contained a mucoprotein and a lipoprotein.

3. *Nature of B-, C- and D-fractions.* Unlike the A- and E-fractions, mobilities of B-, C- and D-fractions were comparable to serum protein fractions, and were thus assumed to be similar to the serum proteins.

The B-fraction migrated at a rate similar to that of serum albumin. However, the evidence for this similarity also came from other sources than their similar mobilities. For instance, it was reported that bilirubin in serum was combined with albumin and α-globulin (12,13,14,15) and bile pigments likewise combined with the B-fraction. Furthermore, Kunkel and Tiselius(9) reported that bromphenol blue combined only with serum albumin when it was added to serum prior to electrophoresis at pH 8.6. Similarly, bromphenol blue migrated with the B-fraction when it was added to bile prior to electrophoresis at pH 8.6.

Summary. 1. Proteins detectable by paper electrophoresis of concentrated bile were classified into A-, B-, C-, D- and E-fractions. 2. The A-fraction migrated more rapidly than any protein fractions in serum. 3. The B-fraction was similar to serum albumin as evidenced by its similar mobility to serum albumin and affinity for bile pigments and bromphenol blue. 4. C- and D-fractions had mobilities similar to serum globulins. 5. The E-fraction was probably a denatured A-fraction, and became prominent only after concentration of bile, associated with a decrease in the A-fraction. 6. Normal and pathological human bile, the bile of sheep and dog contain all these protein fractions. 7. Values of bile protein fractions varied considerably according to species and type of bile.

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Received July 6, 1959. P.S.E.B.M., 1960, v103.

ACTH Releasing Factor Active in the Guinea Pig.* (25487)

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The hypothalamus produces a chemical mediator concerned with ACTH release(1). It is presumably transported by way of neurohypophyseal tracts into the posterior pituitary gland where it is stored(2). There is now much evidence which points to presence of a substance in extracts of posterior pituitary that is capable of causing ACTH release(3,4,5); apparently this is related to the hypothalamic agent. Certain investigations lead to the belief that this substance is vasopressin (6,7,8,9). However, powerful evidence suggests that it differs from vasopressin(10,11,12). This communication presents the results which demonstrate that vasopressin

* This project was supported by grant from U.S.P.H.S.

† This investigation was carried out in partial fulfillment for requirements of degree of Doctor of Philosophy.

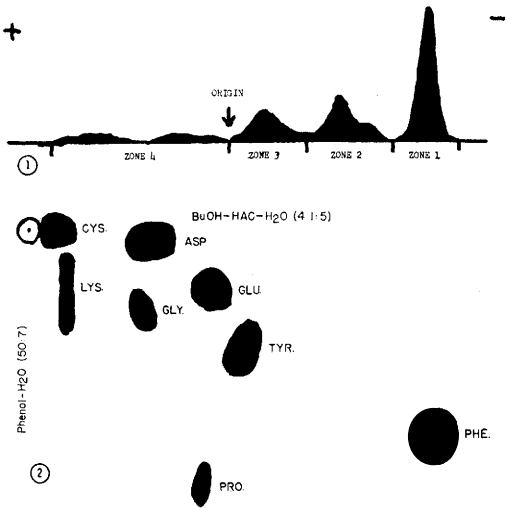

FIG. 1. Commercial pitressin powder fractionated by electrophoresis at pH 5.7, and 0.1 M acetate buffer. 100 volts for 17 hr in Spinco paper electrophoresis apparatus. Stained by spraying with 0.1% ninhydrin, collidine and acetic acid in alcohol(17). The pattern was obtained with the Spinco Analytrol.

FIG. 2. Amino acid and composition of material in zone 1.

causes ACTH release in the guinea pig.

