ratio of antagonist to folic acid necessary to produce deficiency symptoms in rats fed succinylsulfathiazole to be 3000. According to microbiological assay, the basal ration contained at least 0.3 mg of folic acid per 100 g. The data in Table III show that if female rats are depleted of vit. B_{12} they produce litters with a high incidence of hydrocephalus, even though the basal diet is supplemented with folic acid.

Discussion. In our early work casein was the source of protein and the incidence of hydrocephalus was under 2%. When folic acid (O'Dell *et al.*)(2), was added to the diet the incidence of the abnormality was practically zero indicating that folic acid is an important preventive factor. The importance of folic acid was supported by the observation of Hogan *et al.*(3) that when female rats consumed a soybean oil meal diet the addition of a folic acid inhibitor precipitated the occurrence of hydrocephalus. However, the failure of folic acid to reverse the inhibition made it seem doubtful that folic acid was the only factor involved. According to microbiological assay casein usually contains more vit. B_{12} than does soybean oil meal, and this suggested that a deficiency of vit. B_{12} may be implicated in the abnormality. Our recent studies show that when this vitamin is administered to female rats on a soybean oil meal diet which contains low levels of Methyl PGA their offspring are not hydrocephalic. Since the level of inhibitor used is unlikely to induce a marked folic acid deficiency it is uncertain whether or not an adequate level of vit. B_{12} in the absence of folic acid will prevent hydrocephalus. It is certain, however, that folic acid, in the absence of vit. B_{12}, will not prevent the abnormality. Studies are now under way to determine whether both of these vitamins are required.

Summary. After female rats, on a diet that contained soybean oil meal as a source of protein, became depleted of the factor that prevents hydrocephalus, the incidence of the abnormality among the offspring was 28%. The addition of a vit. B_{12} concentrate to the diet or the injection of crystalline vit. B_{12} in the dams during the early stages of gestation prevented the abnormality in the young animals and increased their viability. Hydrocephalus was not prevented by folic acid alone, but it has not been determined whether vit. B_{12} alone is effective or whether both folic acid and vit. B_{12} are required.

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Effect of Treatment with ACTH or Cortisone on Anatomy of the Brain.* (18488)

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Much evidence indicates that adrenocortical hormones exert important effects on the central nervous system. In rats sensitized by unilateral nephrectomy and feeding of high salt diets, Selye(1) induced inflammatory vascular lesions and edema in the brain by the administration of cortisone. In man, euphoria, psychotic reactions, restlessness, convulsions(2), and irregularities in the electroencephalogram(3) have been observed

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following treatment with cortisone or ACTH. Psychotic states have been correlated, also, with decreased adrenal responsivity to adrenocorticotropin(4). Although it has been suggested that the hypothalamus controls the secretion of ACTH by the anterior hypophysis, there is no agreement concerning the localization of this function in the hypothalamus. Hume(5) contended that the integrity of the anterior hypothalamus is essential for the hypophysis to secrete ACTH in response to stress. Harris and De Groot(6) found that lesions in the posterior region of the tuber cinereum and in the mammillary body abolished the lymphopenic response to stress in rabbits. Stimulation of these regions resulted in lymphopenia. On this basis they concluded that secretion of adrenocorticotropic hormone is under neural control via the hypothalamus and the hypophyseal portal vessels. If this hypothesis is correct it would be reasonable to expect that treatment with ACTH or cortisone would produce microscopic evidence of reduced secretory activity in the hypothalamus.

In this investigation we have studied the effect of administration of ACTH and cortisone on the portion of the brain extending from the olfactory bulbs to the region just caudal to the posterior commissure and the mammillary bodies, thus including the cerebral cortex, thalamus and hypothalamus.

