TABLE I. Duration of Pseudopregnancy in Normal and Traumatized Hamsters.

Group	No. of animals	Duration in days 7	8	9	10	11	12	13	14	Mean duration in days*
Normal	52		6	40	5				1	9.08 ± .84
2 loops	22		3	17	2					8.95 ± .48
4 "	22		3	14	4	1				9.14 ± .71
15 "	27	1	5	11	5	2	2	1		9.44 ±1.35

* ± stand. dev.

Results. The duration of pseudopregnancy in the several groups is indicated in Table I. Differences between the means are not statistically significant. The observed duration of normal pseudopregnancy is in agreement with that reported earlier by Deanesly(1).

At the end of pseudopregnancy gross examination of horns with 2 or 4 sutures revealed enlargements at each site of trauma. Horns with 15 sutures were massive, exhibiting many enlargements which encroached on one another. Microscopic examination revealed the enlargements to be the result principally of stromal hypertrophy, often accompanied by evidence of an incomplete, localized decidual response. In some horns the uterine lumen was much reduced and displaced; in others, large detached masses of necrotic tissue occupied the uterine canal. Vaginal smears were not always estrual at the mating terminating pseudopregnancy.

Discussion. The ineffectiveness of uterine trauma in prolonging pseudopregnancy probably is attributable to induction of insufficient competent decidual tissue. No quantitative comparisons were made of the decidual responses elicited among the experimental groups since no statistically demonstrable prolongation had been effected, and the deciduomata were insufficiently differentiated to permit quantitative study. The ineffectiveness of uterine trauma in prolonging pseudopregnancy in mice also has been reported(2).

Conclusion. An 8 to 10-day pseudopregnancy period occurs in most hamsters, the 9-day interval predominating. Hamsters rendered pseudopregnant by sterile mating do not exhibit a statistically significant prolongation of pseudopregnancy following uterine trauma, however massive, by interrupted sutures. The observations indicate species differences in luteal function in pseudopregnant hamsters as contrasted with rats.

1. Deanesly, R., *Proc. Zool. Soc.*, 1938, v108A, 31.
2. Kamell, S. A., Atkinson, W. B., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 415.

Received March 9, 1959. P.S.E.B.M., 1959, v101.

ACTH Releasing Activity *in vivo* of a CRF Preparation and Lysine Vasopressin.* (24848)

ROGER GUILLEMIN, WAYNE E. DEAR, BUFORD NICHOLS, JR. AND H. S. LIPSCOMB
Dept. of Physiology, Baylor University College of Medicine, Houston, Texas

This laboratory has previously reported (1) purification of a substance of hypothalamic and/or neurohypophysial origin, which stimulates release of ACTH from anterior pituitary tissue surviving *in vitro*. From evidence then available, it was proposed that the hypophysiotropic activity observed was due to a substance with characteristics of a peptide and different from any of the several known neurohumors investigated.† It was also concluded that any physiological significance of these findings obtained with simple

* Supported by Contract with School of Aviation Medicine, Randolph Field, Texas and USPHS Grants.

† On basis of similar results obtained independently, Saffran and Schally proposed the name Corticotrophin Releasing Factor (CRF) for this substance.

in vitro methods should have to be ascertained *in vivo*, in preparations in which stress-induced ACTH release can be inhibited by pharmacological means or hypothalamic lesion. The present report will show that crude CRF preparation *fraction D*, characterized on basis of its activity *in vitro*, stimulates release of ACTH with a linear log-dose response relationship in animals with inhibition, by a hypothalamic lesion or administraton of nembutal-morphine, of the discharge of corticotrophin which normally follows upon exposure to stress.

