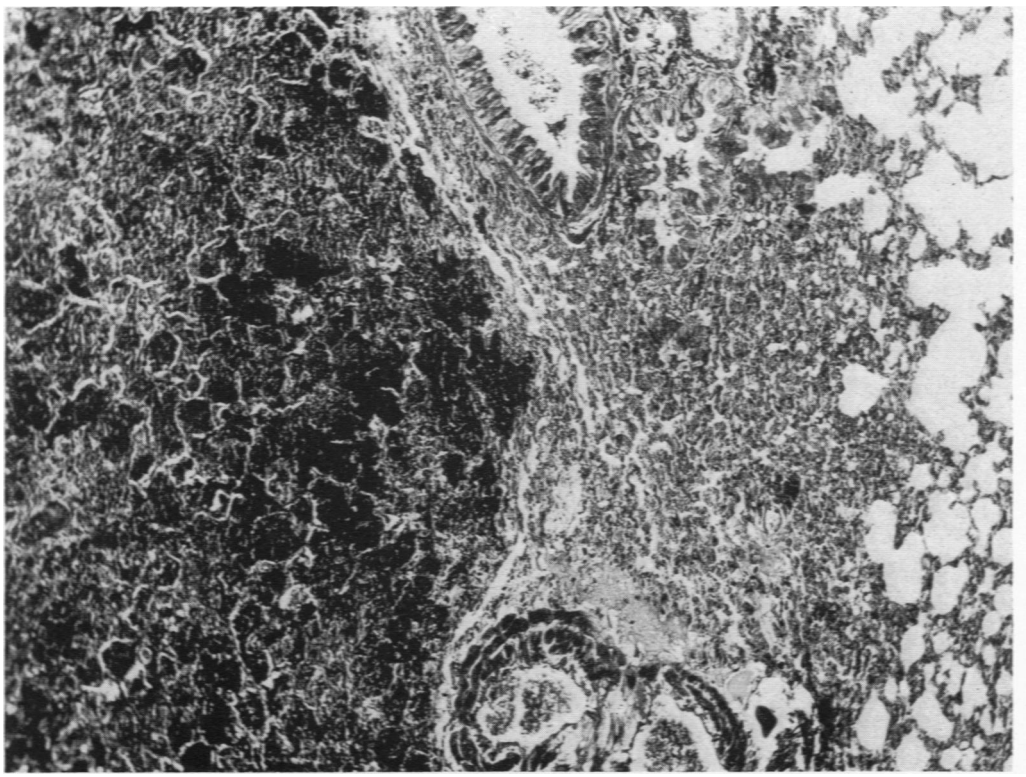
FIG. 13. Pneumonia and pulmonary abscess in lung of mouse bearing transplanted AtT-1. Gram's stain. × 105.

FIG. 14. Gram-positive bacilli in pulmonary abscess seen in Figure 13. Gram's stain. × 1,700.



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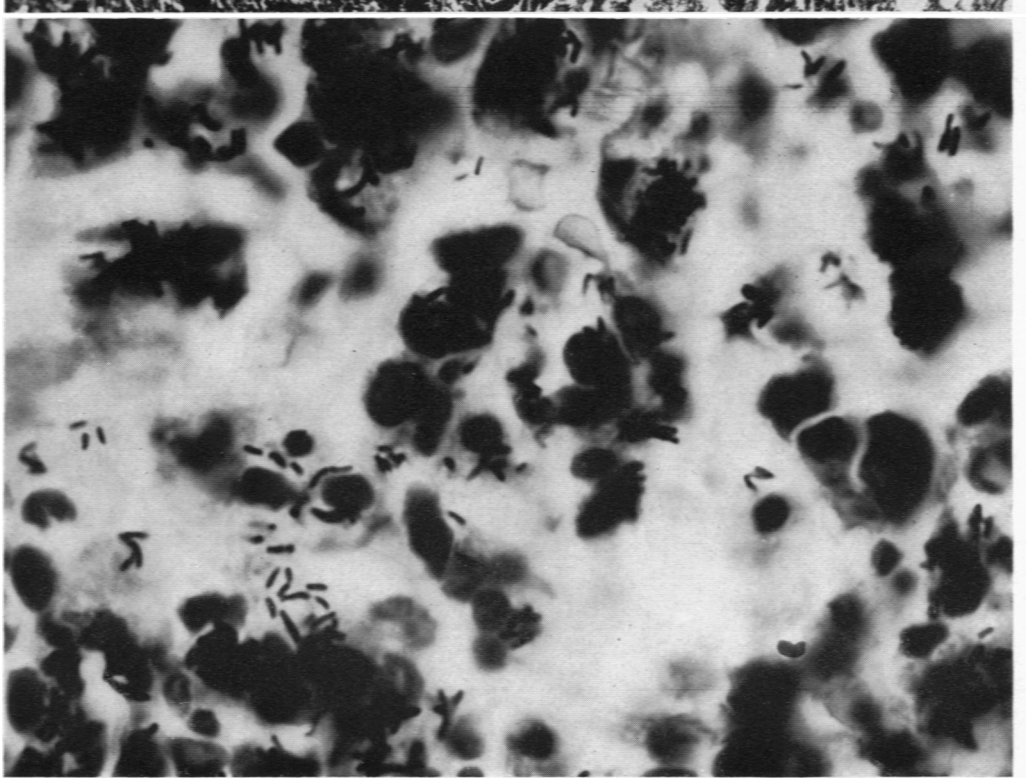

