TABLE I. Yields in Corticotropic Activity in 2 Experiments Expressed as Minimal Dose to Induce a Fall in Adrenal Ascorbic Acid Concentration between 60 and 100 mg % in Hypophysectomized Rats.

Fraction	Sheep		Pig	
	Wt, g	Minimal effective dose, μg	Wt, g	Minimal effective dose, μg
Acetone-dried whole pituitary	20	10		
Acetone-dried anterior lobes			20	2.5
Extract in N. acetic acid	9.2	5	11.6	2.5
Supernatant after adsorption		>50		>50
Eluate in N/10 hydrochloric acid	0.144	0.1	0.320	0.05

The "direct adsorption method," as described here, was subsequently modified for use on fresh frozen sheep pituitaries. The frozen glands (water content approximately 80%) were rapidly broken into pieces of convenient size, and dropped into a macerator containing 320 ml of glacial acetic acid for each 100 g of frozen glands. The macerator was run at low speed until the lumps of frozen glands had sufficiently disintegrated, then at full speed until thorough maceration had been achieved (about 10 minutes). Heating, dilution and adsorption were then done essentially as in the original procedure, but it was found that centrifuging the dilute acid extract was preferable to sedimentation and that the addition of Super Cel was unnecessary and undesirable.

The method as used on acetone-dried pituitary was briefly described in September, 1951 (5), and was successfully applied by Sydnor and Sayers(6) to the extraction of corticotropin from blood.

Summary. A simple procedure is described whereby a good yield of highly active corticotropin can be obtained by direct adsorption from a crude pituitary extract. The method is equally suitable for pituitaries from sheep and from pigs.

1. Payne, R. W., Raben, M. S., and Astwood, E. B., *J. Biol. Chem.*, 1950, v187, 719.
2. Astwood, E. B., Raben, M. S., Payne, R. W., and Grady, A. B., *J.A.C.S.*, 1951, v73, 2969.
3. Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, 1948, v177, 751.
4. Sayers, M., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, v42, 379.
5. Astwood, E. B., Rabin, M. S., and Payne, R. W., *Recent Progress in Hormone Research*, 1952, v7, 1.
6. Sydnor, K. L., and Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 432.

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Mast Cell Resistance to Hormonal Influence.* (20352)

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Since their description by Ehrlich in 1879, the tissue mast cells have been a continual source of curiosity for histologists and physiologists. Over the years, these ubiquitous cells have been accused of some 30 different functions. Currently, evidence has been presented that these cells are controlled, or at least influenced, by endocrine glands, *viz.*: the pituitary-adrenal cortex(3,6,7) or pituitary-thyroid(1,2) systems. Only one of the papers (6) that have come to our attention records numerical counts to substantiate the author's opinion. Other workers have presented subjective impressions in lieu of statistically valid counts. The purpose of this study was to investigate tissue mast cell response to hormonal influence, utilizing numerical counts to indicate increased or decreased numbers of cells. The controversial field of tissue mast cell function will be avoided.

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Materials and methods. Male piebald rats averaging 225 g weight were used throughout. Tissue mast cells were observed in skeletal muscle from the anterior thigh, mesentery, and heart. A whole mount technic was employed to study the mesentery. Two loops of terminal ileum, each about 20 cm in circumference, were ligated, excised, and mounted with tiny springs on wire frames averaging 8 x 10 cm in dimensions. The mesentery was thereby spread out as a sheet or fan with a circumferential rim of ileum. The specimens of mesentery were fixed in absolute methanol and stained in a 35% alcoholic solution of 0.25% toluidin blue. The hearts and specimens of skeletal muscle were fixed in Carnoy's fluid, cut in serial sections 7 μ thick, and stained with 0.5% toluidin blue in a 30% alcoholic solution. The mast cells of the mesentery were counted in avascular areas using a magnification factor of 150. Forty such fields, 20 from each of 2 specimens, were examined and counted in each animal. A field with a magnification factor of 240 was used in examining the heart and skeletal muscle. In each specimen 375 fields were counted through multiple sections. This larger number of counted fields was necessary because of the sparseness of mast cells in these tissues. Naturally, precautions were taken to avoid overlapping of any of the fields. All counts were made by the same worker, using the same microscope. The morphological appearances of the mast cells were simultaneously noted. Thyroxine (Squibb) and Cortone Acetate (Merck) were injected subcutaneously into the appropriate animals. In the thyroxine studies 19 rats were divided into groups of 9, 5, and 5. Nine received 200γ of thyroxine daily for 7 days. Five rats were starved, being given tap water only for a period of 5 days to rule out any inanition effect of the thyroxine. Five rats were used as controls. In the cortisone series, 24 rats were divided into 3 groups of 8. One of these groups received 10 mg Cortone Acetate daily for 6 days. A second group was given 5 mg for a similar period, and the third was the control group. Loss of weight by all experimental animals was interpreted as indicating the potency of the hormone administered. The mean, standard deviation, and standard error were calculated for each group. The results obtained were analyzed for statistical significance using the *t* test of "Student".

