ent method.[3] Not all of the subjects tested showed an elevation in pain threshold following administration of 0.016 g of morphine sulfate, which for the entire group produced a mean increase of only 0.0218 volt.

The fact that for producing analgesic effects in the present experiment ethylchloride had to be applied until pain developed indicated that pain rather than cold was the sensation responsible for elevating pain thresholds. The mechanisms involved in producing the analgesic effects cannot yet be stated with certainty. Distraction, changes in local blood supply and other short lasting effects of induced pain cannot fully explain the phenomenon because the pain induced in our subjects lasted for 2 to 3 minutes at the most while the analgesic effects were noticeable for a period of 90 to 120 minutes.

In additional investigations, the possibility is being examined whether epinephrine might be the agent involved in producing analgesic effects following induced pain. The trend of thought seems logical in view of the facts that epinephrine is released by pain from the adrenal glands[4] and that it possesses marked analgesic properties when injected in man or dog.[5]

Summary. Experiments have been made in normal human subjects which demonstrate that induced pain of short duration produces long lasting analgesic effects.

3 Ivy, A. C., Goetzl, F. R., and Burrill, D. Y., *War Medicine*, 1944, **6**, 67.

4 Cannon, W. B., *Bodily Changes in Pain, Hunger, Fear, and Rage*, New York, D. Appleton and Co., 1929.

5 Ivy, A. C., Goetzl, F. R., Harris, S. C., and Burrill, D. Y., *Quart. Bull. Northwestern Univ. Med. School*, 1944, **18**, 298.

15179

Effect of Adrenocorticotropic Hormone (ACTH) on Hypophysectomized Adrenal-demedullated Rats.*

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Ingle[1] has raised the question of the possible role of the adrenal medulla in the response of the adrenal cortex to adrenocorticotropic hormone. In the present study the problem of the relationship between the two parts of the adrenal has been approached by comparing the effectiveness of adrenocorticotropic hormone as a stimulant of the cortical tissue when administered in the presence or absence of the medullary tissue.

Methods. Male rats were adrenal-demedullated† at 25 days of age and the adrenal cortex was allowed to regenerate for 16 days. The animals were supported for the first 13 days of this period by 1% NaCl in the drinking water. The rats were then hypophysectomized, at 41 days of age. Pure ACTH[2] was injected for 15 days, beginning on the day of hypophysectomy, in an effort to maintain the adrenal cortical tissue. The cortical response of the doubly operated animals was compared with that of an equal number of rats hypophysectomized at the same age, in which the adrenal was intact. The daily dose of ACTH, 0.2 mg, was divided into two intraperitoneal injections of 0.5 cc each.

At autopsy the sella turcica of all hypophysectomized rats was searched under 10-fold magnification for remnants of pituitary tissue.

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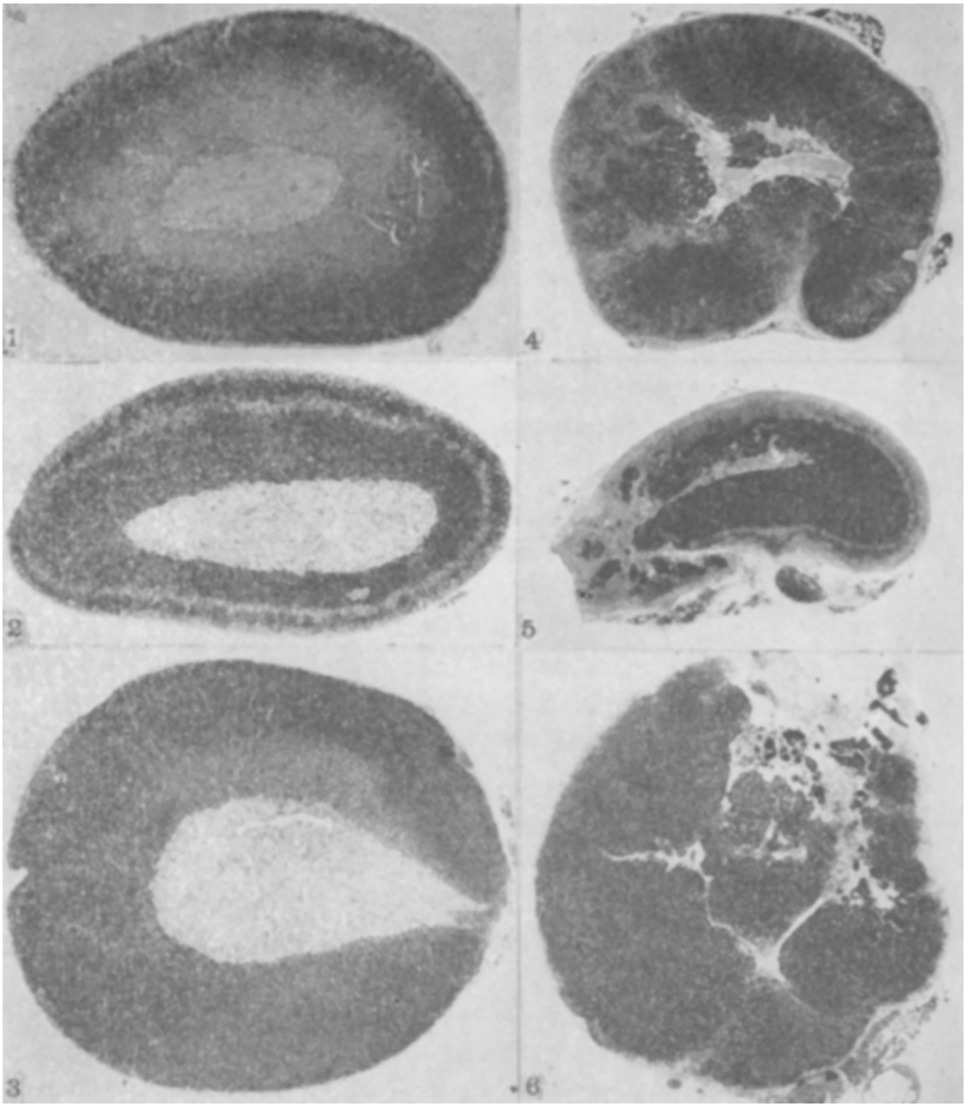
1 Ingle, D. J., *Endocrinol.*, 1942, **31**, 419.

