and in hypercholesterolemic humans, 22,25-diazacholestanol inhibits the biosynthesis of cholesterol by a triparanol-like(9) rather than a cholesterol-like mechanism as suggested on the basis of studies *in vitro*.

Summary. Oral administration of 22,25-diazacholestanol dihydrochloride to rats caused striking lowering of cholesterol and significant accumulation of desmosterol in the serum, liver and adrenals. The findings are similar to those in humans and suggest a triparanol-like mechanism of action.

We wish to thank Mrs. K. Voith for the animal work.

1. Ranney, R. E., Counsell, R. E., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 820.

2. Counsell, R. E., Klimstra, P. D., Ranney, R. E., Cook, D. L., *J. Med. Pharm. Chem.*, 1962, v5, 720.

3. Sachs, B. A., Wolfman, Lila, *Circulation*, 1962, v26, 669.

4. Avigan, J., Steinberg, D., Vroman, H. E., Thompson, M. J., Mesettig, E., *J. Biol. Chem.*, 1960, v235, 3123.

5. Blankenhorn, D. H., Kuzma, O., *Metabolism*, 1961, v10, 763.

6. Abel, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.*, 1952, v195, 357.

7. Siperstein, M. D., Guest, M. J., *J. Clin. Invest.*, 1959, v38, 1043.

8. ———, *ibid.*, 1960, v39, 642.

9. Avigan, J., Steinberg, D., Thompson, M. J., Mosettig, E., *Biochem. Biophys. Research Comm.*, 1960, v2, 63.

Received February 15, 1963. P.S.E.B.M., 1963, v112.

Isolation and Chemical Characterization of a β-CRF from Pig Posterior Pituitary Glands.* (28238)

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We wish to report here the isolation from commercial powders of pig posterior pituitary gland of a basic peptide with ACTH-releasing activity. Amino acid composition showed that this peptide was probably related to lysine-vasopressin; it was thus designated β-CRF in accord with the terminology proposed earlier(1,2). Only the terminal sequences could be determined with the minute quantities of the peptide available.

Methods. The assays for vasopressin, CRF, ACTH and MSH have been described(3,4, 5). Pituitary preparations, chromatographic methods and estimation of peptides have also been reported(4,6,7). The countercurrent distribution and recovery of materials thereafter were performed essentially as in Schally *et al.*(8); n-butanol was freshly redistilled over zinc powder. For paper chromatography of peptides the Partridge system was used (9).

Results and discussion. Fifty grams of a material similar to protopituitrin(4) from *hog* posterior pituitary glands were subjected to the precipitation procedure as in(4); 12.3 g of precipitate B(4) were obtained; about 4.1 g of precipitate B were applied on a preparative column of carboxymethylcellulose (CMC) (0.45 meq/g; 2.9 × 55 cm, equilibrated with 0.02 M pH 4.6 ammonium acetate buffer). Elution was effected by a gradient to 0.1 M, pH 7 buffer, through 2000 ml mixing flask. The fractionation pattern was identical to that shown in Fig. 3 in Schally and Guillemin(4). A large peak of basic peptides had as its main components lys-vasopressin and α-MSH; CRF activity was contained on the ascending edge of this peak. Two such columns yielded after lyophilization 1.4 g of basic peptide material. One gram of this material was distributed for 103 transfers (Fig. 1) in a system of n-butanol/0.03 M p-toluene sulphonic acid containing 0.001 M EDTA and 6.10^{-5} M cystine. CRF

* Supported by USPHS grant.