Materials and methods. 1. *Materials.* a. *CRF Preparation* used was fraction D prepared and characterized *in vitro* according to Guillemin *et al.*(1). This particular batch (No. 300-6) of fraction D has a vasopressor activity of 0.4 U/mg when assayed against USP Posterior Lobe Reference Standard in the rat. b. *Lysine vasopressin (LVP).* A sample of purified vasopressin prepared by the method of Ward and Guillemin(2) was further purified using partition chromatography on paper with system m-cresol/H_2O(3). After elution, the lysine vasopressin (Rf: 0.82) had a specific activity of 287 U/mg confirmed on 2 subsequent 4-point assays in duplicate on lyophilized material. Assays for pressor activity were performed in the rat, against USP Posterior Lobe Reference Standard. 2. *ACTH-release in morphine-nembutal blocked animals.* 200 animals (rats, males, 175-200 g B.W., Holtzman Farms, Houston) were used. Multiple doses of CRF fraction D and of highly purified lysine vasopressin were administered intravenously according to time schedule outlined in Fig. 1. Release of ACTH was assessed by variations of concentrations of plasma free corticosteroids using the fluorometric method of Silber as modified by Guillemin *et al.*(4) using 2 ml of plasma, and also by adrenal ascorbic acid depletion method of Sayers *et al.*(5). In each experiment, whether one or multiple doses of CRF or vasopressin were tested, control animals injected with solvent were introduced in randomized fashion. Administration of nembutal and morphine, according to above schedule, was shown in preliminary experiments to inhibit the discharge of ACTH which normally follows laparotomy, cannulation of carotid artery, or injection in jugular vein. This agrees with original report of Munson and Briggs (6). 3. *ACTH-release in animals with effective hypothalamic lesion.* a. 390 animals (rats, males, 220-250 g B.W., Holtzman Farms), were used. 18 to 19 hrs. after stereotaxic placement of a lesion in median eminence of the hypothalamus (see below), all animals were tested for effectiveness of the block of stress-induced ACTH discharge as follows: they were placed in ether jar for 3 min, allowed to recover, anesthetized again 12 min later and 1.2 ml of blood taken from jugular vein exactly 15 min after beginning of stress period. Corticosteroid levels were determined immediately on 0.5 ml plasma(4). Animals with plasma corticosterone levels <20 μg/100 ml (see 7) were considered to have inhibition of corticotrophic response to stress. Twenty-four hrs. after placement of lesion, animals having shown inhibition of ACTH discharge when exposed to stress, were given Nembutal i.p. (4.5 mg/100 g B.W.)

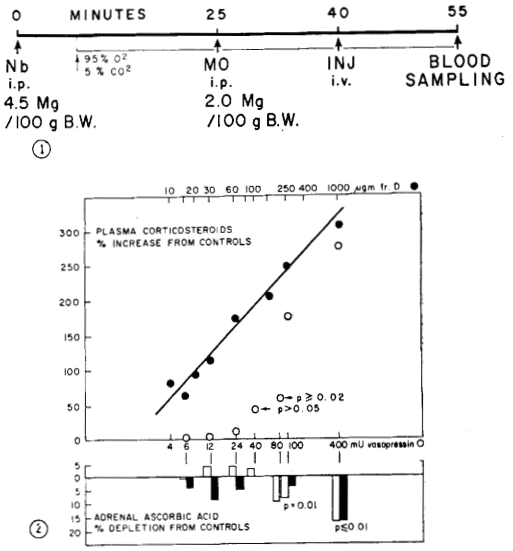

FIG. 1. Schedule of administration of nembutal and morphine, intrav. inj. and blood sampling. When indicated on diagram, animals are placed on an electric blanket at 37.5°C in a 30″ × 15″ lucite box through which a mixture of 95% O_2, 5% CO_2 is circulated at 8 l/min.

FIG. 2. Stimulation of ACTH release by CRF *fraction D* and vasopressin in nembutal-morphine blocked rats. Simultaneous measurements of plasma corticosteroid levels and adrenal ascorbic concentrations.

