# Pituitary Stimulating Substance in Brain Blood of Hypophysectomized Rat Following Electric Shock "Stress".*† (22269)

SHAWN SCHAPIRO, JESSIE MARMORSTON, AND HARRY SOBEL.
*Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.*

Release of ACTH following non-specific physiological stimuli is thought to involve the hypothalamus. However, the role of the hypothalamus in mobilization of ACTH is unclear. Following the ingenious experimental work of Harris and his co-workers (1-3), the concept of a hypothalamic humoral mediator in release of ACTH has gained wide support. The work of Harris *et al.*, Hume(4) and others(5-6), supports the thesis that areas in the posterior hypothalamus contain centers responsible for ACTH release. McCann and Sydnor(7) emphasize importance of anterior hypothalamus and suggest that pitressin, synthesized in this area(8), stimulates ACTH production following stress. The possible role of pitressin in stimulating release of ACTH has been emphasized by our work(9). However, Saffran *et al.*(10) reported that a contaminant of pitressin, but not pitressin itself, will cause ACTH release *in vitro*.

A minority of investigators, chiefly Vazquez-Lopez(11), believe that nerve fibers coursing in the hypothalamic-hypophyseal tract may serve to increase ACTH release by the anterior pituitary. To determine if a humoral substance is released from the hypothalamus following stress, the following experiment was devised: Venous brain blood of stressed hypophysectomized rat (donor animal) was administered into recipient animal in which endogenous mobilization of ACTH was impaired by electrolytic lesions placed in the hypothalamus (Fig. 1).

*Methods. Hypophysectomy*: Adult rats of the Addis-Slonaker strain were hypophysectomized and not used until at least 3 weeks later. The effect of ether anesthesia on eosinophils of several of these animals was determined. After each experiment, the sella was inspected with dissecting microscope for pituitary remnants. If found, the experiment was discarded. *Hypothalamic lesions*: Electrolytic lesions were placed in the hypothalamus by the Horsley-Clark stereotaxic apparatus as modified for the rat by Krieg(12). An indifferent electrode was placed in the rectum. The lesion electrode consisted of #21 gauge hollow tubing into which an entomological pin‡ was soldered at one end. The electrode was insulated with Formvar enamel§ except for 1 mm at tip of protruding pin. A current of 3 ma maintained for 15-20 seconds was used. Bilateral lesion sites were placed 4-7 mm anterior to intra-aural plane, 1 mm lateral and withdrawn 0.5-1 mm from base of skull. This location was generally within 1-2 mm of Bregma. Thirty to sixty % of the animals died within 2 days after the lesion. All lesioned rats surviving 3 weeks or more were subjected to ether anesthesia, found by Sydnor *et al.*(13) and Sayers *et al.*(14) to produce a stress reaction in rats as measured by increase in serum ACTH. Changes in level of circulating eosinophils were determined. Any rat having a decrease in eosinophils > 20% was discarded as this indicated ability of lesioned rat to mobilize ACTH following stress was undisturbed. About 70% of lesioned rats were thus discarded. The remaining rats were used for experimental purposes. In addition, intact and adrenalectomized rats were exposed to ether and changes in eosinophils determined.

*Experimental Procedure.* Electric shock

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‡ No. A-80; Size 1—Clay Adams Co., New York.

§ We wish to thank Mr. A. E. McCotter of Division of General Electric, Schenectady, for generously supplying Formvar enamel No. 9825 and thinner No. 9415.