Procedure. Four rats received ACTH by continuous subcutaneous injection for 21 days at a dosage of 6 mg per day. Since ACTH disappears rapidly from the blood stream(7), this method of administration is necessary in order to secure the maximal physiological effect of the hormone. A fifth rat received 1 mg of ACTH per day in 8 divided subcutaneous injections. Three rats received 10 mg of cortisone acetate (Merck) per day by intermittent injections for 10 days. For each rat receiving hormone a control was injected with the vehicle. Adult, male, Sprague-Dawley rats were used and at the beginning of the experiment the difference between the body weights of the treated and non-treated rats in each pair was less than 10 g. The control rats gained in weight during the experiment whereas those receiving ACTH lost, on an average, 35 g; and those receiving cortisone, 40 g. The ACTH was prepared by the method of Li, Evans and Simpson(8). The animals were fed by stomach tube according to the method of Reinecke, Ball and Samuels(9). The brains were fixed in Carnoy's fluid. Frontal serial sections were cut at 12 and 20 μ and stained with .5% toluidine blue. Insofar as possible, control and experimental sections were matched area for area and the slides stained in pairs. The nuclear masses identified and studied were for the hypothalamus those listed by Rioch, Wislocki, and O'Leary(10) and Gurdjian(11) and for the thalamus those listed by Gurdjian (11). In the cerebral cortex, the areas designated by Krieg's(12) numerical nomenclature were investigated. All of the above named areas in which changes are not described were normal.

Results. ACTH-treated rats. Consistent cytological changes were observed only in the hypothalamic paraventricular nucleus, these occurring in all 5 animals receiving the hormone. There was an over-all diminution in cytoplasmic basophilia throughout the paraventricular nucleus, the decrease in Nissl substance frequently being most marked in the perinuclear areas (Fig. 1-4). Some cells were affected more than others. In those affected most severely, the total cell volume was re-

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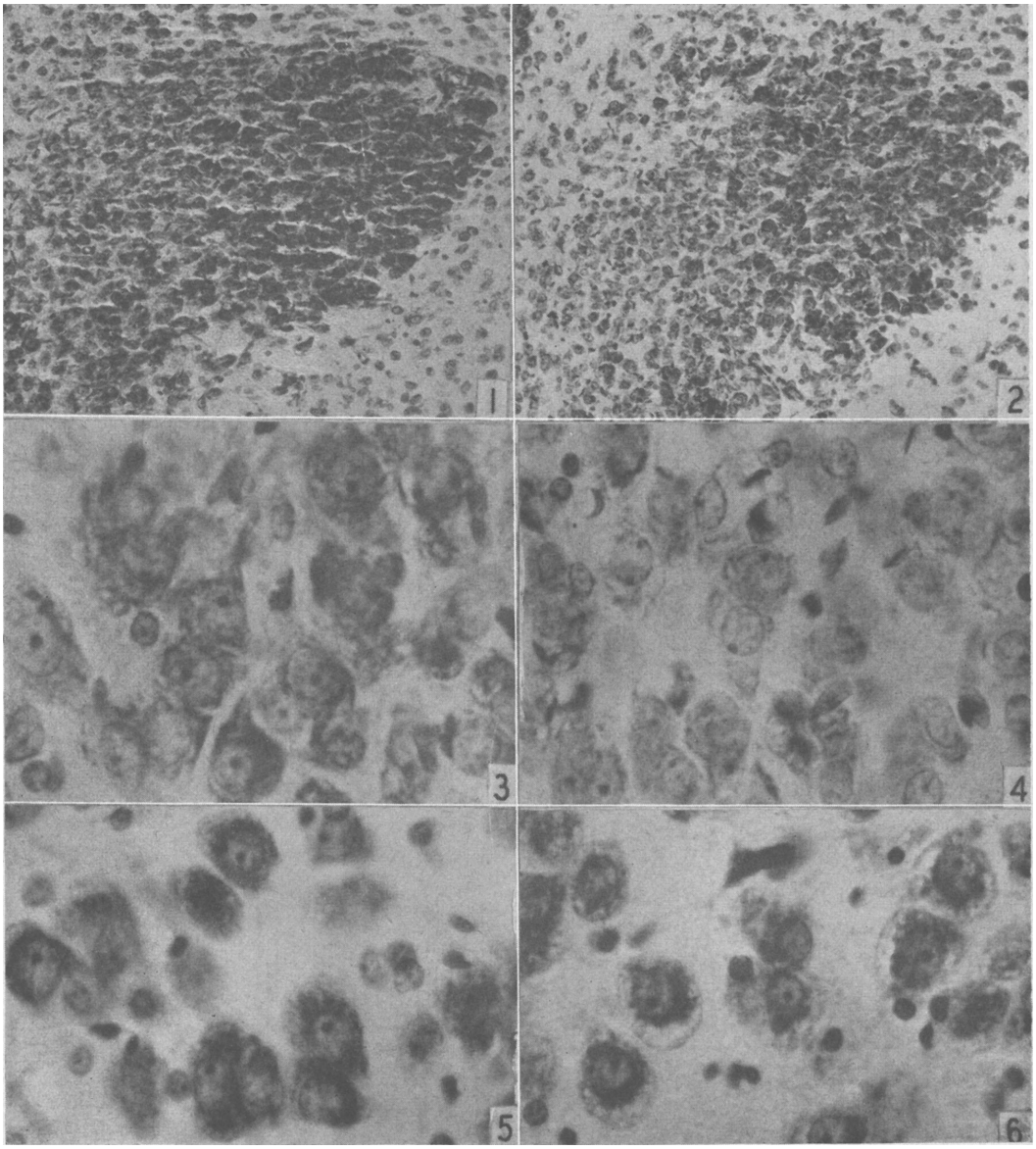