and 25 min. later, fraction D or vasopressin was injected in jugular vein. A sample of blood (1.2 ml) was taken from jugular vein 15 min. later for measurement of plasma corticosteroid levels. Stimulation of ACTH release by injected material was assessed by comparing levels of plasma free corticosteroids of this sample with those of earlier sample obtained after exposure to stress. The sensitivity to ACTH of the rat with effective hypothalamic lesion was determined 24 hrs. after placement of lesion according to same protocol, using 0.5, 1.0 and 1.5 mU ACTH USP Standard. b. *Hypothalamic lesion.* A minute lesion of the posterior (bulbar) part of median eminence was made, using a high frequency generator, the electrodes placed with a modified Krieg-Johnson stereotaxic instrument. The brains of *all* animals were preserved at autopsy, and reconstructions of lesion from frozen sections are available for each animal. A detailed study of lesions will be reported separately along with various physiological findings obtained in these animals. 4. *Possible potentiation of circulating ACTH by fraction D.* 24 hrs. after hypophysectomy, 24 animals (rats, males, Holtzman, 150-180 g B.W.) were given 0.5 mU ACTH USP Standard in 0.01 N HCl-saline followed exactly 60 sec later by 10 μg or 50 μg of fraction D, both substances being administered intravenously. Adrenocorticotrophic activity was assessed 15 min after injection of ACTH by measuring levels of plasma free corticosteroids(7).

Results. 1. *Stimulation of ACTH-release in nembutal-morphine "blocked" animal.* The results summarized in Fig. 2 show that injection of CRF fraction D stimulates release of ACTH with a linear log-dose response relationship. Injection of lysine vasopressin is ineffective *i.e.* does not stimulate release of ACTH, until a threshold of approximately 60-80 mU pressor activity is reached. A sudden discharge of ACTH is then observed, which seems to increase linearly with dose of vasopressin. The release of endogenous ACTH, stimulated by injection of fraction D as measured by variations of plasma corticosteroid levels, can be obtained without changes of adrenal ascorbic acid concentration when these 2 variables are measured in the same animals. Depletion of adrenal ascorbic acid occurs only upon administration of a dose of pressor activity of approximately 80-100 mU. ACTH-sensitivity of nembutal-morphine blocked rat is of the order of the 24-hr. hypophysectomized rat, when tested at multiple doses of ACTH USP standard, ranging from .025 mU to 1.5 mU.

2. *Stimulation of ACTH-release in the rat with an "effective" lesion of median eminence of hypothalamus.* The results are summarized in Table I. Of 390 animals with a lesion confirmed by reconstruction of the brain, only 107 had complete inhibition of stress-induced ACTH release according to our criteria *i.e.*, measurements of plasma corticosteroid levels. The block bore no relationship to presence or absence of diabetes insipidus. 24 hrs. after placement of hypothalamic lesion, animals with complete block of response to stress have a sensitivity to ACTH which is definitely inferior to that of the 24 hr. hypophysectomized rat; the smallest effective dose of ACTH is 1 mU *vs.* 0.1 mU for the hypophysectomized rat.

Injection of multiple doses of CRF fraction D stimulates release of ACTH, with a linear log-dose response relationship. Additional data are necessary to ascertain whether the responses at higher doses of fraction D are really on a plateau, or simply scattered about the regression line. This observation may be related to the minor resistance to ACTH of adrenals of the 24-hr. hypothalamic lesioned rat. Lysine vasopressin does not stimulate discharge of ACTH in the hypothalamic-lesioned rat until a dose of 60-80 mU pressor activity is reached. Injection of 10 or 50 μg of fraction D, 60 sec after administration of 0.5 mU of ACTH USP Standard in the 24-hr. hypophysectomized rat, did not alter (potentiate or diminish) the response of adrenals to the injected ACTH. Injection of multiple doses of fraction D (15, 30, 250 and 500 μg) in the 24-hr. hypophysectomized rat showed the material to have no direct adrenocorticotrophic activity or ACTH contamination using plasma corticosteroid or adrenal ascorbic acid concentrations as indicators of corticotrophic stimulation.

Discussion. The results would indicate

TABLE I. Effects of Various Doses of CRF Fraction D and Lysine Vasopressin (LVP) on Plasma Corticosterone Levels of Rats with Median Eminence Lesion.