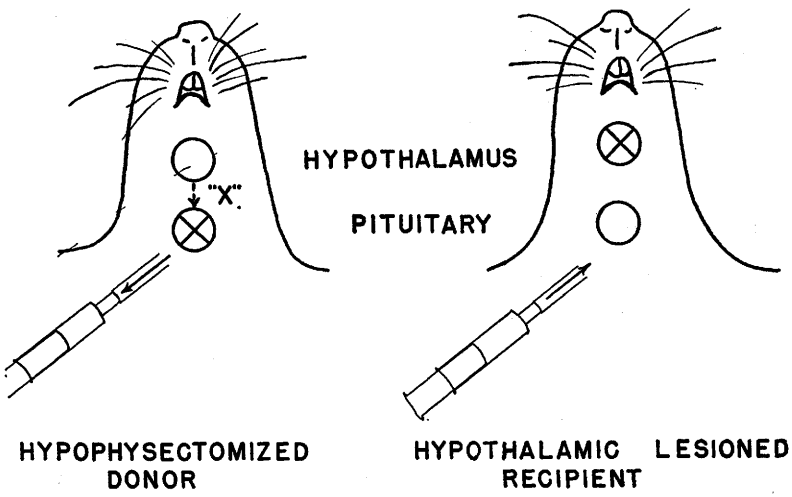


FIG. 1. Schematic representation of experimental design. The hypophysectomized rat is shocked with electricity and its venous brain blood collected in a syringe. This blood is injected intra-arterially into the hypothalamic lesioned rat. The dotted arrow represents a presumed secretion of the "Hypothalamic Hormone(s)" in response to the shock. Circles with × through them indicate removal of the designated areas.

stress was selected, for this could be controlled as to duration and intensity. An Ene-volt transformer¶ was employed. One lead was connected to each front foot and a current of approximately 24 volts administered at 3 per second for 2 minutes; 15 seconds on—5 seconds off. This constituted a severe stress, for the rats would struggle and convulse.

The experimental design is shown schematically in Fig. 1. At 9:00 A.M. a hypophysectomized donor rat was anesthetized with ether. The posterior facial vein (PF vein)** on one side was cannulated peripherally. The opposite PF vein was isolated and 0.2 cc heparin injected intravenously. The rat was maintained under light anesthesia for 60-90 minutes, then ether was withdrawn. Now the lesioned rat was anesthetized with ether and a blood sample immediately taken from free-flowing tail blood for eosinophil determinations. One carotid artery was cannulated peripherally; electric shock stress was then administered to the hypophysectomized donor rat. The isolated PF vein was ligated and about 2 cc of blood was withdrawn, during a 2-3 minute period, from the cannula in the contralateral PF vein. This blood was immediately injected into the lesioned recipient by means of the carotid cannula. Generally $<15$ minutes elapsed between anesthetizing the lesioned rat and injection of blood from the hypophysectomized rat. Eosinophils in the lesioned rat were then counted 30 minutes and 4 hours after injection of brain blood from the hypophysectomized rat. They were stained with propylene glycol-phloxine solution(15) and plated on a Fuchs-Rosenthal counting chamber. Four chambers were counted under low power. There was no evidence of blood incompatibility as determined by mixing 2 drops of blood from both donor and recipient animals.

*Controls.* Hypophysectomized rats were anesthetized with ether and the posterior facial vein was cannulated as described above. After 90 minutes, blood was withdrawn into the cannula and injected into the carotid artery of the same lesioned rats, which had received, or were subsequently to receive blood from "stressed"†† hypophysectomized rats.

¶ Correl & Correl, Chicago Heights, Ill.

** Dr. H. Hoagland(16), has shown that 70-80% of venous brain blood of the rat leaves the head via this vein.

†† Hypophysectomized rats shocked with electricity are hereafter referred to as "stressed" hypophysectomized rats.

TABLE I. Changes in Circulating Eosinophils in Rats 4 Hr after Exposure to Ether.

| | (10) Normal | (8) Lesioned | (6) Hypophysectomized | (6) Adrenalectomized |
|---|---|---|---|---|
| % change in eosinophils | —58* | —11, —10, —20, —15, +13 | +8, +43 | —16 |
| | —41 | —20, —22 | —15 | — 3 |
| | —35 | +83 | —25 | +17 |
| | —55 | —19 | —13, —15, +9 | — 7 |
| | —69 | +18 | —22 | — 8 |
| | —50 | +16 | — 8 | —10 |
| | —43 | + 7 | | |
| | —53 | +14 | | |
| | —38 | | | |
| | —55 | | | |

* (—) % decrease; (+) % increase.

Injection of blood from "unstressed"‡‡ hypophysectomized rats occurred either a week before or after injection of brain blood from "stressed" hypophysectomized rats. Brain blood from "stressed" hypophysectomized rats was injected into adrenalectomized rats and hypophysectomized rats prepared as indicated above for the lesioned rat.

