determine, except roughly, their melting points and other physical properties.

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PARTIAL PURIFICATION AND AMINO ACID CONTENT OF VASOPRESSIN FROM HOG POSTERIOR PITUITARY GLANDS

Sir:

A highly purified vasopressin preparation (400–500 pressor units per mg.) from beef posterior pituitary glands has recently been prepared[1] by countercurrent distribution of concentrates between *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. Analysis of hydrolysates by chromatography on starch columns[2] showed phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine and cystine in approximately equimolar amounts, plus three moles of ammonia per mole of any one amino acid. The preparation and the amino acid analysis have been verified on several batches of posterior pituitary material of bovine origin.

We wish to report here an unexpected result encountered when pressor concentrates from hog posterior pituitary lobes were used as starting material for vasopressin preparation. A mixture of pressor fractions obtained by a solvent fractionation procedure (fractions "e" and "f" of Kamm, *et al.*[3]) were subjected to a twenty-transfer countercurrent distribution at room temperature in an all-glass machine[4] in the system *s*-butyl alcohol and 0.1% acetic acid. The material from tubes 1–4 inclusive was then submitted to a fifty-transfer countercurrent distribution at 5–10° in the system *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. The peak of pressor activity seemed to be in the vicinity of tube 20, which indicated a distribution constant of 0.66. The vasopressin of bovine origin had a distribution constant of 1.25 in this solvent system.[1]

Material from tubes 10–24 inclusive was subjected to a 150-transfer distribution in the same solvent system. Analysis of the distribution pattern by quantitative ninhydrin reaction[5] on aliquots of the lower phase showed a peak at tube 59 (distribution constant 0.65) which corresponded to the peak of pressor activity. The combined material from tubes 54–65 inclusive had a potency of approximately 175 pressor units per mg. This potency probably does not represent the highest obtainable for this principle. We have reason to believe that some inactivation has occurred in the process of working up the material.

Analysis of a hydrolysate of this material by starch column chromatography showed a pattern similar to that of vasopressin of bovine origin except that arginine was absent and a peak occupying the position of lysine was present.[6] If calculated as lysine this peak represented approximately one mole per mole of each of the other amino acids. One-half mg. of this preparation gave a negative Sakaguchi test both before and after acid hydrolysis. The same amount of a purified beef vasopressin preparation or an equimolar amount of arginine gave a strong positive test. Two-dimensional paper chromatograms of a hydrolysate with arginine or lysine added showed clearly that the basic amino acid present was not arginine, and was inseparable from lysine under these conditions. Microbiological assay of a hydrolysate for L-lysine[7] gave a value in reasonable agreement with the value from the starch column analysis.

Efforts are being continued toward further purification of lysine-vasopressin. Additional efforts are being made to ascertain whether lysine-vasopressin can be found in beef glands and arginine-vasopressin in hog glands, or whether this interesting and unexpected result represents a qualitative species difference.

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(8) Public Health Service Postdoctorate Research Fellow of the National Institutes of Health.

(9) Appreciation is expressed to Lederle Laboratories, American Cyanamid Company for a grant-in-aid and to Parke, Davis and Company and Armour and Company for gifts of material.

STREPTOLIN. THE STRUCTURE AND SYNTHESIS OF ISOLYSINE

Sir:

Hydrochloric–formic acid hydrolysis of the antibiotic streptolin[1] followed by chromatographic separation on Dowex-50 has given five major fractions; the last to be eluted possesses the empirical formula $C_6H_{14}O_2N_2$ for the free base and is designated as "*iso*-lysine." This substance, which is also a hydrolysis product of viomycin[2] and streptothricin,[3] we have characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate)[4] (I), dec. 243.5–244°, $[\alpha]^{25}D$ +6.5 ± 1 (alc.) (found: C, 50.91; H, 5.08; N, 12.00) and the dipicrate,[4] m.p. 200–201° (found: C, 35.38; H, 3.63; N, 18.06).

Isolysine gave a positive hydroxamic acid test[5]

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