Methods. The procedure for measuring ACTH release depends upon increase in corticoid excretion by the guinea pig(13,3). Young guinea pigs (300-450 g) of either sex were used. Urine was expressed from bladder and the material for testing was injected intraperitoneally in 5 cc of saline. Blocking agents were not used since it has already been demonstrated that pitressin is active in causing increased corticoid excretion in presence of morphine(14). Animals were placed in urine collection cages for 6 hours. Bladders were again emptied and specimens frozen until used. Corticoids were determined by procedure of Silber and Porter(15). Pressor activity was determined on cats anesthetized with nembutal and in which the carotid artery was catheterized. All injections were given into femoral vein in saline and followed by saline washes. A recovery period of 30 minutes was allowed before the next injection. Recordings were made on smoked drum kymograph. The pressor test was used rather than ADH assay because following thiosorbitol treatment according to procedure of Croxatto(16), pressor and ACTH releasing activities are destroyed while ADH activity is retained.‡

Results. Commercial pitressin powder§ was subjected to electrophoresis at pH 5.7 in 0.1 M acetate buffer according to conditions developed by Taylor *et al.*(17). Four zones were obtained (Fig. 1). The pattern appears identical with that obtained by Taylor *et al.* (17) with similar material obtained from beef. The foremost cathodic zone (fraction 1) had a mobility which suggested that the material was vasopressin(17). Vasopressin is unique in that its isoelectric point is 10.9 which makes it one of the most basic natural substances known(17).

For preparative work sheets of Whatman #3 mm, 12½ x 11¼ inches were used and 20 mg of commercial powder was applied across the sheet. The zones were identified by staining of lateral strips. Individual zones were eluted with 0.25% acetic acid. Resulting solutions were dried by lyophilization and the products, which contained in excess of 97% of salt, were used without further purification. The material from each fraction was assayed for ACTH releasing activity and pressor activity (Table I). Only zone 1 was active in these tests.

The amino acid composition of zone 1 was determined as follows: The lyophilized ma-

TABLE I. Activity of Fractions Obtained by Electrophoresis.

	No.	Guinea pig corticoid exer., μg/6 hr	Cat pressor response increase, mm Hg
Control	18	75 ± 10*	
Zone 1	18	173 ± 22[1]	28[2]
2	19	77 ± 13[1]	8[3]
3	19	66 ± 9[1]	0[4]
4	18	66 ± 11[1]	0[5]

[1] 100 μg of lyophilized material * Stand. error
[2] 40 *Idem*
[3] 280 "
[4] 280 "
[5] 350 "

‡ Sobel, H., and Schapiro, S., unpublished data.

§ Obtained through generosity of Dr. D. A. McGinty of Parke, Davis & Co. This material assayed 70 U/mg. Commercial pitressin may be of bovine (arginine vasopressin) or porcine (lysine vasopressin) origin. Pitressin refers to commercial mixture, vasopressin to isolated peptide.

terial was heated in sealed tubes for 6 to 8 hours with 0.02 cc of constant boiling H Cl/mg at 150°C. The hydrolysate was chromotographed on Whatman #1 paper according to procedure of Levy and Chung(18) except that the phenolic phase contained no m-cresol. Only 8 amino acids were detected and the material corresponded in composition with that of lysine vasopressin (Fig. 2).

It has been shown that mild acid hydrolysis of pitressin causes loss of ACTH releasing activity‖(3). A study was made of the effect of mild alkaline and acid treatment upon pressor and ACTH releasing activity of the material in zone 1. After numerous trials, the conditions employed were as follows: Material from the active zone was dissolved in solution of HCl (pH 1.2) and NaOH (pH 11.55). The solutions were placed in boiling water bath for following times: in acid 10, 20 and 30 minutes, in alkali 5, 10 and 20 minutes. After the required intervals the solutions were immediately placed in ice water and pH adjusted to 7.4 for injection. The results are shown in Table II. Under both conditions decrease in ACTH releasing activity was paralleled by decrease in pressor activity.

The effect of iodination was investigated. A solution of iodine in KI was added to pitressin in acetate buffer pH 4.6 and phosphate buffer pH 7.4 until a brownish tinge remained after stirring. The solution was placed in refrigerator 1 hour to complete iodination. The solution in acetate buffer was brought to 7.4 before injection. Both ACTH releasing and pressor activities were destroyed.

Placenta is capable of destroying pressor activity of pitressin(21). A study was made of the effect upon ACTH releasing activity.

TABLE II. Effect of Treatment upon ACTH Releasing and Pressor Activity of Material from Zone 1 and Pitressin.

