tosterone (ethisterone) would be about 4 times higher(4). Thus the comparative values agree reasonably well in rabbit, monkey, and man(2,3).

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Cytological and Weight Changes in Pituitary Gland of the Severely Stressed Rat.* (22282)

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Numerous cytological studies have been made of the anterior pituitary gland following the application of different types of stress. Finerty and Briseno-Castrejon(1) and Brokaw *et al.*(2) reported an increase in the percentage of acidophils as shown by azocarmine after unilateral adrenalectomy in rats. Daughaday, Perry and McBryde(3) associated the acidophils with ACTH secretion in their studies of increased adrenal function in acromegaly. Finerty, Hess and Binhammer (4) reported no increased percentage of acidophils or basophils at intervals of 1, 3, 12 and 24 hours after stress with the azocarmine staining method but did describe an increase in numbers of acid hematein positive cells. No change in the percentage of the basophils by staining with PAS or with aldehyde fuchsin was found. Recently, Knigge(5) reported an increase in percentage of acidophils and argyrophilic basophils 12 hours after severe stress, and concluded that the latter were most probably associated with ACTH production since response to stress occurs in thyroidectomized animals which are characterized by absence of acidophils. Kraus(6) reported a decrease in the number of basophils in the anterior pituitary of Addison's disease patients. Further evidence for the basophils as the cytological source of ACTH was noted by Koneff(7) who found a decrease in the basophils after bilateral adrenalectomy and after ACTH injection in rats. D'Angelo *et al.*(8) noted adrenal hypertrophy during prolonged periods of inanition in the guinea pig. This was accompanied by loss of weight of the pituitary and hypertrophy and hyperplasia of the basophils, increasing with the severity of the inanition. Marshall(9) found ACTH antibody coupled with fluorescent dye to be localized in the basophil cells of the hog pituitary. Recently, Wilson *et al.*(10) reported the characteristic Crooke's hyaline cytoplasmic change of the pituitary basophils associated with hyperfunction of the adrenal cortex, and a progressive loss of acidophils in 100 routine human necropsies.

It was the purpose of the present experiments to reinvestigate histological changes and to study changes in weight of the pituitary gland in order to gain further insight into the effects of a severe stress.

Method. Male rats of the Holtzman strain weighing between 110-130 g were used. They were stressed by immersion (except for the head) in water (containing Aerosol) at 70°C for 5 seconds while under Nembutal anesthesia. All animals were sacrificed by decapitation. The pituitary gland was removed immediately and weighed on a Roller-Smith torsion balance. About half of the glands were then placed in appropriate fixative for future histological study. The rest were placed on previously weighed coverslips, dried in an oven at 30°C for 48 hours and re-

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TABLE I. Number of Cells per Field* in Anterior Pituitary after Severe Stress Using Various Staining Methods.

Hr after stress	No. of animals	Acid hematein	Azocarmine			Aldehyde fuchsin		
			Acid.	Baso.	Chrom.	Beta	Delta	PAS
Unstressed	30	21.1 ± .5†	15.7 ± .6	7.0 ± .2	41.9 ± .5	4.6 ± .5	3.6 ± .4	7.8 ± .6
1	35	18.5 ± .5	16.6 ± 1.0	7.7 ± .4	33.5 ± .9	4.2 ± .3	4.4 ± .2	8.0 ± .4
3	32	20.0 ± .8	17.3 ± 1.3	9.8 ± .7	39.3 ± 1.6	3.7 ± .4	5.2 ± .3	8.3 ± .4
12	32	29.4 ± .8	25.5 ± .6	9.9 ± 1.0	51.4 ± 2.5	3.1 ± .4	6.4 ± .5	9.9 ± .5

* Oil immersion 1.25 mm lens and a 20× eyepiece.
† ± stand. error.

weighed. Glands used for histological study were stained by 5 different methods: fixation in Bouin's fluid and stained with hematoxylin for nuclei counts; fixation in Zenker-formol solution and staining with a modified azocarmine stain(11) for cell counts; fixation in formol-sublimate and staining with periodic acid Schiff method for basophils(12); fixation in formol-calcium and staining with acid hematein for phospholipids(13); and fixation in Susa-picric acid and staining with aldehyde fuchsin to distinguish beta and delta cells(14). Cell counts were made on 3 serially cut sections selected at approximately the ¼, ½ and ¾ levels of the gland. The cells were counted in every tenth field for 10 consecutive fields. A standard field was obtained with a 1.25 mm oil immersion Spencer lens and 20x eyepiece. Cell counts were recorded as the number of cells per field.