*Results. Effect of ether stress on circulating eosinophils.* Eosinophil changes occurring in 10 normal rats following exposure to ether are shown in Table I. A marked decrease occurred in each animal. This eosinopenia did not develop in 6 hypophysectomized rats exposed to ether, nor in 6 adrenalectomized rats similarly treated. This indicates that eosinopenia, following exposure to ether anesthesia, depends upon an intact pituitary-adrenal axis. In 8 lesioned rats anesthetized with ether eosinopenia also did not occur (Table I). Thus, it appears that a functional hypothalamic-pituitary-adrenal axis is necessary for the eosinopenic effect of ether. Several rats cited above were tested more than once for their response to ether.

*Injection of brain blood from "stressed" and "unstressed" hypophysectomized rats into lesioned rats.* The 8 lesioned rats which indicated no eosinopenia, following ether (Table I), were injected with brain blood from "stressed" and "unstressed" hypophysectomized rats. Changes in eosinophils are shown in Fig. 2. Lesioned rat #1 received blood from 2 different "stressed" hypophysectomized rats twice during 2 weeks, while the other lesioned rats received one injection of brain blood from a "stressed" hypophysectomized rat, and one injection of brain blood from an "unstressed" hypophysectomized rat. Thus, each rat served as its own control. The decrease in eosinophils in lesioned rats following injection of brain blood from "stressed" hypophysectomized rats was 65-90%. Following injection of brain blood from unstressed hypophysectomized rats into these same lesioned rats, the decrease was generally 25-35%; in one animal, eosinophils actually increased. In each case, regardless of whether the lesioned rat received "stressed" or "unstressed" blood, the 30 minute count was higher than the initial count.

*Injection of brain blood from "stressed" hypophysectomized rats into adrenalectomized rats and hypophysectomized rats.* This procedure was undertaken to determine whether eosinopenia following injection of brain blood from "stressed" hypophysectomized rats, was mediated by the adrenal-pituitary axis. In 4 adrenalectomized rats injection of brain blood from "stressed" hypophysectomized rats caused a decrease in eosinophils of only 15%, 1%, 19% and 11%. In 4 hypophysectomized rats into which brain blood from "stressed" hypophysectomized rats was injected, there was a decrease in eosinophils of 26%, + 27%, 23% and 4%.

*Location of lesion sites.* The brain was fixed in 10% formalin and several weeks later, sectioned through the hypothalamic area and stained in hematoxylin and eosin. Lesions were of massive nature, as intended, for we desired to block mobilization of ACTH without regard to minute hypothalamic areas. In 4 rats in which lesion sites were determined, the following areas were involved:§§ Rat #1 posterior hypothalamus including mammillary bodies, tuber cinereum and mammilothalamic tract. Rat #2 posterior hypothalamus including mammillary bodies, pitui-

‡‡ Hypophysectomized rats not shocked with electricity are referred to as "unstressed" hypophysectomized rats.

§§ We wish to thank Dr. John D. Green for localizing the lesion sites.

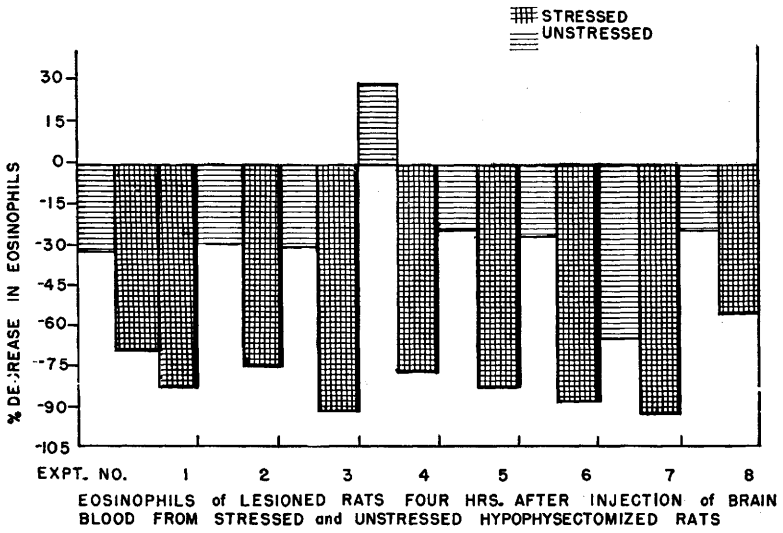


FIG. 2.

tary stalk, supraoptic and ventral hypothalamus. Rat #3 ventral hypothalamus including tuber cinereum; ventromedial nucleus and dorsomedial nucleus. Rat #4 posterior hypothalamus including tuber cinereum and mammillary bodies.

