responsible for the peculiar behavior of the inhibition indices of these compounds at intermediate concentrations of folic acid, since A-ninopterin, which is much less inhibitory, behaves as one would expect of a strictly competitive inhibitor. Since the amounts of A-ninopterin are relatively large, it is of little consequence whether or not small amounts are deaminated.

Summary. The antifolics, 4-amino-9-methylpteroylglutamic acid (A-ninopterin), 4-amino-10-methylpteroylglutamic acid (A-methopterin) and 4-amino-9,10-dimethylpteroylglutamic acid (A-denopterin) are all inhibitory to *Tetrahymena*. The inhibition produced by A-ninopterin is strictly competitive, being released over wide ranges of concentration of folic acid. The inhibition indices of the other two analogs decrease as the amount of folic acid in the medium increases until a certain level is reached, at which they become constant. This shifting index is tentatively explained on the basis of the ability of *Tetrahymena* to deaminate the 4-position of the analogs.

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Effect of Estrogen-Treatment and Castration on ACTH Content of Rat Adenohypophysis.* (18985)

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In a previous paper(1) we were able to show that the ACTH content of the rat's adenohypophysis is not affected by the severe depletion of acidophilic and beta-granular material which follows thyroidectomy. In the present study we sought to determine the effect of radical changes in the amount of the third described type of adenohypophysial secretory granulation, that of the delta cells, on the ACTH stores of the pituitary. For this purpose, the hypophyses of estrogen-treated and castrated male rats were assayed for their ACTH content against those of controls. Massive doses of estrogen were previously found to lead to a dramatic reduction in the number of delta cells(2), whereas castration is characterized by a progressive hypertrophy and hyperplasia of this cell type(3).

Method. The pituitaries of 46 young adult male Sprague-Dawley rats were used in this experiment. Twelve rats received daily injections of 62.5 μg α-estradiol in sesame oil over a period of 32 days, 10 rats were castrated and kept for 30 days and 24 intact animals served as controls. At the time of their sacrifice the estrogen-treated rats weighed 215-245 g (mean 226 g), the castrates 288-345 g (mean 313 g) and the controls 288-350 g (mean 328 g). All animals were killed by decapitation. The pituitaries of 4 castrates, 4 estrogen-treated and 6 intact rats were saved for quantitative histological examination. The remaining hypophyses were prepared for ACTH-assay by the method of Richards and Sayers(4). The simultaneous assay of the 3 groups of pooled donor pituitaries was carried out on hypophysectomized rats by the adrenal ascorbic acid depletion method of Sayers *et al.*(5). The recipients, the administered doses of the extracts, the procedure for the determination of the adrenal ascorbic acid concentration and the method for the statistical analysis of the assay data, as well as the

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TABLE I. Pituitaries of Estrogen-Treated and Castrated Rats Assayed for ACTH Content Against Those of Controls.

Dose	Estrogen-treated donors (8)* No. of recipients	Response	Control donors (18) No. of recipients	Response	Castrated donors (6) No. of recipients	Response
1.25†	8	26.2‡ ± 1.5§	10	27.2 ± 2.5	8	24.1 ± 1.9
.625	7	17.1 ± 2	8	19.6 ± 1.1	7	15.3 ± 2
.3125	7	8.8 ± 2	8	15.2 ± 2.2	9	7.7 ± 2.8
Total	22		26		24	
Slope of log dose-response curve		29.3		21.2		27.4
Significance of diff. between slopes			P = .2		P = .3	

For estrogen-treated vs. control $M = \overline{1}.8723$. $S_M = .06771$. For castrate vs. control $M = \overline{1}.7988$. $S_M = .07940$.

With that of controls at 100%, ACTH content of estrogen-pituitaries = 75%, range of potency at 95% level of confidence = 55-101%. ACTH content of castrate-pituitaries = 63%, range of potency at 95% level of confidence = 44-90%.

* No. of donor rats.

† % donor pituitary per 100 g recipient.

‡ Mean of differences between ascorbic acid concentration in left and right adrenal of individual recipients, expressed as % of concentration in left adrenal.

§ Standard error.

TABLE II. Pituitary Weights and Cell Counts.