Results. Exp. I. Ninety-nine pituitaries were prepared for histological study from rats which were sacrificed at intervals of 1, 3 or 12 hours post-stress. Thirty unstressed animals served as controls. Table I shows no significant differences in number of anterior pituitary cells per standardized microscopic field among control, 1 and 3 hour post-stress animals by any of the staining methods. However, 12 hour post-stress animals demonstrated a significant increase in acid hematein positive cells per field. There also were significant changes in the number of acidophils, basophils and chromophobes per standard microscopic field in the 12 hour post-stress animals stained by azocarmine stain as compared to controls. PAS staining of pituitaries of the 12 hour post-stress animals showed an increase in number of basophils per field. The 12 hour post-stress pituitaries showed an increased number of delta cells and a slight decrease in beta cells when stained by Halmi's aldehyde fuchsin method (14). In the 12 hour post-stress groups all cell counts (except beta cells) after various methods of staining show a significant increase in number of cells per field as shown in Tables I and II.

Calculations of percentage of cells stained by the azocarmine method show that there is no significant change in percentage of each type of cell after severe stress. The nuclei counts are similar to the total cell counts after azocarmine staining as shown in Table II. Table I shows total number of basophils after staining by aldehyde fuchsin, azocarmine or PAS to be similar also. The acid hematein stain for acidophils gives consistently higher numbers than for acidophils

TABLE II. Relation of Fresh Pituitary Weight to Total Cells per Field* after Severe Stress.

Hr after stress	No. of animals	Body wt, g	Fresh pituitary wt		Total nuclei/field*	
			Absolute, mg	Relative, mg/100 g	Hematoxylin	Azan
Unstressed	30	119 ± 1.2†	4.11 ± .03	3.44 ± .03	65.6 ± .7	64.7 ± .9
1	35	118 ± 1.2	4.06 ± .02	3.44 ± .02	60.1 ± 1.4	57.4 ± .8
3	32	111 ± 1.6	3.71 ± .03	3.40 ± .02	61.9 ± .9	66.5 ± 1.2
12	32	112 ± 2.0	3.40 ± .03	3.05 ± .02	84.6 ± 2.1	87.2 ± 1.4

* Oil immersion 1.25 mm lens and a 20× eyepiece.
† ± stand. error.

TABLE III. Fresh and Dry Weight of Pituitary after Severe Stress.

Hr after stress	No. of animals	Body wt, g	Rel. wt/100 g body wt		
			Fresh, mg	Dry, mg	Liquid lost in drying, mg
Unstressed	31	121 ± 1.2*	3.41 ± .03	.75 ± .009	2.66 ± .01
1	11	122 ± 1.6	3.46 ± .04	.77 ± .01	2.69 ± .02
12	28	128 ± 2.4	3.01 ± .04	.62 ± .01	2.39 ± .02
Diff. between unstressed and 12 hr post-stress			.40	.13	.27
Relative fluid and solid loss (in %)				32.5	67.5

* ± stand. error.

stained by the azocarmine method as shown in Table I.

General observations of the 12 hour post-stress pituitaries indicated few mitotic figures, and an apparent reduction of cytoplasm in the cells, resulting in an increased nuclear-cytoplasmic ratio.

Results in Table II show no significant change in fresh weight of the pituitary of 1 and 3 hour post-stress animals as compared with controls. There is also no significant change in relative fresh weight per 100 g body weight of 1 and 3 hour post-stress animals. The 12 hour post-stress pituitaries show a significant absolute decrease in weight which is also apparent relative to body weight.

Exp. II. Thirty-nine male rats were stressed and sacrificed at 1 and 12 hours later. Pituitaries were removed, weighed and dried. Determinations of relative weight per 100 g body weight were made of fresh weight, dry weight and weight of fluid lost in drying. The same determinations were made on 31 control animals.