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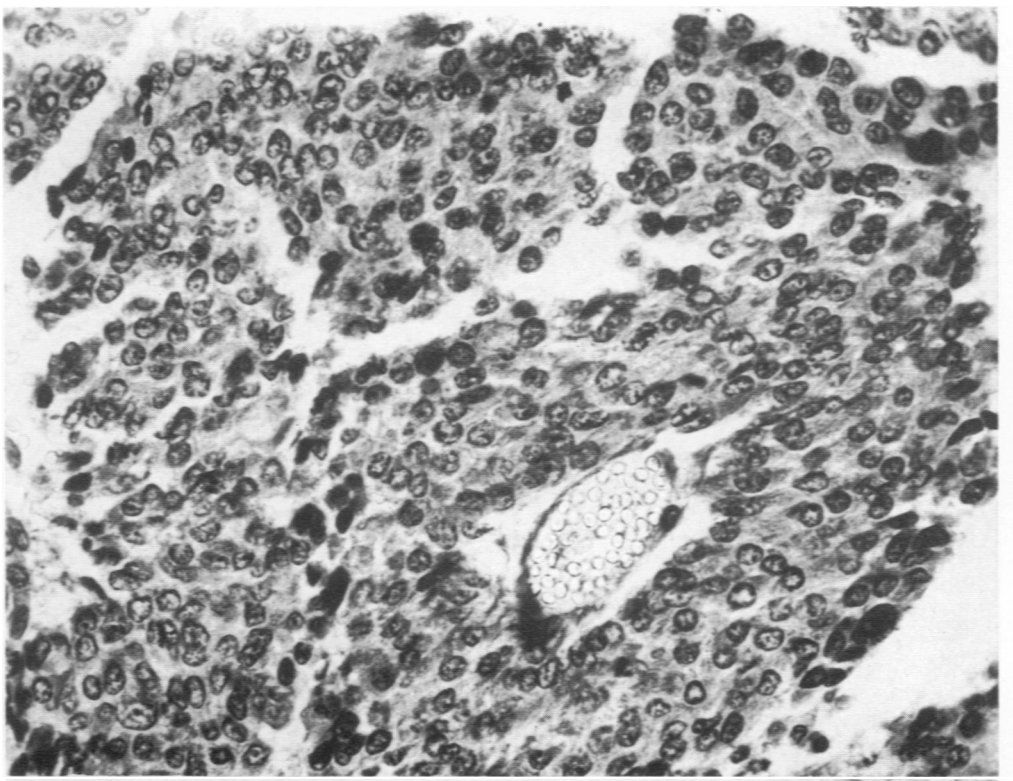
FIG. 15. Transplanted adrenocorticotropic tumor 2 (AtT-2) dissected from thigh of adult LAF_1 mouse. The nuclei are large, vesicular, and moderately pleomorphic. No specific chromophilic granules can be identified in the cytoplasm. The stroma is sparse. Periodic acid-Schiff and hematoxylin stains. × 550.

