tidyl serine is mixed with phosphatidyl-ethanolamine. There is a significant increase in % of total lipide phosphorus in the necrotic kidney in phosphatidylethanolamine-serine and phosphatidylinositol fractions (Table II). However, a decrease occurs in the lecithin fraction. The increase in lipide phosphorylation in the necrotic kidney apparently involves only phosphatidylethanolamine-serine and sphingomyelin fractions. These results demonstrate that phospholipides of the kidney are involved in production of kidney necrosis from administration of a single dose of DL-Serine. These phospholipide changes may be a reflection of the degenerative process since these lipides make up 16-32% dry weight of the cellular mitochondria, microsomes and nuclei (12).

Summary. 1) The effects of a single dose of DL-Serine on lipide phosphorylation in kidney were studied in the rat. 2) A statistically significant decrease occurred in phospholipide phosphorus of the necrotic kidney as compared to controls. However, there was a 3-fold increase in lipide phosphorylation as shown in the relative specific activities values. 3) A statistically significant increase occurred in % of total lipide phosphorus in phosphatidylethanolamine-serine and phosphatidylinositol fraction, in the necrotic kidney as compared to controls. However, a decrease occurred in the lecithin fraction. This increase in lipide phosphorylation in the necrotic kidney apparently involved only the phosphatidylethanolamine-serine and sphingomyelin fractions.

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Isolation of α- and β-Melanocyte Stimulating Hormones (MSH) on a Preparative Scale.* (25812)

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The need for large amounts of α- and β-MSH for investigation of their physiological significance in mammals and particularly for the study of effects of β-MSH on the central nervous system(1) and for further studies on biosynthesis of ACTH from α-MSH(2), prompted us to develop still other methods for isolation‡ of these 2 substances on a preparative scale.

Methods and materials. Starting materials for purification were an MSH concentrate

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‡ For review on isolation and structure of α- and β-MSH, see Harris(3).

(Armour and Co.) of porcine origin(4) with potency of 3-5 x 10^5 MSH U/mg and Pitressin Intermediate(5,6), 1.5 x 10^5 MSH U/mg (Parke, Davis & Co.). Reagent grade diethylaminoethyl cellulose (DEAE) anion exchange adsorbent (0.88 m equiv/g) purchased from Brown & Co., was treated before chromatography(7). Other details of chromatography, including operation of columns and methods for following peptide concentration have been described(5,6,8). Bioassay for MSH activity is the *in vitro* assay of Shizume *et al.* (11) with minor modifications, using some of their original MSH Standard. As utilized here the assay is highly reproducible as exemplified by standard errors for composite slope b and index of precision λ, calculated(12) for 22 assays in triplicate or quadruplicate ($b_c = 1.41 \pm 0.14$; $\lambda_c = 0.247 \pm 0.027$). Other bioassays as described in(6).

Results. The first step in purification of α- and β-MSH is chromatography on CMC as in the method of Steelman and Andersen(4), but using different conditions. Fig. 1 shows chromatographic pattern and biological activities obtained on column 2.8 x 52 cm with hold-up volume of 160 ml. MSH concentrate (5 g) was applied on column equilibrated with 0.005 M, pH 4.6 ammonium acetate. Gradient to 0.1 M, pH 7, buffer through 1000 ml mixing flask was started at tube No. 29, and 20 ml fractions were collected. Fractions 91-102 yielded after lyophylization 1.7 g β-MSH with specific activity of 0.8 x 10^6 U/mg. Approximately 300 mg of α-MSH similarly obtained from fractions 130-154 had a potency of 2 x 10^6 MSH U/mg and pressor activity of 50 U/mg.

β-MSH could be further purified on DEAE cellulose (Fig. 2). The material (1.7 g) dissolved in 55 ml glass distilled water was adjusted to pH 7 with NH_4OH before chromatography. The column 1.6 x 87 cm contained 31 g DEAE equilibrated with 0.0075 M, pH 7 ammonium acetate, with hold-up volume of 120 ml. The gradient to 0.175 M, pH 7 buffer through 1000 ml flask was started after 2 hold-up volumes and 10 ml fractions were collected. Lyophylization of fractions 54-64 yielded 200 mg of β-MSH with specific potency of 4-5 x 10^6 U/mg. Overall recovery of β-MSH was slightly higher than 20% of total MSH activity present in concentrate. It was pointed out by Lerner and Lee(9) that α-MSH is the main MSH component of hog pituitary gland. β-MSH was electrophoretically homogeneous and relatively stable on storage *in vacuo*.