All photographs are preparations fixed in Carnoy's fluid and stained with Toluidine blue.

FIG. 1. Hypothalamic paraventricular nucleus of a control rat. ×125.

FIG. 2. Paraventricular nucleus of a rat treated with 6 mg of ACTH daily for 21 days by continuous injection. The amount of Nissl substance is reduced. ×125.

FIG. 3. Cells of the paraventricular nucleus of a control rat showing the distribution of Nissl material in the cytoplasm and large size of the nucleolus. ×680.

FIG. 4. Cells of the paraventricular nucleus of a rat treated as for Fig. 2. The cells, nuclei, and nucleoli are smaller and the cytoplasm is depleted of Nissl substance. ×680.

FIG. 5. Nissl material in the cells of the anterodorsal nucleus of the thalamus of a control rat. ×575.

FIG. 6. Chromatolysis and vacuolation of the cells in the anterodorsal nucleus of the thalamus after treatment with cortisone. ×575.

duced, the nucleus was smaller, less chromatic and contained a smaller nucleolus. In the anterior hypothalamic area, there was some suggestion that the number of cells containing Nissl material was reduced in the treated rats.

In 2 of the 4 rats treated with the 6 mg

dose of ACTH, the cells of the *N. reuniens* and *N. rhomboidalis* of the thalamus were swollen and distorted with some chromatolysis and vacuolation of the cytoplasm. One rat which received 6 mg of the hormone daily showed swelling and chromatolysis of the large ganglion cells in the stratum ganglionare throughout most of the cerebral neocortex (Krieg's(12) areas 4, 6, 8, 10, 24, 1, 2, 2a, 3, 39, 40, 17, and 18a).

Cortisone-treated rats. These animals were in poor condition at autopsy because of the high dosage of hormone given, and the histological changes in the brain were more widespread and of a character different from that induced by ACTH. In the cells of the hypothalamic paraventricular and supraoptic nuclei, cortisone caused a reduction in the Nissl material and that which remained was aggregated into clumps which were located in the peripheral part of the cell. In some cells there was moderate vacuolation of the cytoplasm. Nissl material was reduced in the anterior, lateral, ventromedial and posterior hypothalamic nuclei and in the *N. periventricularis posterior pars ventralis.*

Widespread chromatolysis and cytoplasmic vacuolation occurred throughout the thalamus, the following nuclei being affected in all treated rats: *N. anterodorsalis* (Fig. 5 and 6), *N. anteroventralis, N. lateralis thalami, N. lateralis pars posterior, N. parataenialis, N. medialis ventralis, N. paracentralis, N. reuniens, N. paraventricularis anterior, N. centralis,* and the commissural nuclei. In 2 of the treated rats the following nuclear groups were altered in a similar manner: *N. anteromedialis, N. posterior thalami, N. parafascicularis, N. rhomboidalis,* and *N. paraventricularis posterior.*

No hemorrhage, edema, liquefaction necrosis, or glial proliferation was observed in the cerebral cortex.