FIG. 16. Invasion of thigh muscles by transplanted adrenocorticotropic tumor 2 (AtT-2). For comparison with Figure 15. Periodic acid-Schiff and hematoxylin stains. × 105.



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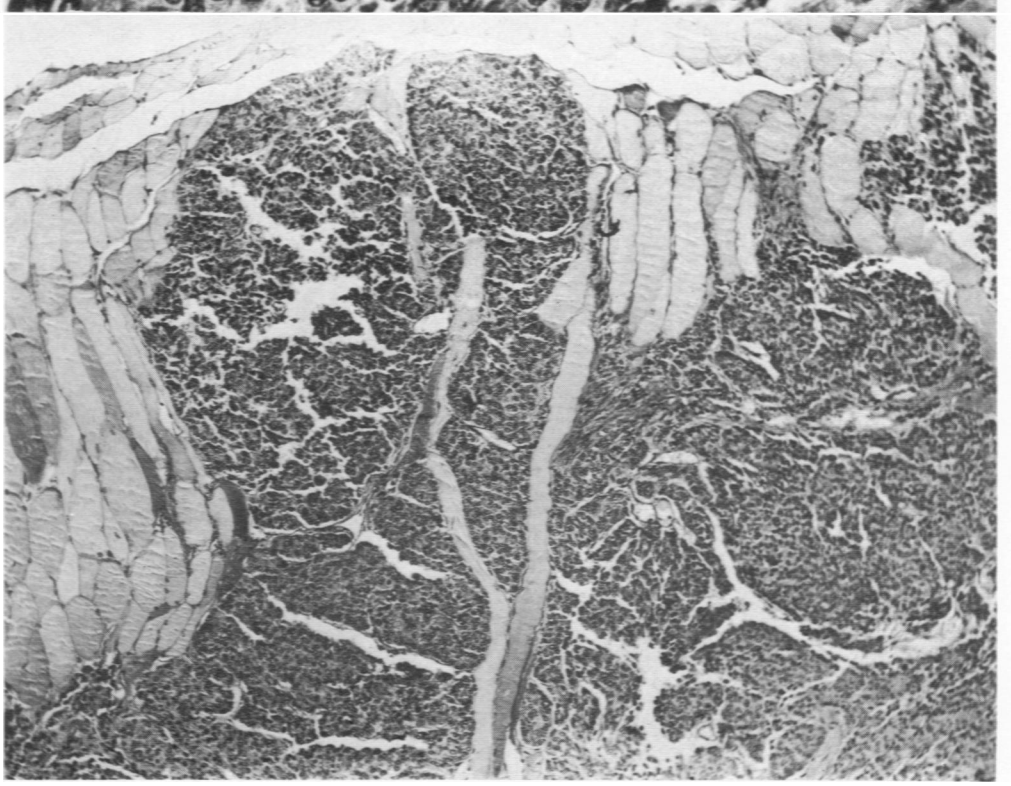

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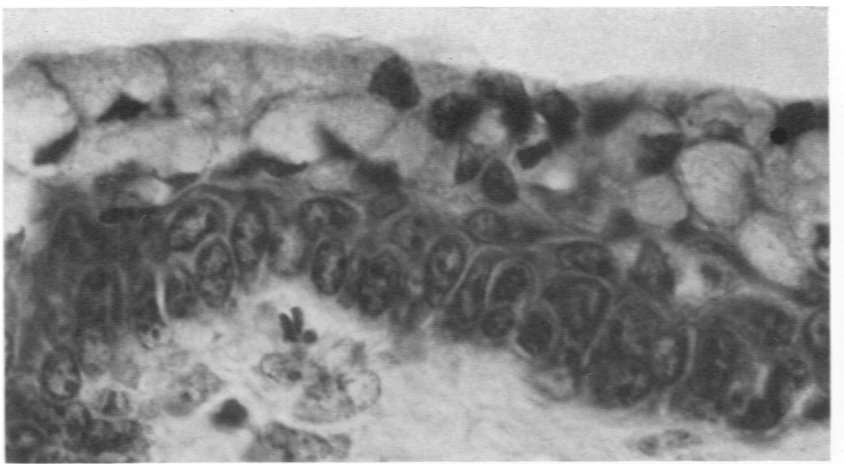
FIG. 17. Vagina of mouse bearing transplanted AtT-2. Mucin ladened cells compose the superficial layers of the epithelium. Hematoxylin and eosin stain. × 1,580.

