H., *Endocrinol.*, 1946, v38, 345.
7. Harris, G. W., *J. Physiol.*, 1948, v107, 418.
8. Brooks, C. McC., Beadenkopf, W. G., and Bojar, S., *Endocrinol.*, 1940, v27, 878.
9. Kasdon, S. C., *Endocrinol.*, 1949, v44, 211.
10. Everett, J. W., and Sawyer, C. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 696.
11. Everett, J. W., *Anat. Rec.*, 1951, v109, 291.
12. Warren, D. C., and Scott, H. M., *Poul. Sci.*, 1935, v14, 195.
13. Fraps, R. M., Riley, G. M., and Olsen, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 313.
14. Rothchild, I., and Fraps, R. M., *Endocrinol.*, 1949, v44, 134.
15. Fraps, R. M., and Dury, A., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 346.
16. Meyer, R. K., Leonard, S., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1929, v27, 340.
17. Jacobson, A., Salhanick, H. A., and Zarrow, M. X., *Am. J. Physiol.*, 1950, v161, 522.

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Cytologic Changes in Rat Adenohypophysis Following Administration of Adrenocorticotrophin or Cortisone. (19342)

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An increased pituitary basophile count has been found at post mortem examination of patients dying after receiving pituitary adrenocorticotrophic hormone (ACTH)(1) or cortisone(2). In addition, the hyaline changes described by Crooke(3) were seen in many of the basophile cells after the administration of both hormones(1,4). These changes have usually been considered to be characteristic of Cushing's syndrome. The appearance of these findings following the administration of adrenal cortical hormones strongly supports the theory that the pituitary alterations in Cushing's syndrome are a secondary manifestation of increased adrenal cortical activity. An attempt was therefore made to reproduce these lesions in experimental animals and to define the circumstances under which the changes occur.

Experimental. Male Sprague-Dawley rats were treated with ACTH* or with a microcrystalline suspension of cortisone (Cortone, Merck) according to the schedule in Table I. The daily dose of ACTH was administered subcutaneously in 3 portions—one-fourth of the total at 8:00 A.M. and at noon, and one-half at 5:00 P.M. The phenol control solution was given in a similar manner, in the same volume as the ACTH solution (.025 ml/100 g at 8:00 A.M. and noon; .05 ml/100 g at 5:00 P.M.). A group of normal rats was examined without having been subjected to any trauma. The effects of "stress" were also studied in animals fasted for 15 hours in individual cages in a cold-room at 4°C. At the end of the experimental period, the animals were anesthetized with intraperitoneal pentobarbital. The adrenals were removed, cleaned and weighed on a Roller-Smith torsion balance to the nearest milligram.

The pituitary glands were removed by the dorsal approach. They were fixed in 10% solution of USP formaldehyde, embedded in paraffin and sectioned serially at 5 μ. Multiple sections were stained for differential counting by Mallory's 1% acid fuchsin, orange G, aniline blue stain(5). Selected sections were also stained by a modification of the periodic acid Schiff technic(6). A minimum of 6000 cells of the pars distalis were counted from each gland by the method of Rasmussen(7). In all but two instances, 3 or 4 sections from different areas were included in the count. Two sections from each gland were evaluated for Crooke's hyaline change of the basophile cells. The identity of the slides was unknown at the time of these observations which were repeated on 3 separate occasions.

* Wilson's Corticotrophin, kindly supplied by Dr. David Klein of the Wilson Co.

TABLE I. 4 Rats in Each Experiment.

Procedure	Initial wt (g)	Final wt (g)	Adrenal wt (mg)	% basophiles in ant. pituitary
ACTH, 1 mg/100 g/day, 10 days	227 ± 10	308 ± 19	63 ± 4.6*	7 ± 1.2
Cortisone, 3 mg " " 10 "	290 ± 11	233 ± 9*‡	25 ± 1.1*	11.5 ± 1.3‡
Control, .5% phenol, 10 "	229 ± 15	312 ± 14	43 ± 3.6	9.9 ± 1.9†
Normal, no treatment		328 ± 11	48 ± 3.3	5.2 ± .6
Cold-room, 15 hr, fasting		328 ± 13	48 ± 2.2	10.9 ± .8‡

* Significantly different from control ($p < .01$).
† Probably different from normal ($p < .05$).
‡ Significantly different from normal ($p < .05$).

TABLE II. Distribution of Crooke's Change. Two slides were examined for each pituitary on 3 different occasions, and each slide graded from 0 to 4+. Each group consisted of 4 animals.

	Total No. + per group
Normal	7
Phenol control	12
ACTH	63
Cortisone	66
Cold stress	35

Results. The effects of the various treatments on the body weight and pituitary basophile counts are shown in Table I. The effectiveness of the doses of ACTH and cortisone are demonstrated by the increased adrenal weight after ACTH and the decrease after cortisone. The animals receiving cortisone lost weight. whereas all of the other groups gained significant amounts of weight during the experiment.