Discussion. The observation that ACTH exhibits its greatest effect on the paraventricular nucleus of the hypothalamus correlates well with the results of other studies dealing with this area. Scharrer and Scharrer(13) postulated on the basis of the presence of cytological evidence of secretion by the constituent nerve cells, that they perform both a secretory and nervous function. The failure of dogs with bilateral lesions in the lateral hypothalamic area to show the expected drop in blood eosinophiles during stress combined with other experimental data, suggested to Hume and Wittenstein(14) that the paraventricular nucleus serves as a central area which controls the secretion of ACTH by the anterior hypophysis. Unfortunately, the location of their lesions was not proven by microscopic examination of the brain. Since the supraoptic nucleus gives rise to nerve fibers passing to the hypophysis, it is of interest that destruction of this nucleus in the experiments of Hume and Wittenstein did not interfere with the eosinopenic response and also, that in our experiments ACTH did not modify its structure. These workers concluded that the paraventricular nucleus exerts its control over the hypophysis by humoral rather than nervous means. This conclusion correlates nicely with the cytological changes described here since the reduction in the amount of Nissl material and in size of the nucleolus (structures containing large quantities of ribonucleoprotein) as well as the reduction in cellular and nuclear size might be interpreted in terms of a lowered rate of synthesis of a protein secretion(15). Also, these changes might indicate diminished capacity for discharge of nervous impulses.

Heinbecker and Pfeiffenberger(16) observed degeneration in the paraventricular nucleus in a case of Cushing's syndrome and interpreted this change as being the primary cause of the disease. Our observations on the action of ACTH on the paraventricular nucleus indicate that these workers were dealing with the result of hyperadrenocortical activity rather than with the cause of the disease.

The important rôle played by the hypo-

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thalamus in the regulation of emotional expression is well-known. Hence, these observations may be of some assistance in understanding the changes in personality which sometimes are observed clinically following treatment with these hormones. Since the anatomical modifications induced by cortisone were more general and the rats so treated were in poor condition at the time of autopsy, further studies are being carried on to determine the specificity of the changes elicited by cortisone in the hypothalamus and in the thalamus.

Summary. Administration of ACTH caused chromatolysis in the cells of the paraventricular hypothalamic nucleus. Cortisone affected this nucleus but induced more widespread chromatolysis and vacuolation of thalamic and hypothalamic nerve cells.

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Intraspinal Inoculation of Mice in Experimental Poliomyelitis. (18489)

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A large proportion of the experimental work accomplished in the field of poliomyelitis has been through the use of mice inoculated intracerebrally with the Lansing rodent-adapted virus(1). Low titers, prolonged incubation periods and irregular responses of individual animals have continued to present difficulties in the use of this intracerebral technic(2). The apparent hypersusceptibility of the anterior horn cells of the spinal cord has made it seem obvious that direct inoculation of virus in this location should give better results. Melnick, Horstmann and Ward(3) recognized this and used an intraspinal technic for inoculating monkeys with poliomyelitis materials. However, in spite of a consistently shorter incubation period they concluded that this technic was not practical since a large number of undesirable side reactions occurred and there was evidence that it was a less sensitive method in monkeys than intracerebral inoculation. Howe and Bodian have reported the successful intraspinal inoculation of monkeys with central nervous system virus(4). Other workers in unpublished experiments have attempted intraspinal or intracisternal inoculation of mice through the skin with negative results due to technical difficulties. Using dye solutions the following technic in mice has been developed and its possible application to poliomyelitis research evaluated.

Technic of intraspinal (I.S.) inoculation. Under ether anesthesia the middle of the back of the mouse is wetted down with alcohol and a transverse incision through the skin is made with small iris scissors. The mouse is then held in one hand in such a way that the vertebral column is slightly flexed. Using a ¼ inch 27 gauge short bevelled needle and a 0.25 ml tuberculin syringe the needle point is introduced between the vertebrae at the level of the lumbar enlargement of the cord, slightly to the right of the midline. The direction of the pathway of the needle as slight pressure is applied is at about a 45° angle toward the head and a very slight angle toward the midline. As the point of the needle enters the spinal canal a definite "giving" sensation is felt by the hand holding the syringe. A volume of 0.02 ml is then injected and the needle removed along the same pathway by which it was introduced. No appli-

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