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activity was concentrated in tubes 31-45 (β-CRF) and in tubes 79-99 (a_1-CRF). The purification of a_1-CRF from a-MSH peak will be reported elsewhere. The β-CRF area had an average pressor activity of 45 u/mg; 64.0 mg of this material after lyophilization were applied on an analytical CMC column (1 × 51 cm) equilibrated with pH 5.8, 0.03 M ammonium acetate; a gradient to pH 7, 0.1 M buffer through 250 ml mixing flask was applied after 40 ml went through and 2 ml fractions were collected (Fig. 2). CRF activity was now entirely localized in a sharp peak preceding the peak of vasopressin (Table I). This material had no ACTH-like activity when tested at the same dose level in the 24 hour-hypophysectomized rat. CRF and pressor activities decreased markedly on standing. Some of the pressor activity could be inherent

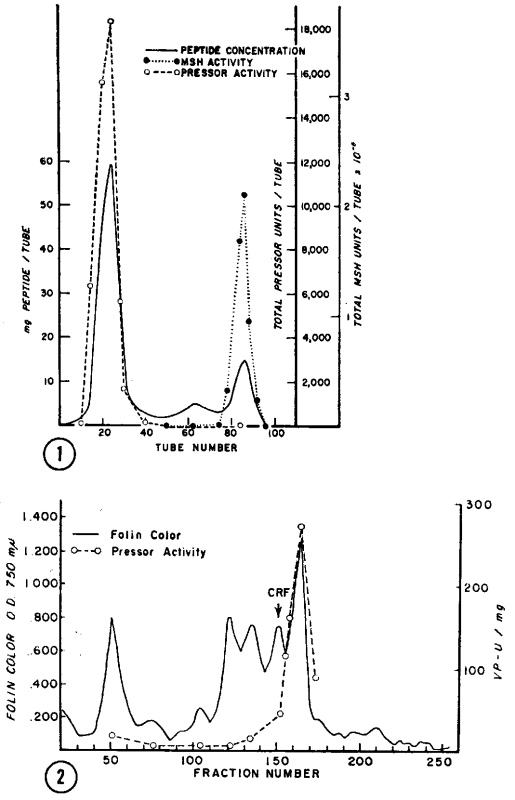

FIG. 1. Countercurrent distribution of basic peptide area from CMC. Folin-Lowry color on 50 μl aliquots.

FIG. 2. Separation of β-CRF area from CCD on CMC. 50 μl aliquots were used for Folin-Lowry analyses.

TABLE I. Biological Activities of the β-CRF Peak.

Tube No.	Vasopressor activity (u/mg)	CRF activity; cpd B (μg/100 ml plasma)
104†	6.7	9.8 ± 1.67 —
122	6.7	7.7 ± 1.83 —
134	14.8	5.4 ± .96 —
152	44.7	25.5 ± 3.77 **
NaCl		5.2 ± 1.30

† All materials injected in the assay for CRF at 30 mu pressor activity/rat.

** $p = 0.01$; levels of statistical significance calculated from NaCl level by multiple comparison test of Dunnett(13). Analysis of variance on the whole experiment showed the variance ratio for treatments to be highly significant ($F = 13.54$ *** for 13/4 d.f.).

and some could be due to a minute contamination with the peak of lys-vasopressin. On paper chromatography (Fig. 3) β-CRF had a different R_f from lys-vasopressin and a-MSH and appeared to be at least 80% pure. Lyophilization yielded 4.9 mg of peptide material. The amino acids were determined on a 200 μg sample after HCl hydrolysis by paper chromatography according to Levy and Chung(12) (Fig. 4). No special measures were taken during the lyophilization or chromatography so that most cystine became oxidized to cystic acid and traces of methionine to methionine sulphone. The amino acids present in major amounts were cysteic acid, aspartic acid, glutamic acid, serine, glycine, lysine, histidine, tyrosine, valine, phenylalanine, proline. There was a lesser amount of alanine, leucine or isoleucine and arginine; these, and the traces of threonine and methionine sulfone also detected, can be considered as impurities. Since the peak (tubes 129-140) which preceded the β-CRF area contained major amounts of arginine, methionine, alanine, and threonine, these amino acids could be impurities derived from it.