TABLE I. Effect of Thyroxine and Starvation upon Mast Cell Counts in the Rat's Mesentery.*

Treatment	200 γ thyroxine daily	Starved	Controls
Duration	7 days	5 days	—
No. of animals	9	5	5
Mean value	538.6	608.4	485.6
S. D.	± 106.0	± 101.4	± 49.1
S. E.	± 35.3	± 45.5	± 21.9

* Units: No. of cells in 20 fields (× 150).

Results. The experimental data are recorded in Tables I and II.

Statistical evaluation shows that no significant difference exists between the tissue mast cell counts of the thyroxine-treated rats and their corresponding controls. The same holds for the cortisone-treated animals and their control group. Only the starved group of rats shows a statistically significant change in number (an increase) when compared with the associated controls.

A preliminary investigation using desoxycorticosterone acetate failed to reveal any changes in mast cell number.

Typical so-called degenerative changes, *i.e.*, vacuolation, granule conglomeration and irregularity, cellular bursting with granule dispersion, and loss or change in degree of metachromasia, were very frequently observed. However they were seen as frequently in the controls as in the experimental animals.

Discussion. We were unable to effect any alterations of tissue mast cell numbers except in the starved group of animals. Here the increase was probably more apparent than real. It was observed that in this group most of the perivascular fat in the mesentery had been resorbed. This meant that the fields counted probably encroached upon these perivascular mast-cell-rich areas, which in other specimens would have been avoided because the thickness of the fat makes accurate counting impossible.

The figures presented for the cortisone-treated heart specimens are somewhat more impressive when it is realized that 2 of the members of the 5 mg group and one of the 10 mg group gave low counts because the sections were washed out. Thus many mast

TABLE II. Effect of Cortisone upon Tissue Mast Cell Counts in the Mesentery, Skeletal Muscle, and Heart of the Rat.

Tissue	Mesentery*			Heart†			Skeletal muscle†		
Daily dose, mg	10	5	—	10	5	—	10	5	—
No. of animals	8	8	6	8	8	8	8	8	8
Mean value	604.9	568.9	568.8	15.91	14.51	21.44	20.46	16.51	18.39
S. D.	±83.7	±106.7	±78.2	±5.37	±5.59	±7.79	±7.02	±6.31	±3.26
S. E.	±29.6	± 37.7	±27.6	±1.90	±1.98	±2.75	±2.48	±2.23	±1.15

* Units: No. of cells in 20 fields (× 150).
† " " " " " 25 " (× 240).

cells were undoubtedly missed in the examination of these 3 specimens.

Although numerical counting of tissue mast cells is difficult, and not an ideal method of investigating their response to various stimuli, their capricious distribution renders simple estimation of numbers by impression alone worthless. One essential step in making numerical counts is to run a control group with each experimental series. It was found early in this investigation that counts vary significantly from batch to batch of rats, apparently because of age and weight differences. We could attribute none of the "degenerative changes" to treatment the rat had received. It was our impression that these appearances were a function of the fixation-staining-mounting technic. It is, therefore, our opinion that tissue mast cell response can only be adequately measured by actual numerical counts evaluated statistically.

Using such a criterion we were unable to find any suggestion of mast cell susceptibility to hormonal influence. These observations oppose those of Asboe-Hansen(3), Cavallero and Braccini(6), Bloom(5), and Stuart(7), who have reported a decrease in the number of mast cells following cortisone treatment. Baker *et al.*(4) found no morphological or real numerical alteration in the mast cells of the thymus after ACTH therapy, although no counts were presented. Asboe-Hansen(2) has reported differences in mast cell numbers and appearance in patients suffering from myxoedema and thyrotoxicosis. Arvy and Gabe(1) claimed to observe an increase of the mast cells in all tissues following thiourea administration. No counts were offered in either of these articles. We were unable to demonstrate any effect on mast cell numbers following thyroxine treatment.

Summary and conclusions. The effect of cortisone and thyroxine and starvation on the numbers of tissue mast cells in the rat mesentery, heart, and skeletal muscle was investigated by making numerical counts of the cells. A whole mount technic was employed for the mesentery, while other tissues were prepared by the usual histological routine. All were stained with an alcoholic solution of aqueous toluidin blue. No significant changes in mast cell numbers were effected by either cortisone or thyroxine. Starvation produced a statistically significant increase. The alleged degenerative types of mast cells were seen as frequently in controls as in treated rats. This group of experiments therefore has failed to provide any evidence supporting the contention that tissue mast cells are affected by thyroxine or cortisone.

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1. Arvy, L., and Gabe, M., *Experientia,* 1950, v6, 23.

2. Asboe-Hansen, G., *Acta Derm.-Vener.,* 1950, v30, 221.

3. ———, PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 677.

4. Baker, B. L., Ingle, D. J., and Li, C. H., *Am. J. Anat.,* 1951, v88, 313.

5. Bloom, F., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 651.

6. Cavallero, C., and Braccini, C., PROC. SOC. EXP. BIOL. AND MED., 1952, v78, 141.

7. Stuart, E. G., *Anat. Rec.,* 1951, v109, 351.

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