The effect of ethyl chloride spray probably can not be ascribed to reflex circulatory changes in the myofascial structures of the reference zone since in other experiments(16) we found that the spray relieves deep pain due to ischemic muscle work while the circulation to the region remains occluded. Gammon and Starr(15) observed that subcutaneous pain also was relieved by intermittent cold during occlusion of the circulation.

Summary. Thirty-six tests were carried out on 7 patients with effort angina but a normal resting electrocardiogram, in whom intravenous injection or ergonovine (0.1 to 0.4 mg) induced angina and electrocardiographic changes. Nitroglycerin, 0.4 or 0.6 mg given sublingually just before ergonovine, prevented pain and electrocardiographic changes in most patients. When angina had been induced by ergonovine and ethyl chloride spray was applied to pain areas of chest, shoulders, arms or neck (reference zone of cardiac pain in each patient), pain was uniformly relieved. Relief of ergonovine angina by ethyl chloride spray required less time than by nitroglycerin. When ethyl chloride spray was applied to pain areas just *before* ergonovine was injected, pain failed to occur in the majority, and the onset of pain was delayed in the remaining patients. In combination with ethyl chloride spray, ergonovine caused depression of the S-T segment even in the absence of pain. These results afford additional evidence of the previously reported effectiveness of ethyl chloride spray for relief of the somatic component of cardiac pain.

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1. Travell, J., and Rinzler, S. H., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 480.

2. Rinzler, S. H., and Travell, J., *Am. Heart J.*, 1948, v35, 248.

3. Travell, J., *Circulation,* 1951, v3, 120.

4. Rinzler, S. H., *Cardiac Pain,* Charles C. Thomas, Springfield, Ill., 1951, pp 23-25; 76-87.

5. Travell, J., *Connective Tissues,* Transactions of the Second Conference, Josiah Macy, Jr. Foundation, New York, 1952, pp 86-125.

6. Stein, I., *Am. Heart J.*, 1949, v37, 36.

7. Stein, I., and Weinstein, J., *J. Lab. Clin. Med.*, 1950, v36, 66.

8. ———, *New York State J. Med.*, 1953, v53, 1454.

9. Travell, J., *Arch. Phys. Med.*, 1952, v33, 291.

10. ———, *New York State J. Med.*, 1952, v52, 1550.

11. Weiss, S., and Davis, D., *Am. J. Med. Sci.*, 1928, v176, 517.

12. Lindgren, I., *Cardiologia,* 1946, v11, 207.

13. Theobald, G. W., *Lancet,* 1949, v2, 41.

14. Russek, H. I., Urbach, K. F., Doerner, A. A., and Zohman, B. L., *J.A.M.A.*, 1953, v153, 207.

15. Gammon, G. D., and Starr, I., *J. Clin. Invest.*, 1941, v20, 13.

16. Travell, J., and Rinzler, S. H., *Fed. Proc.*, 1949, v8, 339.

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Presence of Antidiuretic Material in Blood of Hypophysectomized Rats. (20872)

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It has generally been accepted that removal of the posterior pituitary results in disappearance of antidiuretic hormone. Several investigators, using different assay technics (1-3), have reported the absence of antidiuretic material from the blood of hypophysectomized rats.

In the course of studies of turn-over of water and metabolism of posterior pituitary hormone of hypophysectomized rats which were a month or more postoperative, it was observed that an appreciable number of the animals had antidiuretic material in their blood. This was not found in the blood of

recently hypophysectomized animals. This report describes these findings and other evidence which supports the concept that this antidiuretic material may be formed and released by the hypothalamus.