Further chemical characterization of the lyophilized peptide material was accomplished by Dr. R. A. Boissonnas (Sandoz Ltd., Basle, Switzerland). Semi-quantitative amino acid analysis was performed by 2-dimensional separation (electrophoresis at pH 1.9 followed by ascending chromatography in n-butanol/acetic acid/water 7:1:2) and ninhydrin-copper staining of hydrolysates (HCl 6N; 16 h; evacuated tube) of performic acid

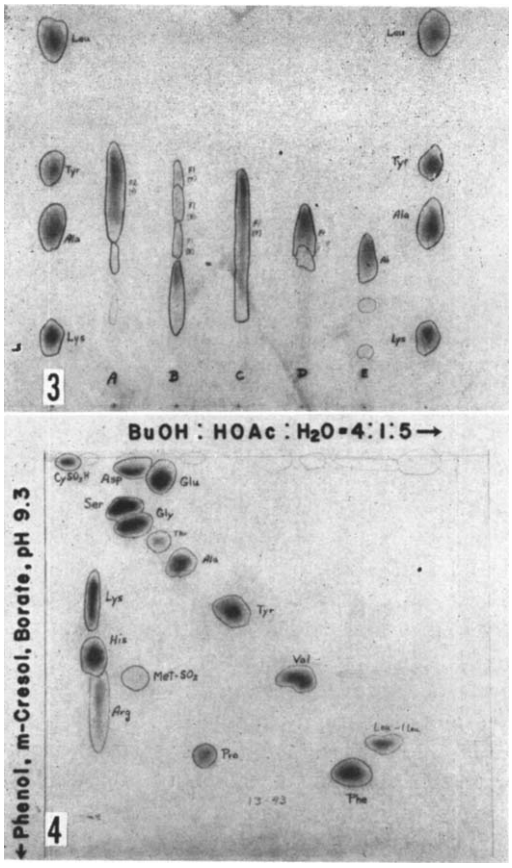

FIG. 3. Paper chromatography of β-CRF Spot D in butanol:acetic acid:water (4:1:5) on Whatman #1 for 24 hr. Solvent front not shown, but leucine has an $R_f = 0.6$. A: α-MSH; B: α_1-CRF; C: α_2-CRF; D: β-CRF; E: lys-vasopressin.

FIG. 4. Two-dimensional chromatography of an acid hydrolysate of β-CRF.

oxidized and non-oxidized samples of the peptide material. The results confirmed the amino acid composition we had obtained by the method of Levy and Chung. Furthermore the presence of tryptophane was ascertained by the shape of the UV spectrum of the unhydrolyzed material and by its positive reaction with p-dimethylamino benzaldehyde. Free tryptophane could be identified after digestion by chymotrypsin followed by carboxypeptidase. One of the fragments liberated by chymotryptic digestion migrated to the anodic side. It was negative to ninhydrin, positive to the Folin reagent, gave serine and tyrosine on acid hydrolysis, liberated tyrosine by digestion with carboxypeptidase and had the same mobility on paper chromatography (Partridge system) and on paper electrophoresis (pH 5.8) as an authentic sample of acetyl-seryl-tyrosine. Dinitrofluorobenzene reacted with the ε-amino group of the lysine present in the material. Tryptic digestion gave glycinamide and subsequent carboxypeptidase digestion liberated lysine. It is therefore most likely that the sequence Ac-Ser-Tyr- occupies the N-terminal position and the sequence -Lys-Gly-NH_2, the C-terminal position. It is interesting that the sequence at the N-terminus is the same as in α_2-CRF which apparently is an analog of α-MSH(11).

β-CRF appears to be an extremely labile molecule. The mechanism of inactivation is not clear: in many cases lyophilization led to a loss of biological activity with similar preparations of β-CRF. For denaturation to occur with such a small molecule is of course a very unusual phenomenon, though Ressler may have encountered it with oxytocin(12). The isolation of β-CRF has been further complicated by the fact that only 2 batches of protopituitrin out of approximately 20 examined yielded sizable amounts of β-CRF after the separation procedures. It is not known whether this is related to depletion of CRF due to the stress of slaughtering or to inactivation and subsequent change in chemical and biological properties during the multiple stages of purification.