The administration of cortisone was associated with a significant increase in the pituitary basophile count as compared with the normal rats. No other manipulation significantly affected the proportion of basophiles in the pituitary, except for cold stress, which also raised the basophile count significantly compared to the normal.

The only reliable criterion of Crooke's change was degranulation and the appearance of a deeply cyanophilic homogeneous cytoplasm. This was not necessarily associated with enlargement of the cell. The cytoplasmic masses stained for glyco-protein, as previously described by Laqueur(8). Vacuolization of the cytoplasm, sometimes regarded as a part of Crooke's change(3) was not used as a diagnostic finding as it did not correlate with the cytoplasmic hyalinization.

Discussion. Increased cyanophilia and Crooke's hyaline change have been described in human pituitary glands under conditions known to inhibit the secretion of ACTH. Before the use of ACTH and cortisone in clinical medicine, these changes were seen only in patients with Cushing's syndrome, where the increased blood corticosteroid levels might be expected to inhibit the secretion of ACTH(9). The observation of similar changes in patients receiving ACTH or cortisone appeared to be due to the same mechanism. In our previous communication, we suggested that increased basophilia and Crooke's hyaline change might be morphologic manifestations of storage of ACTH in the pituitary(1).

Differential cell counts were made of the pituitary and a search was made for Crooke's hyaline change in rats under experimental conditions of altered demand for endogenous secretion of ACTH. The release of ACTH was inhibited by the administration of exogenous cortisone or ACTH, and was encouraged by the stress of exposure to cold. Alterations in demand for ACTH were not correlated with changes in the basophile count. Animals which received cortisone or 0.5% phenol, or were exposed to the cold all showed increased basophile counts. The animals which received ACTH, however, showed no significant increase. The lack of response of the pituitary basophiles to ACTH is in accord with the observations of Baker(10), who also failed to find changes in the pituitary basophile count after the administration of this hormone in large quantities.

Marked hyaline change of the basophiles was observed in animals receiving either ACTH or cortisone. Changes of almost equal degree, however, occurred in animals sub-

jected to cold stress. Whereas, the administration of 0.5% phenol caused increased basophilia without Crooke's change, ACTH caused hyalinization without any increase in the basophile count. In the rat, therefore, the two morphologic findings are not correlated. Moreover, Crooke's hyaline change can be found whether the gland is producing increased amounts of ACTH because of stress, or ACTH secretion is depressed by the administration of cortisone. The data indicate that one is unable to deduce the functional state of the rat pituitary gland from the total number of basophiles or the presence of Crooke's hyaline change. These findings are in agreement with the observations of Halmi and Bogdanove (11), who found no correlation between the basophile count and the ACTH content on bioassay of rat pituitaries.

Purves and Griesbach(12) have divided the pituitary basophiles into two groups, "thyrotrophs" and "gonadotrophs", on the basis of response to endocrine manipulation. They showed that the two types of cells have a characteristic anatomic distribution in the pars distalis. In our experiments, the increase in basophiles appeared to involve all areas of the gland. Crooke's change was also noted in all areas of the pars distalis. The morphologic changes which occurred in these experiments did not resemble those described by Purves and Griesbach following administration of estrogens or thyroxine.

It is apparent that our original hypothesis concerning the nature of Crooke's change and increased basophilia is untenable. The fact that the hyaline cytoplasmic masses of Crooke's cells stain for glycoprotein in both the human and rat pituitary is strong evidence that the cytoplasmic substance is not ACTH itself, although it could be a glycoprotein to which ACTH is attached. The purest preparations of ACTH now available do not appear to be a protein nor do they contain carbohydrate (13).

We are unable to relate Crooke's change to alteration of ACTH content of the basophile cells. Possibly the change reflects the storage of a pituitary hormone other than ACTH.

In the human being, prolonged and fatal stress fails to cause the appearance of Crooke's change or of increased basophilia(14). In this respect, therefore, the human differs significantly from the rat. It seems possible that much of the confusion in the literature pertaining to the specific function of the various pituitary cell types may arise from failure to take into account important species differences.

Summary. 1. Adult male rats were treated with adrenocorticotrophic hormone or cortisone, or subjected to stress at 4°C. One control group received 0.5% phenol, and another was untreated. The percentage of pituitary basophile cells was calculated and the basophiles were examined for Crooke's hyaline change. 2. Animals receiving cortisone and those exposed to cold stress were found to have a significant increase in the percentage of pituitary basophiles. The ACTH and 0.5% phenol-treated animals failed to show this response. Crooke's change was prominent in animals receiving ACTH or cortisone and was also seen in the cold stress group but not in either control. 3. In the rat, the percentage of anterior pituitary basophiles and the presence of Crooke's change cannot be correlated with increased or decreased demand for endogenous adrenocorticotrophin. The fact that basophilic granules and the hyaline masses of Crooke's cells stain histochemically for glycoprotein makes it apparent that they are not ACTH. 4. The absence of increased numbers of basophiles or Crooke's change in human pituitaries following prolonged stress points to important species differences in the occurrence of these findings.