α-MSH could be further fractionated by zone electrophoresis in pyridine acetate buffer in a large vertical apparatus(10). α-MSH (565 mg) was introduced on column of ethanolyzed cellulose 4 x 160 cm, containing 0.1 M, pH 4.9 pyridine acetate as an electrolyte. After electrophoresis at 2300 volts (200-250 mA) for 50 hr at 3°C, the column (1600 ml) was displaced and collected automatically in 10 ml fractions. α-MSH emerged in fractions 49-62 and was freed from components with a lower cathodic mobility and from the bulk of vasopressin moving ahead of α-MSH. After lyophylization 73 mg of α-MSH with a potency up to 1 x 10^7 MSH U/mg were obtained. Pressor contamination of this material was 3-4 U/mg.

It is difficult to separate α-MSH and lysine vasopressin on CMC on analytical or preparative scales with a gradient of 0.02 M, pH 4.6 to 0.1 M, pH 7 ammonium acetate, as α-MSH emerges on the descending slope of vasopressin (6). A somewhat better separation can be achieved using different conditions for chromatography: Precipitate B (a concentrate of posterior pituitary peptides(6) from Pitressin Intermediate) was chromatographed routinely in 5 g amounts on CMC; the basic peptide fraction (0.6-0.7 g) contains as its main components vasopressin and α-MSH, emerging as a single peak. Chromatography of 40 mg of this α-MSH-vasopressin peak on CMC on an analytical scale is shown in Fig. 3. The column, 1 x 62 cm was equilibrated with 0.03 M, pH 5.7 ammonium acetate. A gradient to pH 7, 0.1 M buffer through 250 ml flask was applied after 2 hold-up volumes and 2 ml fractions were collected. Under these conditions the relative order of elution is reversed, α-MSH emerging first, followed by lysine-vasopressin. These peptides however are still contaminated with each other (Fig. 3).