The location of massive lesions which blocked ACTH release, as measured by eosinopenia, were in the posterior and ventral hypothalamus and included mammillary bodies and tuber cinereum. McCann and Sydnor (7) observed that only anterior hypothalamic lesions, which destroyed the median eminence, blocked adrenal ascorbic acid depletion following laparotomy and subcutaneous epinephrine. Our lesion sites are in general agreement with Harris(1-3), Hume(4), Slusher & Roberts(6), and Porter(5), who similarly found areas in posterior hypothalamus responsible for ACTH mobilization. None of our lesioned rats demonstrated marked diabetes insipidus although daily water consumption records exceeded that of normal controls.

*Discussion.* Our data indicate that venous brain blood of "stressed" hypophysectomized rats will induce eosinopenia when injected into rats bearing hypothalamic lesions. This eosinopenia depends upon presence of intact pituitary-adrenal axis in recipient animal. Eosinopenia did not occur when brain blood from "unstressed" hypophysectomized rats was injected into these same lesioned rats. The assumption therefore appears justified that following "stress," brain blood of hypophysectomized animal contains a pituitary stimulating substance(s). These observations are compatible with the hypothesis of hypothalamic humoral control over ACTH secretion, and point to hypothalamus as the most probable site of hypophysiotropic principle demonstrated here. The demonstration that eosinopenia occurs only if pituitary and adrenal glands are present in recipient animal, further suggests that brain blood from "stressed" hypophysectomized rats exerts an eosinopenic effect via pituitary-adrenal axis, and has little, if any, direct effect upon level of circulating eosinophils.

The level of circulating eosinophils has been used by many workers investigating functional integrity of the hypothalamic-pituitary-adrenal axis(1,4,5). In some cases, however, eosinopenia may develop in adrenalectomized animals(17-18), following laparotomy and other severe stresses, although most investigators have found that this is not the case. We did not notice eosinopenia in adrenalectomized or hypophysectomized rats exposed to ether stress and minor neck surgery, nor did it occur in our rats with hypothalamic lesions. Thus under our conditions, eosinopenia depends upon intact hypothala-

mic-pituitary-adrenal axis.

*Summary.* Brain blood from "stressed" and "unstressed" hypophysectomized rats was injected into rats bearing hypothalamic lesions, and also into hypophysectomized and adrenalectomized rats. When brain blood from stressed hypophysectomized rats was injected into lesioned rats, a marked eosinopenia occurred; this did not occur when unstressed brain blood was injected into those same lesioned rats. This eosinopenia depended upon the presence of an intact pituitary-adrenal axis.

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## Effects of Vitamin E, N,N$^1$-Diphenyl-Para-Phenylene Diamine, and Fish Liver Oil on Reproduction in Turkeys.*† (22270)

LEO S. JENSEN, J. S. CARVER, AND JAMES MCGINNIS.

*Department of Poultry Science, State College of Washington, Pullman.*

A deficiency of vit. E has recently been reported in turkey breeder hens fed practical rations(1,2). The lenses of the eyes of embryos from the eggs laid by hens fed diets deficient in vit. E were cloudy in the central portion(3). In vit. E studies with chicks, Singsen *et al.*(4) found that the unsaturated fat level of the diet markedly influenced the development of encephalomalacia and that the antioxidant N, N$^1$ diphenyl-para-phenylene diamine (DPPD) was about as effective as vit. E in preventing the disease. The effects of DPPD, fish liver oil and alpha tocopheryl acetate on the reproductive performance of turkeys are reported herein.

*Procedure.* Broad Breasted Bronze turkey hens were distributed into 12 pens of 11 hens each. These birds had been fed a practical diet during the growing period and were reared on range. A basal diet of the following percentage composition was used: 66 ground corn, 18 soybean oil meal, 2.5 dehydrated alfalfa, 3 herring fish meal, 3 dried whey, 3.7 limestone, 3.5 steamed bone meal, 0.3 iodized salt, 0.0125 manganese sulfate and a vitamin mix supplying 680 I.C.U. vit. D, 1135 I.U. vit. A, 1 mg riboflavin, 6.2 mg pantothenic acid and 2.5 mg vit. $B_{12}$ per lb of

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† Scientific Paper No. 1463 Washington Agric. Exper. Stations, Pullman. Project No. 715.