No. of animals	Plasma cpd. B after stress, μg/100 ml	Amount CRF fr. D inj. in μg	Pressor equivalent in mU	Plasma cpd. B after CRF, μg/100 ml	Variations plasma cpd. B in μg/100 ml	p value‡	% variations from control level
8	10.2 ± 2.3*	20.0	8.0	16.9 ± 1.9	+ 6.6 ± 2.0†	=.01	+ 65
8	11.3 ± 1.7	40.0	16.0	26.3 ± 1.9	+14.9 ± 2.4	<.01	+132
8	10.9 ± 2.6	80.0	32.0	31.6 ± 2.7	+20.7 ± 3.0	<.01	+190
8	12.8 ± 2.1	160.0	64.0	32.9 ± 1.6	+20.1 ± 3.5	<.01	+157
5	4.2 ± 1.0	320.0	128.0	27.4 ± 1.7	+23.3 ± 2.0	<.01	+555

No. of animals	Plasma cpd. B after stress, μg/100 ml	Pressor amount of LVP in mU	Plasma cpd. B after LVP, μg/100 ml	Variations plasma cpd. B in μg/100 ml	p value‡	% variations from control level
9	11.5 ± .7*	4.0	14.5 ± 2.9	+ 3.0 ± 2.2†	>.2	0
6	11.5 ± 2.5	8.0	8.6 ± 2.0	- 2.9 ± 1.9	=.2	0
9	12.0 ± 1.2	16.0	10.5 ± 1.2	- 1.5 ± 1.7	=.4	0
5	10.9 ± 1.2	32.0	19.3 ± 3.8	+ 8.4 ± 3.3	>.05	0
9	12.0 ± 1.2	64.0	20.5 ± 2.4	+ 8.4 ± 1.9	<.01	+ 70
4	12.3 ± 1.0	128.0	26.3 ± 3.2	+14.0 ± 2.0	<.01	+114

* Stand. error of the mean. † Stand. error of the mean difference.
‡ p calculated from t on values for plasma cpd. B levels before and after CRF or vasopressin.

that a substance in fraction D, different from vasopressin stimulates release of ACTH *in vivo,* in animals with hypothalamic lesion or pharmacological blockade of the discharge of ACTH induced by stress. These results are in agreement with earlier conclusions regarding corticotrophin releasing activity *in vitro* of fraction D and vasopressin(1). The failure of McCann(8) to observe stimulation of ACTH release in a similar series of experiments with materials corresponding to fraction D seems to have been due to his using adrenal ascorbic acid depletion test as a criterion for ACTH stimulation: the same results were observed here with this method, while in the same animals definite stimulation of corticoidogenesis due to endogenous ACTH release was evidenced (Fig. 2). The difference of sensitivity of the 2 methods (see also 7) may have accounted for these divergent results, or the 2 parameters (ascorbic acid depletion *vs.* corticoidogenesis) may partake from different regulatory mechanisms(9). The dynamics of adrenal responses (ascorbic acid/corticoidogenesis) of the 24-hr. hypophysectomized rat to exogenous ACTH(7) and that of the 24-hr.-hypothalamic lesioned rat to CRF are remarkably similar. If the ratio for these responses (ascorbic acid/corticoidogenesis) were different in the normal animal when exposed to stress, the question would be raised anew(10) as to whether ACTH alone can account for the whole adrenal response upon exposure to stress. This point deserves undoubtedly attentive investigation.

The argument of McCann (in 8) on differences of specific activity of fraction D and vasopressin in stimulating release of ACTH is difficult to follow. Such comparisons are significant only when dealing with *pure* materials. Fraction D is a crude peptide mixture made by a single chromatographic separation and which, upon further purification by electrophoresis, reveals multiple components one of which only (fraction D-delta) has hypophysiotropic activity and accounts for about 1/100th of the weight of fraction D(1). No such material was available for our experiments. However, a purified CRF prepared by carboxymethylcellulose chromatography has been reported by this laboratory, more potent in releasing ACTH in nembutal-morphine blocked rat than its equal weight of lysine vasopressin(11). Comparison of respective specific ACTH-releasing activity of CRF and vasopressin will have to await availability of pure CRF. Stimulation of ACTH release by large doses of vasopressin in animal with an effective hypothalamic lesion confirming the original observations of McCann(12) is best explained by attributing inherent CRF-activity to the molecule of vasopressin. This overlapping of physiological activities (pressor,

oxytocic, hypophysiotropic) for vasopressin and CRF may be tentatively related to possibly close structural relationships between the 2 molecules(2,13). Available results do not eliminate the possibility(14) that these large doses of vasopressin may have potentiating effects on circulating ACTH and/or direct effects on the adrenal cortex.