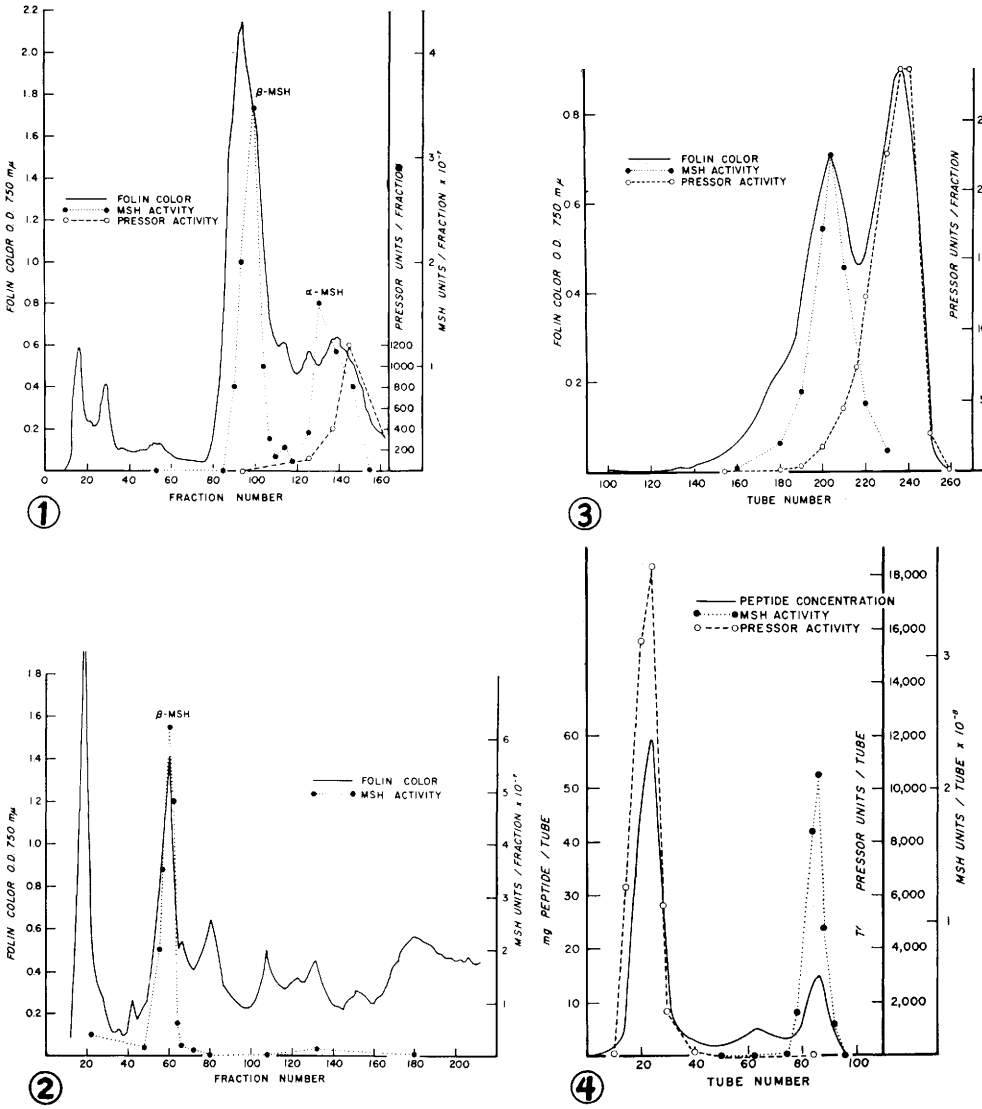

FIG. 1. Separation of α- and β-MSH on CMC. 20 μl aliquots used for Folin-Lowry analysis.

FIG. 2. Chromatography of β-MSH on DEAE. 20 μl aliquots were analyzed with Folin-Lowry reagent.

FIG. 3. Separation of α-MSH and vasopressin on CMC. Folin-Lowry color obtained with 50 μl aliquots.

FIG. 4. CCD of lysine vasopressin and α-MSH.

For complete separation of pressor and MSH activities, countercurrent distribution was the method of choice. The basic peptide fraction from CMC was subjected to 100 transfers in all glass CCD apparatus, using the system of n-Butanol, 0.03 M p-toluene sulfonic acid, with 10^{-3} M EDTA. The material (1 g) was dissolved in 20 ml of lower phase and placed along with 20 ml upper phase in the first 2 cells of the CCD apparatus. Fig. 4 shows that there was a complete separation of lysine vasopressin and α-MSH, K values differing greatly in this system, 0.32 *vs.* 6. Tubes 15-30 were pooled for recovery of vasopressin and 76-98 for α-MSH. Material in upper phase was displaced into the lower phase by addition of 3-5 vol. of benzene and was deionized by passage through Amber-

lite CG-45 resin in acetate form. Yields were 500 mg of lysine vasopressin (≧300 U/mg) and 200 mg of α-MSH (1.3-1.5 MSH U/mg, pressor activity 0.2 U/mg). Data from experiments with synthetic materials led us to believe that this minimal pressor activity may be inherent to the molecule of α-MSH.

α-MSH could be freed of small amount of fast moving contaminant by re-chromatography on CMC. Potency of final material was 1.5-2.0 x 10^7 U/mg. This α-MSH preparation was homogeneous on electrophoresis and chromatography in BuOH: HOAc:H_2O = 4:1:5, R_f = 0.6.

Tryptophan was detected spectrophotometrically and only the other 11 amino acids of α-MSH were found after acid hydrolysis. Overall recovery of α-MSH after precipitation with organic solvents(6), CMC chromatography and countercurrent distribution was 40% of total MSH activity in Pitressin Intermediate.

Summary. α- and β-MSH were isolated on preparative scale with high recovery of starting activity. The initial step in purification of both hormones is chromatography on carboxymethylcellulose. β-MSH is further purified (4-5 x 10^6 U/mg) by use of DEAE cellulose. α-MSH was not completely freed of vasopressin and other contaminants by zone electrophoresis on large vertical column. Countercurrent distribution of concentrate of vasopressin and α-MSH, yields α-MSH with a potency of 1.5 x 10^7 U/mg, free of vasopressin.

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Studies of Gastrointestinal Viral Flora of Cholera Patients.*† (25813)

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Pathogenesis of cholera is poorly understood. Presence of the organism in the environment or even in the individual person does not necessarily result in the clinical disease(1). In the disease state, cholera vibrio appears to exert a profound effect on the gastrointestinal tract without entering into the tissue deeply enough to be found in the blood stream(2). Many theories have been postulated to explain the pathogenesis of this disease. Among them has been presence of symbiotic or synergistic microorganisms including viruses. With the newly developed tissue culture technics for isolation of virus, and the discovery that large numbers of virus inhabit the gastrointestinal tract, a study was under-

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