FIG. 18. Ovary of mouse bearing transplanted AtT-2. There is atrophy of the interstitial tissue and atresia of follicles. No cystic follicles or corpora lutea are present. Hematoxylin and eosin stain. × 73.

FIG. 19. Adrenal cortex of mouse bearing transplanted AtT-2. The zonae fasciculata and reticularis are hypertrophic and depleted of lipids. Lipids remain in outer rim of zona glomerulosa. Frozen section and oil red O stain. × 105.

FIG. 20. Spleen of mouse bearing transplanted AtT-2. Megakaryocytes are present. Lymphoid follicles are atrophic. Hematoxylin and eosin stain. × 100.



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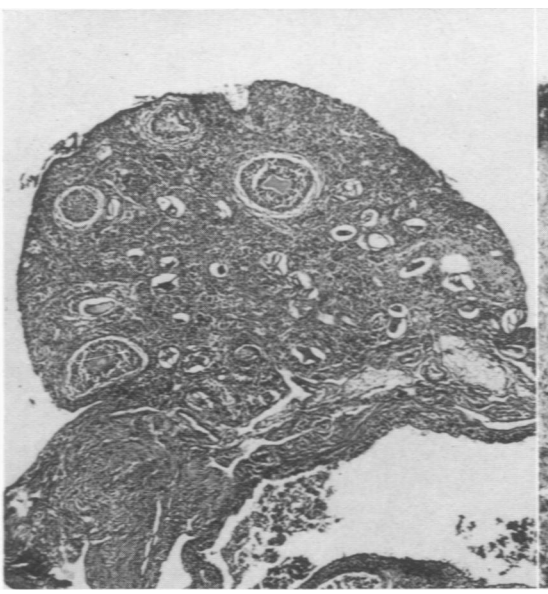

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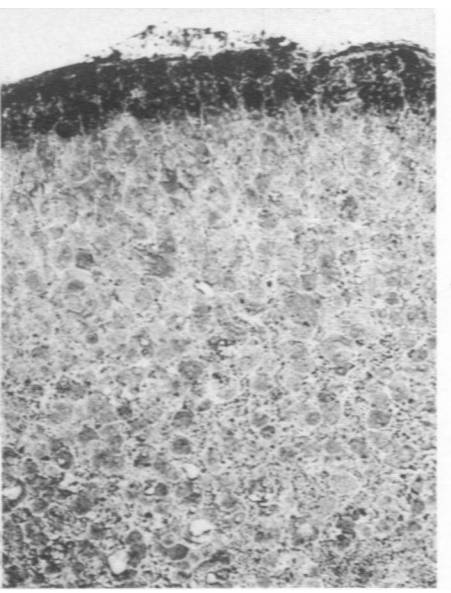

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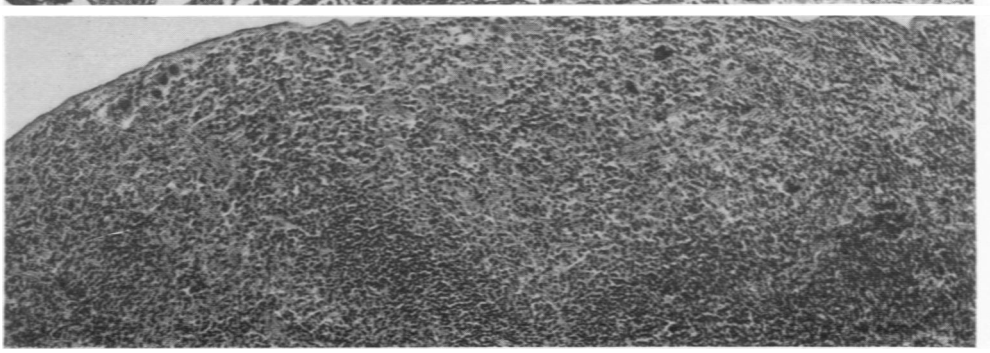

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