1. Golden, A., Bondy, P. K., and Sheldon, W. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 455.

2. Golden, A., unpublished data.

3. Crooke, A. C., *J. Path. Bact.*, 1935, v41, 339.

4. Laqueur, G. L., *Science,* 1950, v112, 429.

5. Mallory, F. B., *Pathological Technic,* 1938, 153.

6. Lillie, R. D., *Histopathologic Technic,* 1948, 188.

7. Rasmussen, A. T., *Am. J. Path.*, 1929, v5, 263.

8. Laqueur, G. L., *Stanford Med. Bull.*, 1951, v9, 75.

9. Sayers, G., *Physiol. Rev.*, 1950, v30, 241.

10. Baker, B. L., Proc. of the Laurentian Hormone Conference—Recent Progress in Hormone Research, 1952, v7.

11. Halmi, N. S., and Bogdanove, E. M., PROC.

SOC. EXP. BIOL. AND MED., 1951, v77, 518.

12. Purves, H. D., and Griesbach, W. E., *Endocrinol.*, 1951, v49, 244.

13. Geschwind, I. I., Hess, G. P., Condliffe, P. G., and Williams, B. S., *Science*, 1950, v111, 625; Li, C. H., Evans, H. M., and Simpson, M. E., *J. Biol. Chem.*, 1943, v149, 413.

14. Rasmussen, A. T., *Endocrinol.*, 1936, v20, 673.

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Demonstration of *Escherichia coli* 055 and 0111 Antigens by Means of Hemagglutination Test.* (19343)

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It has been shown by Keogh, North and Warburton(1,2) that certain bacterial antigens may be adsorbed onto red blood cells, thus rendering the erythrocytes agglutinable by the anti-bacterial antibodies. Subsequently this indirect hemagglutination test was used for the demonstration of antibodies against tubercle bacilli (Middlebrook and Dubos)(3), *H. influenzae* type b (Warburton, Keogh and Williams)(4), streptococci (Kirby)(5) and *Pasteurella pestis* (Amies)(6). Furthermore, Hayes and Stanley(7) showed that the somatic antigen (polysaccharide) isolated from an untyped strain of *Escherichia coli* was operative in producing hemagglutination by the homologous bacterial antiserum. The experiments to be described in this communication revealed that the hemagglutination test can be used for the identification of the 0 antigens of two groups of *E. coli*, namely, serogroups 055 and 0111. These antigenic groups of *E. coli* are of particular interest, since they are closely associated with, and, indeed, may be considered as a cause of, epidemic and sporadic diarrheal disease of infants. Furthermore, the observations reported here are of interest in connection with the problem of the nature of the phenomenon of bacterial inagglutinability.

Material and methods. Several strains of *E. coli* serogroups 055 and 0111, isolated from infants suffering from diarrheal disease, were used; in addition, one strain each was kindly supplied by Dr. Joyce Wright, London, England. The organisms were maintained on brain veal agar and transferred weekly. For the hemagglutination test brain heart infusion cultures were used, after the pH had been adjusted to 7.0. Group-specific antisera were prepared in rabbits by injection of suspensions of the organisms; and, for control purposes, *E. coli* 055 B5 and 0111 B4 antisera, made available through the courtesy of Dr. Wm. H. Ewing, Communicable Disease Center, were employed. For the hemagglutination test both human and rabbit red blood cells were washed in physiological saline solution. To the sediment of such a suspension were added *E. coli* infusion broth cultures or filtrates thereof to make a 2.5% red blood suspension. The mixture was kept for 2 hrs at 37°C and stirred repeatedly. The cells were then washed three times with physiological saline solution. The 2.5% cell suspension (vol. 0.25 ml) was added to 0.25 ml of various *E. coli* antisera in serial dilutions and to normal human and rabbit sera, serving as controls. The mixtures were incubated in the waterbath at 37°C and the resulting agglutination was read grossly at various intervals up to 2 hrs.

Results. In the first experiments human red cells were treated with broth cultures of several strains of *E. coli* 0111. Agglutination of these treated cells occurred only irregularly and with relatively concentrated amounts of group-specific antiserum. It was then decided to study the activity of *E. coli* cultures and filtrates which had been heated at 100°C for 2 hrs. The data obtained in one representative experiment, read after 2 hrs. at 37°C, are summarized in Table I.

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