Methods and materials. Antidiuretic material was assayed by the method of Jeffers (4) which employs the alcohol anesthetized and hydrated rat and the intravenous injection of the material being tested. Details of the technic were as published by Ames and van Dyke(2), except that it was found that a diuresis could be more dependably produced when the interval between the administration of the anesthetizing dose of alcohol and the hydrating dose of water was increased to approximately an hour. In some animals hydration was maintained by the administration of small amounts of water and alcohol through a plastic stomach tube left in place throughout the assay. Hypophysectomized Sprague Dawley males were purchased from the Hormone Assay Laboratories. Careful dissection of the region of the sella turcica or histological sections of this region have not revealed detectable remnants of hypophysis. The marked atrophy of the endocrine system characteristic of hypophysectomized animals occurred in each case. A number of animals were demonstrated to have the inability to excrete water which is found in the hypophysectomized animal. The water test was not carried out in every animal because of the high mortality from water intoxication. In the initial experiment the animals were fed Purina Dog Chow, milk and vegetables *ad libitum*. All of these animals developed the marked cachexia of the hypophysectomized rat. Subsequently, other animals were fed a high protein, high calorie diet in which the principal source of protein was ground horsemeat. These animals gained weight although their endocrine organs all showed the usual posthypophysectomy atrophy. The two diets produced no differences in the amount of antidiuretic substance in the blood. Blood from hypophysectomized animals was obtained by heart puncture, under pentobarbital anesthesia. The assay was usually carried out on serum although the plasma levels were essentially the same. The blood was immediately placed in iced tubes and kept iced during separation of the serum. The serum was injected into the assay animal within 10 minutes after the blood was drawn. The hypothalami were obtained by taking a block of tissue about 5 mm wide from the floor of the third ventricle, extending from anterior of the optic chiasm to posterior of the mammillary bodies. This whole block of tissue was homogenized and diluted with 25 cc of physiological saline solution. Two-tenths cc of this solution was given intravenously. A comparable amount of cerebral cortex from one of the lateral lobes was used as a control. This tissue was never found to have antidiuretic activity.

TABLE I. Antidiuretic Activity of Serum of Hypophysectomized Rats.

Postoperative interval	No. of animals	ADS of serum, γu of pitressin per cc
$<$ 7 days	6	$<$ 30
$>$30 "	29	60–400

Results. The antidiuretic content of the blood of the hypophysectomized animals is shown in Table I. The blood of 29 hypophysectomized animals which were at least one month postoperative was tested and the blood of 21 was found to contain antidiuretic activity. Detectable antidiuretic activity was found in .05 to .3 cc of serum. An amount of antidiuretic substance equivalent to 20 microunits of pitressin was usually encountered in 0.1-0.2 cc of serum *i.e.* 60 to 400 microunits of pitressin per cc of serum. This is essentially the same amount as has been found in the blood of intact animals. Six recently hypophysectomized animals were tested. No activity was found in serum in amounts up to .5 cc.

The hypothalami of 7 animals hypophysectomized for more than 30 days were tested and found to contain activity equivalent to more than 5 milliunits of pitressin. The antidiuretic activity of the hypothalami of 7 recently hypophysectomized animals was considerably less than 5 milliunits, but the exact amount of antidiuretic activity has not yet been determined.

Discussion. The presence of antidiuretic

activity in the blood of hypophysectomized animals suggests either that the posterior pituitary has not been removed or that the structures remaining have reorganized to permit release of antidiuretic material.

Although Bodian and Maren(5) have reported the persistence of neurohypophyseal tissue in hypophysectomized rats, any remnants in the animals used in this study must have been extremely small. The absence of antidiuretic activity in blood soon after hypophysectomy and its appearance somewhat later suggest that a functional reorganization has taken place permitting formation and release of antidiuretic hormone.

There is considerable evidence to support the concept of neurosecretion by the hypothalamus. This hypothesis holds that antidiuretic hormone is formed in the nuclei of the hypothalamus and that it progresses along nerve cells to the posterior lobe from which it is released(6). The data presented here tend to support the concept that the hypothalamus forms antidiuretic hormone and in addition, they suggest that under appropriate circumstances, it releases antidiuretic hormone.

The finding of larger amounts of antidiuretic hormone in the hypothalami of the 30-day postoperative animals suggests that a reorganization has taken place permitting the hypothalamus to accumulate antidiuretic hormone. These data confirm the finding by Sato(7) in the dog of an increased amount of antidiuretic hormone after hypophysectomy. As pointed out by Melville and Hare(8), who found a decline of antidiuretic activity in the supraoptic region after hypophysectomy, the median eminence was included in Sato's extract. It was also included in our extracts. This area has been shown to contain pituicytes which produce vasopressin(9) and these cells are said to hypertrophy after hypophysectomy (10). It is probable that the increase in antidiuretic content of the hypothalamus occurs as a result of increases in activity of cells in the region of the median eminence and that the content of antidiuretic hormone in the supraoptic nuclei is actually decreased.