Summary. A basic peptide with corticotropin releasing activity, no inherent ACTH-like or MSH activity and a pressor activity of 45 u/mg was isolated from pig posterior pituitary glands by the Kamm procedure followed by chromatography on carboxymethylcellulose (CMC), countercurrent distribution and rechromatography on CMC. The amino acid composition showed that the peptide is related to lysine-vasopressin, but with additional amino acids being present; the peptide belongs thus to a group designated β-CRF. The N-terminal and C-terminal amino acid sequences show sequential similarities with those of α-MSH and lysine vasopressin, respectively. This could perhaps explain inherent pressor activity of CRF and CRF activity of LVP and α-MSH analogs (α_2-CRF).

Protopituitrin used in this study was prepared by Drs. W. F. White and J. D. Fisher at Armour and Co. We wish to acknowledge the collaboration of Dr. H. S. Lipscomb, Dr. R. N. Andersen, and Dr. W. E. Dear in various phases of this work.

1. Guillemin, R., Schally, A. V., Andersen, R. N., Lipscomb, H. S., Long, J. M., *C. R. Acad. Sci.,* 1960, v250, 4462.

2. Schally, A. V., Andersen, R. N., Lipscomb, H. S., Long, J. M., Guillemin, R., *Nature,* 1960, v188, 1193.

3. Guillemin, R., Schally, A. V., Lipscomb, H. S., Andersen, R. N., Long, J. M., *Endocrinology,* 1962, v70, 471.

4. Schally, A. V., Guillemin, R., *Tex. Rep. Biol. Med.,* 1960, v18, 133.

5. Guillemin, R., Long, J. M., *Experientia,* 1961, v17, 132.

6. Schally, A. V., Guil'emin, R., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 138.

7. Schally, A. V., Lipscomb, H. S., Guillemin, R., *Biochim. Biophys. Acta,* 1959, v31, 250.

8. Schally, A. V., Andersen, R. N., Long, J. M., Guillemin, R., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 290.

9. Partridge, J. M., *Biochem. J.,* 1948, v42, 238.

10. Levy, A. L., Chung, D., *Anal. Chem.,* 1953, v25, 396.

11. Schally, A. V., Lipscomb, H. S., Guillemin, R., *Endocrinology,* 1962, v71, 164.

12. Ressler, C., *Science,* 1958, v128, 1281.

13. Dunnett, C. W., *Am. J. Statistical Assn.,* 1955, v50, 1096.

Received February 20, 1963. P.S.E.B.M., 1963, v112.

Lack of Action of Serum Opsonins in Phagocytosis of Inert Particles by Cells of Reticuloendothelial System. (28239)

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Normal or immune serum opsonins play a determinant role in the phagocytosis of bacteria either by isolated phagocytes or by the fixed cells of the reticuloendothelial system (R.E.S.). The situation is completely different as far as the phagocytosis of inert colloid is concerned.

Studies on the kinetics of carbon particles uptake by the R.E.S. have shown that rate of phagocytosis is determined by the balanced interplay of 2 factors acting in opposing directions(1):

1) Actual concentration of particles in the blood, which is proportional to the rate of phagocytosis.

2) Degree of R.E. cell saturation produced by the engulfed particles which slows down the rate of phagocytosis of the colloid remaining in the blood.

These facts have been confirmed with different inert colloids in various animal species (2,3) and in man(4). On the basis of these findings, we were able to establish the principles governing phagocytosis of inert particles by the R.E.S. in terms of the two above mentioned factors, *i.e.,* the amount of particles respectively in blood and in R.E. cells. It appears therefore that the kinetics of phagocytosis can be fully explained by the sole interplay of these 2 factors excluding the intervention of limiting serum factors.

Recently Jenkin and Rowley(5) have suggested that rate of phagocytosis of carbon particles by R.E. cells may be related, at least in part, to the level of serum factors acting as opsonins on the following basis:

a) The rate of carbon phagocytosis in normal mice is increased when the colloid is pre-incubated *in vitro* with different heterologous sera. Adult or fetal pig serum was found to be the most active. A similar effect was also observed with serum from mice treated with a bacterial lipopolysaccharide.

b) Injection of a large amount of carbon (R.E.S. "blockade") reduces the rate of phagocytosis of a second dose of the same colloid. This effect disappears if the carbon