† The adrenal medulla and all cortical tissue except a few cells adhering to the capsule were enucleated.

2 Li, C. H., Evans, H. M., and Simpson, M. E., *J. Biol. Chem.*, 1943, **149**, 413.



Sudan orange stain, X 21.5.

FIG. 1. Adrenal of a normal 41-day-old male rat, illustrating the condition of the adrenal cortex at the time of hypophysectomy. Note the even distribution of fine lipid droplets, with a slightly greater concentration in the glomerulosa and a faint subglomerular zone of somewhat depleted lipid content. Weight of 2 adrenals 25 mg.

FIG. 2. Adrenal of a 56-day-old hypophysectomized male rat, 15 days postoperative. The cortex is markedly reduced in width. The lipid occurs in coarse droplets and there is a wide subglomerular sudanophobe zone. Weight of 2 adrenals 10 mg.

FIG. 3. Adrenal of a 56-day-old hypophysectomized rat treated with 0.2 mg of pure ACTH daily for 15 days, beginning on the day of hypophysectomy at 41 days of age. The cortex has been completely maintained and even slightly hypertrophied, as shown by the strikingly uniform distribution of lipid throughout its entire width, obliterating even the narrow subglomerular zone of the normal male. Weight of 2 adrenals 22 mg.

FIG. 4. Regenerated adrenal cortex of a 41-day-old male rat which was adrenal-demedullated at 25 days of age. The cortical tissue has reorganized in the normal zonal relationships around a central vein. The narrow subglomerular zone of lipid depletion characteristic of the normal male is discernible. Weight of cortical tissue 12 mg.

FIG. 5. Regenerated adrenal cortex of a 56-day-old male rat which was adrenal-demedullated

at 25 days of age and hypophysectomized at 41 days of age. During the 15 days elapsing since the second operation, the typical pattern of hypophysectomy has been superimposed upon the regenerated cortex. Weight of cortical tissue 8 mg.

FIG. 6. Regenerated adrenal cortex of a 56-day-old male rat which was adrenal-demedullated at 25 days of age, hypophysectomized at 41 days of age and treated, beginning immediately after operation, with 0.2 mg of pure ACTH daily for 15 days. The cortical lipid distribution is entirely comparable to that seen in the injected hypophysectomized animal with intact adrenals, and the same evidence of hypertrophy is seen in the obliteration of the subglomerular zone (Fig. 3). Reorganization of the regenerated cortical tissue has advanced as much under the influence of ACTH as in the presence of the pituitary. Weight of cortical tissue 17 mg.

Any questionable tissue was serially sectioned and examined. Atrophy of the thyroid and gonads gave additional evidence for complete removal of the pituitary. The adrenal tissue of the "demedullated" rats was sectioned serially and examined for remnants of medullary tissue. Any animals in which fragments of pituitary or adrenal medullary tissue had been left at operation have been eliminated in reporting the results of the experiment.

A total of 42 rats was used. It was necessary to provide appropriate controls for each important period in the experiment, in order to establish: (1) the extent of cortical regeneration 16 days after adrenal demedullation, at which time hypophysectomy was performed and injections were begun; (2) the condition of the adrenal cortex of the normal animal at that time; (3) the effect of hypophysectomy upon both regenerated and normal cortical tissue, as seen 15 days postoperatively, at the end of the experiment; (4) the amount of additional cortical regeneration which had taken place in the demedullated animal possessing a pituitary, during this 15-day period. Accumulated data on normal 56-day-old rats were available for the controls at the end of the experiment.