Summary. The CRF preparation *fraction D* stimulates release of ACTH in animals with hypothalamic lesion or pharmacological blockade, at doses where pressor equivalents as pure lysine vasopressin are inactive. Hypophysiotropic activity of fraction D observed *in vitro* is therefore confirmed *in vivo* and should be attributed to a substance different from vasopressin.

1. Guillemin, R., Hearn, W. R., Cheek, W. R., Housholder, D. H., *Endocrinol.*, 1957, v60, 488.

2. Ward, D. N., Guillemin, R., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 568.

3. Schally, A. V., Saffran, M., Zimmerman, B., *Biochem. J.*, 1958, v70, 97.

4. Guillemin, R., Clayton, G. W., Smith, J. D., Lipscomb, H. S., *J. Lab. Clin. Med.*, May, 1959.

5. Sayers, M. A., Sayers, G., Woodbury, L. A., *Endocrinol.*, 1948, v42, 379.

6. Munson, P., Briggs, N., *Rec. Prog. Horm. Res.*, 1955, v11, 83.

7. Guillemin, R., Clayton, G. W., Smith, J. D., Lipscomb, H. S., *Endocrinol.*, 1958, v63, 349.

8. McCann, S. M., *ibid.*, 1957, v60, 664.

9. Slusher, M. A., *ibid.*, 1958, v63, 412.

10. Fortier, C., Skelton, F. R., Constantinides, P., Timiras, P. S., Herlant, M., Selye, H., *ibid.*, 1950, v46, 21.

11. Schally, A. V., Guillemin, R., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 138.

12. McCann, S. M., Brobeck, J., *ibid.*, 1954, v87, 318.

13. Guillemin, R., *Endokrinologie,* 1957, v34, 193.

14. Royce, P. C., Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 70.

Received March 9, 1959. P.S.E.B.M., 1959, v101.

Goat Red Blood Cells in Agglutination Test for Infectious Mononucleosis.* (24849)

SAN-PIN WANG AND J. THOMAS GRAYSTON (Introduced by Robert A. Phillips)
U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan (Formosa) and Dept. of Medicine, University of Chicago School of Medicine, Chicago, Ill.

Taiwan is one area of the world in which sheep red blood cells (rbc) are not readily available. Sheep are not economical animals to raise on this island because of high mortality from the liver fluke (*Dicrocoelium dendriticum*). Goats, however, are available. This investigation was undertaken to determine whether goat rbc could be substituted for sheep rbc in agglutination test for infectious mononucleosis(1). The study was designed solely to compare agglutination of goat cells with sheep cells when tested with serum containing heterophile antibody and not to evaluate the clinical significance of these reactions. The ox cell hemolysin test was also performed, since this test had been recommended as a substitute for sheep cell agglutination(2,3), and ox cells can be easily obtained on Taiwan.

Materials and methods. Serums: A total of 161 serums were tested; 61 were from patients and 100 were considered normal controls. Of the patient serums 42 came from the U.S. military dispensary, Taipei and 19 were obtained from the Univ. of Chicago Clinics (through the courtesy of Dr. Ross Benham, Director of the Clinical Microbiology Laboratory). In each case the serum had been submitted for determination of heterophile antibodies, indicating that infectious mononucleosis was being considered in the differential diagnosis. No effort was made to classify these patients by clinical criteria for diagnosis

* This study was supported in part by contract between Office of Naval Research and Univ. of Chicago. The opinions and assertions contained herein are those of the authors and are not be construed as official or reflecting the views of the Navy Dept. or Naval Service at large.