Group	No. rats	Wt, mg	Acidophil	Beta	Delta	Vacuolated delta
			%			
Control	6	7.2	45.10	1.84	8.50	.37
Castrate	4	9.5	40.05	2.55	17.85	.95
Estrogen-treated	4	7.5	52.62	1.48	.48	.00

histological staining technic applied to the pituitaries, were the same as in our previous study(1).

Results. The results of the assay are summarized in Table I and the pituitary cell counts in Table II. Since the histological findings on the castrate and estrogen-hypophyses are in good agreement with earlier observations on similarly treated rats(2,3), there is no reason to believe that they are not representative of the cellular composition of the assayed glands as well. It should be emphasized that the extremely low delta count of the estrogen-pituitaries still does not give an idea of the actual degree of delta-granule depletion from these hypophyses, as even the remaining few delta cells are reduced in size and poorly granulated. The elevated number of delta cells in the castrate pituitaries is a similarly poor measure of the plethora of delta granules in these glands, since the delta cells are not only hyperplastic in this condition, but also hypertrophic and loaded with granules.

The mean adrenal weight (per 100 g body weight) was 12.5 mg in the controls, 16.9 mg in the castrates, and 21.0 mg in the estrogen-injected group.

Discussion. Whereas previous observations failed to suggest any parallelism between delta cell count and adrenocorticotrophic function of the rat hypophysis(3), the present results indicate a similar lack of correlation between the amount of stored ACTH and delta granular material in the pituitary. The slight reduction in the ACTH content of the estrogen-pituitaries is by no means proportionate to the conspicuous degranulation of the delta cells and the enlarged castrate pituitaries, which contain an overabundance of delta granules, appear to have even lower ACTH stores. It seems justified to conclude that the delta granule can not be the cytological sign of ACTH storage.

High titers of pituitary ACTH having been found in coexistence with a large-scale depletion of the acidophilic, the beta(1), and, as the results of this paper show, the delta granulation, none of the 3 types of secretory granules in the hypophysis of the rat can be regarded as the morphological equivalent or even the site of ACTH storage. Since, on the other hand, the secretory granulation is the only reliable distinguishing mark between pituitary cell types, it is hard to conceive how the exclusive role of any given class of cells in the production and storage of ACTH could be convincingly established with our present histophysiological technics. Theoretically, two possibilities exist: (a) ACTH is produced by only one type of cell, or (b) it is elaborated by several or all classes of adenohypophysial cells, possibly including the chromophobes. In either case its storage does not appear to take place in the form of microscopic particles. The connection between ACTH and the uniform microsomes observed by Fernández-Morán and Luft(6) in basophil, acidophil and chromophobe cells of the rat pituitary remains to be explored.

The reduction in the amount of pituitary ACTH in both the castrates and the estrogen-treated rats of this study seems to have been due to an accelerated release rather than a block of formation, since the adrenals were found to be enlarged in both conditions. The lowered ACTH level in the castrate pituitaries might explain why Szego and White(7) observed a diminished adrenocorticotrophic response to the stress of starvation in castrated rats.

Emery and Winter(8), in contradiction to our observations, found no difference between the adrenal-stimulating potency of the pituitaries of castrated and intact male rats. As these authors did not use hypophysectomized recipients, the reliability of their assay method is open to serious doubt.

Summary. (1) The ACTH content of the pituitaries of estrogen-treated and castrated male rats was assayed against that of controls by the adrenal ascorbic acid-depletion method. (2) The estrogen-hypophyses were found to contain 75% and those of the castrates 63% of the amount of ACTH encountered in the controls. (3) Since no parallelism was observed between the ACTH content and the amount of delta granules present in these pituitaries, it is concluded that adenohypophysial ACTH is not associated with the delta granules. (4) Further histophysiological and physiological implications of these findings are discussed.

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Sinusoidal Dilatation in Liver and Adrenal Cortex of Tumor-Bearing Rats.* (18986)

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The weights of the liver, spleen, adrenals and other organs of tumor-bearing rats and mice are reported as significantly increased over that of normals(1,2). In some instances this increase in total weight of the organ is associated with increased water con-

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