The ability of the hypothalamus of the hypophysectomized animal to secrete antidiuretic hormone is suggested by the demonstration by Mirsky *et al.*(11) of an increase in antidiuretic activity in the blood of hypophysectomized animals following noxious stimuli. Anatomical evidence suggesting this possibility has been found by Laqueur(12) who has demonstrated structural reorganization in the region of the median eminence of rats after hypophysectomy suggesting the possibility that this tissue may be able to secrete. This concept would help to explain the demonstration of antidiuretic substances in the blood of some humans with diabetes insipidus(13). Although the antidiuretic activity of the blood of these patients tended to be lower than normal, there was appreciable activity detectable in several patients who had severe diabetes insipidus. It was suggested that in these cases the diabetes insipidus might be due not only to a decrease of an antidiuretic factor, but also to an increase in diuretic substances.

Conclusion. 1. The blood of rats hypophysectomized for a month or more contains antidiuretic activity in amounts similar to those found in normal animals. 2. In the blood of animals hypophysectomized for less than one week there is no detectable antidiuretic material. 3. The hypothalami of recently hypophysectomized animals contain less antidiuretic activity than the hypothalami of animals hypophysectomized for a longer time.

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1. Birnie, J. H., Jenkins, R., Eversole, W. J., and Gaunt, R., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 83.

2. Ames, R. G., van Dyke, H. B., *Endocrinol.*, 1952, v50, 350.

3. Ginsburg, M., and Heller, H., *J. Endocrinol.*, 1953, v9, 274.

4. Jeffers, W. A., Livezey, M. M., and Austin, J. H., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 184.

5. Bodian, D., and Maren, T. H., *J. Comp. Neur.*, 1951, v94, 485.

6. Scharrer, E., and Scherrer, B., *Physiol. Rev.*, 1945, v25, 171.

7. Sato, G., *Arch. f. Exper. Path. w. Pharmakol.*, 1928, v131, 45.

8. Melville, E. V., and Hare, K., *Endocrinol.*, 1945,

v36, 332.
9. Blount, R. F., PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 629.
10. Gersh, I., and Brooks, C. McC., *Endocrinol.*, 1941, v28, 6.
11. Mirsky, I. A., 19th International Physiol. Congress, Montreal 1953, Abst. p. 621.
12. Laqueur, G. L., personal communication.
13. Lloyd, C. W., and Lobotsky, J., *J. Clin. Endocrinol.*, 1950, v10, 318.

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Variations in Sensitivity to Anaphylaxis and to Histamine in Inbred Strains of Mice.* (20873)

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(Introduced by Richard Thompson.)
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Following a study demonstrating that there was genetic variation in the ability of mice to form circulating antibody(1), experiments were designed to determine whether or not similar variation could be demonstrated for the ability to react with anaphylactic shock; and to determine the extent that anaphylaxis was dependent on circulating antibody. This further led to an inquiry concerning the basic mechanism of anaphylaxis in the mouse, especially to a re-examination of the possibility of a relationship between anaphylaxis and histamine sensitivity.

Materials and methods. Anaphylaxis. Every attempt was made to operate under conditions identical to those prevailing in the study on circulating antibody(1). The same 5 inbred strains of mice—DBA/1 Jax, BALB/C Jax, C3H/Jax, C57BL/6 Jax, and A/He Jax, all sufficiently inbred to insure genetic uniformity in succeeding generations, were used. They were of mixed sexes, and over 4 months old. The amount of alum precipitated egg white, the intra-abdominal route of injection, and the time interval did not vary between the 2 experiments.

Mice were given a challenging intravenous injection of 15 mg of crystalline egg albumin in 0.25 ml of 0.85% NaCl, an amount estimated to furnish an antigen excess. The mice were carefully observed for the characteristic signs of anaphylaxis, and the findings were recorded in accordance with the following: convulsions with or without death, *positive,* severe; muscular spasms or paralysis without death, *positive,* mild; no effect or very slight agitation, in absence of above, *negative.* The time of death was recorded for each animal

TABLE I. Effect of Circulating Antibody on Sensitivity to Anaphylaxis.

	DBA	BALB/C	C3H	C57BL/6	A/He
No. with circulating antibody* / No. injected	28/28	23/27	22/22	26/27	23/24
Rank*†	Excellent-good	Excellent	Good	Excellent	Fair
Anaphylaxis:					
Severe with death	4	12	4	5	24
" without death	0	3	0	0	8
Mild shock	0	0	1	1	0
Negative	28	10	26	36	1
Total	32	25	31	42	33

* Results from (1).
† Based on a statistical analysis considering both number positive and titer.

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