	No.	Urinary corticoids, μg/6 hr	ΔC	ΔP	ΔC/ΔP
Control	13	62 ± 5*			
Hydrolysis pH 1.2					
0 min.	9	126† ± 16	64	38¶	1.7
10	8	121 ± 19	59	32	1.5
20	8	102 ± 15	40	26	1.5
30	7	97 ± 13	35	23	1.5
Hydrolysis pH 11.55					
0 min.	8	122† ± 15	60	21**	2.9
5	9	120 ± 21	58	20	"
10	8	103 ± 15	41	14	"
20	9	80 ± 9	18	7	2.6
Control	10	62 ± 9			
Pitressin (pH 4.6)	10	163‡ ± 11			
Idem, iodinated	11	68 ± 9			
Control	9	48 ± 12			
Pitressin (pH 7.4)	9	125 ± 21			
Idem, iodinated	9	63 ± 9			
Control	4	81 ± 8			
Pitressin	5	266§ ± 16			
Placental extract‖	5	91 ± 20			
Idem + pitressin	5	73 ± 19			
Boiled extract + pitressin	5	323 ± 30			

ΔC = Difference from control corticoid value.
ΔP = *Idem* blood pressure.
* Staud. error.
† 100 μg of fraction I/100 g body wt.
‡ 1 unit pitressin/100 g body wt.
§ 2 units *Idem* .
‖ Placental extract placed after incubation in boiling water bath for 3 min. Denatured protein removed.
¶ .058 mg fraction I.
** .048 *Idem* .

An extract was made from fresh uninfarcted placental tissue according to procedure of Hawker(21). Pitressin was incubated with the extract for 2 hours. This treatment is known to inactivate pressor activity. ACTH releasing activity was lost.

Discussion. Our findings suggest that lysine vasopressin or another substance very similar to it in composition and stability may cause ACTH to be released by the guinea pig. The latter (remote) possibility must be considered since only simple electrophoretic separation was used. This requires the existence of a substance of similar electrophoretic behavior at pH 5.7, a highly basic polypeptide which responds similarly to partial hydrolysis at pH 1.2 and pH 11.55, to iodination at pH

‖ It has been suggested that vasopressin does not act through ACTH release but rather that it acts directly upon adrenals(19). The conclusion that this may be the mode of action of pitressin is inconsistent with previous findings that pitressin is not effective in hypophysectomized guinea pigs(3). Furthermore other substances, e.g. serotonin (20) may act directly upon the adrenal. It is possible that direct actions are due to vascular phenomena. The evidence now seems overwhelmingly in favor of an ACTH releasing mechanism.

4.6 and pH 7.4, to placental extract and thiosorbital treatment. It is possible that an amide grouping on the glycine moiety accounts for the lability to dilute acid as it does in oxytocin(22).

Several investigators prepared a pressor-free substance from extracts of posterior pituitary(10,11). The preparation of Schally *et. al*(11) contains 10 amino acids including alanine, serine and histadine, in addition to those present in lysine vasopressin, but excluding tyrosine. Furthermore the substance is remarkably resistant to acid hydrolysis. This suggests that this substance is similar to lysine vasopressin, differing from it only in that histidine has replaced tyrosine in the ring and alanine and serine have replaced amide groups. This latter replacement would make the molecule much more acid resistant.

Guillemin *et al.*(23) report that 40 μg of their fraction D, a crude preparation which contains 1/100 of its weight of fraction D-delta (the active component) causes a corticoid response in the rat, equal to that produced by 128 mU (.44 μg) of their purified lysine vasopressin. Although a pressor-free substance exists which is capable of increasing ACTH release, the physiological significance of vasopressin in this regard must not be ignored. It is proposed that in an anatomical system like the portal veins a non-pressor amount of vasopressin might activate ACTH release.

Summary. Lysine vasopressin was obtained from commercial pitressin powder by electrophoretic separation. The ACTH releasing activity in the guinea pig and the pressor activity in the cat were compared following various chemical manipulations. Following mild acid and alkaline hydrolysis, iodination, and incubation with placental extract both activities are altered to the same extent. It was concluded that lysine vasopressin causes ACTH release in the guinea pig. This is consistent with the concept that it may be a hypothalamic mediator in this species.

The authors are grateful to Dr. H. Bergman for the presser assays.

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Received July 22, 1959. P.S.E.B.M., 1960, v103.