Table III shows no significant changes from controls in the relative fresh weight, relative dry weight and relative weight of fluid lost in the 1 hour post-stress animals. In the 12 hour post-stress animals there was a decrease in relative fresh weight of the pituitary per 100 g body weight similar to that in Exp. I. The relative dry weight and relative weight of fluid lost in drying of the 12 hour post-stress animals show a decrease compared to controls. Table III also shows that 32.5% of the difference in weight between the controls and 12 hour post-stress animals is due to loss in solids and 67.5% of the difference is due to a loss in fluid.

To determine the effects of a severe stress on the weight of an organ of similar anatomical position which has little endocrine function, the brains of controls and 12 hour post-stress animals were removed, weighed, dried and reweighed. No change in fresh or dry weight of the brain was found as a result of severe burns suggesting that the two organs respond differently.

Discussion. Our results confirm previous reports of increase in number of acid hematein-positive cells after stress with no change in percentage of azocarmine staining acidophils(4). This severe burn stress after 12 hours induces some increase in number of all cells per microscopic field, regardless of staining method. This is probably a result of general shrinkage of the pituitary gland, as indicated by weight reduction and increased nuclear-cytoplasmic ratio. Such a finding points out a variable factor which must be considered in interpretation of pituitary cytological studies whenever there is a possibility of pituitary anhydremia.

Measurements of water content of pituitaries after stress indicate that about 68% of the weight reduction at 12 hours post-burn is a result of dehydration. This is compatible with hemoconcentration usually seen in burned patients due to edema and resulting anhydremia. Since the same fluid changes were not found in the brain it is concluded that the pituitary is one of the organs specifically affected by anhydremia, possibly because of its oral epithelial origin.

About 32% of pituitary weight reduction at 12 hours post-burn is a result of loss of solids. Since solids make up only one-fifth of total weight of the gland, it is apparent

that their loss is a significant aspect of the response to stress, amounting to an actual loss of 17% of all solids present. Possible factors responsible for the loss of solids may be loss of diffusible cellular proteins, loss of mineral ions to the blood, or excess secretion of pituitary hormones.

Summary. Effects of severe stress, immersion in water at 70°C for 5 seconds, were studied on the male albino rat in relation to response of the anterior pituitary. Using 5 different staining methods, no significant changes in *percentage* of acidophils, basophils or chromophobes were found 1, 3 or 12 hours after stress, although there were marked increases in *number of cells per field* in the 12 hour post-stress rats. Fresh weight of the pituitary showed no significant change in 1 and 3 hour post-stress animals, but the 12 hour group displayed a marked reduction, which was evident on both an absolute and a relative to body weight basis. Comparison of relative dry weight and amount of fluid lost in drying in unstressed and 12 hour stressed animals revealed that the weight decrease consisted of both fluid loss and reduction in solids.

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Hemolysis of Human Erythrocytes by a Sulfhydryl Inhibitor, p-Chloromercuribenzoic Acid.* (22283)

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Although sulfhydryl groups have a function in the metabolism and integrity of erythrocytes, its nature is poorly defined. Ingbar (1) reported that cysteine caused sickling of erythrocytes from patients with sickle cell anemia. It was prevented by the addition of heavy metals or p-chloromercuribenzoic acid. Benesch and Benesch(2) have shown that either phenylmercuric hydroxide or mersalyl (salyrgan) caused hemolysis of sheep erythrocytes in a fairly consistent pattern; and that this hemolysis was blocked by sulfhydryl groups but not by sulfur in the disulfide linkage or other forms of sulfur. Dimant, *et al.* (3) more recently have been concerned with the question of *de novo* synthesis of glutathione by erythrocytes. In our previous studies(4) on the feeding of methionine, prothrombin and accelerator globulin decreased in all 3 human subjects. Increased erythrocyte destruction was demonstrated in only one subject.

This present study concerns the relation of certain factors of the red cell environment to hemolysis caused by p-chloromercuribenzoic acid.

Method. The sodium salt of p-chloromer-

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