The distribution of animals into experimental and control groups may be seen in Table I. Initially there were 6 animals in each group.

Results. As will be seen in Table I, there was no difference in cortical weight response to ACTH conditioned by the presence or absence of medullary tissue. The dose used, 0.2 mg daily, was adequate to maintain the adrenal weight at the time of hypophysectomy in both types of experimental animal. The thymus was also weighed as a further measure of cortical response, and its reduction was found to have been equally drastic in both types of animal receiving ACTH.

Frozen sections of the adrenals, stained with Sudan orange, are illustrated in Fig. 1 to 6, and the salient features of cortical lipid distribution are described there. The regenerated cortical tissue in demedullated adrenals was protected against the effects of hypophysectomy by this dose of ACTH as completely as was the cortex of intact adrenals (Fig. 3 and 6). Although the weights had not been increased above the operative level, the adrenal cortex in both hypophysectomized and doubly operated animals showed some evidences of hypertrophy. Vividly sudanophilic lipid, in droplets slightly larger than normal, was distributed evenly throughout the entire width of the cortex, obliterating even the narrow subglomerular zone characteristic

TABLE I.
Response of Hypophysectomized Rats to ACTH in the Presence or Absence of the Adrenal Medulla.

	Adrenals intact				Adrenals demedullated			
Treatment	No. of rats	Body wt, g	Adrenals wt, mg	Thymus wt, mg	No. of rats	Body wt, g	Cortical tissue, mg	Thymus wt, mg
Controls at time of hypophysectomy, 41 days old	6	133	25	364	6	139	17	402
Hypophysectomized, ACTH for 15 days	6	105	24	63	6	115	17	91
Hypophysectomized controls at autopsy, 15 days postoperative	6	115	10	196	6	124	10	199
Controls at autopsy, 56 days old	54	215	34	451	5	215	22	436

of the normal male.‡ The cortical tissue of the ACTH-treated doubly operated animals seemed, in addition, to have undergone reorganization beyond that seen at the time of hypophysectomy; it resembled more closely the condition in the demedullated controls at autopsy, in which the cortical tissue had been continuing regeneration under the influence of the animal's own pituitary.

Conclusions. The stimulating action of ACTH on the adrenal cortex of hypophysectomized rats, as far as could be judged by adrenal weights and by morphology as seen in lipid stains, was the same in the absence of the medulla as in the intact adrenal.

‡ This is the typical picture of adrenal hypertrophy which has been obtained upon administration of adequate doses of ACTH to normal, hypophysectomized, gonadectomized, hypophysectomized-gonadectomized or hypophysectomized-thyroidectomized male rats.

15180 P

Hypertension Produced in Dogs by Unilateral Ligation of Periadrenal Blood Vessels and Tissue.

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The possibility that adrenal ischemia may cause hypertension was investigated in the present experiments which are concerned with the effect of decreased adrenal blood supply upon systemic blood pressure.

Method. Mongrel dogs were employed. Systolic blood pressure was determined indirectly by a mercury manometer attached to an inflated cuff wrapped around the hind leg just above the ankle of trained unanesthetized dogs. Systolic pressure was indicated by the pressure at which pulsation of the dorsalis pedis artery was first palpable when the air in the cuff was released. The cuff was inflated 15-30 successive times to determine the range of systolic pressure. These observations were checked by direct measurements with the Hamilton manometer with readings from the femoral artery and showed agreement within 5-15 mm Hg. Animals were housed in separate cages. Blood pressures were recorded daily for a preliminary period of 3-6 weeks. Then either a sham operation in the adrenal area or one to decrease adrenal blood flow was performed on one adrenal. Thereafter blood pressures were taken daily. Urine analysis and blood NPN levels were obtained before and after operation.

Operation. The left adrenal was chosen because it was more easily exposed than the right. Blood vessels come to the left adrenal from the aorta to the hilum, from the diaphragm to the superior pole and from below to the inferior pole. Many arteries enter the adrenal from pericapsular fat and connective tissue. Because of the extreme vascularity of the tissue around the adrenal it seemed that an operation which would constrict or obstruct large numbers of vessels around the capsule would be most favorable for diminishing blood supply. Two ligatures of No. 13 white surgical silk braid were tied about vascular fat and connective tissue adjacent to the adrenal. One was placed around the hilar artery and vein and the other around grossly visible arteries and veins at either the superior or inferior pole. The area involved in the ligature was about 1.0 x 0.5 cm. The procedure just described was used on 5 dogs. In a sixth, No. 391, an artery to the inferior pole and the hilar vein were stripped of fat and connective tissue and ligated with No. 11 surgical silk. To rule out non-specific effects of these procedures, sham operations were performed in 3 dogs, 2 of which had subsequent operations for producing adrenal ischemia. A ligature of