

In a few instances,⁵ configurational relationships between α -amino and α -hydroxy acids have been established by the quasi-racemate method of derivatives of type I and II. We have now found that possibly the simplest manner for establishing such a relationship can be a comparison of the rotatory dispersion curves of a N-dithiocarbalkoxy α -amino acid (dithiocarbamate of α -amino acid) (I) and an α -hydroxy acid alkyl dithiocarbonate (xanthate of α -hydroxy acid) (II). Here, again, the confusion caused by opposite signs of $[\alpha]_D$ values of the parent substance is completely eliminated as was exemplified (see also Fig. 1) by the positive Cotton effect curves of the ethyl dithiocarbonates of L-(+)-lactic acid, L(-)-malic acid and L-(+)-mandelic acid.

Details and further applications of this rotatory dispersion approach will be reported in our full papers.

(8) Postdoctorate research fellow (1958-1959) on leave from the University of Uppsala.

DEPARTMENTS OF CHEMISTRY
WAYNE STATE UNIVERSITY
DETROIT, MICHIGAN
UNIVERSITY OF UPPSALA
UPPSALA, SWEDEN
STANFORD UNIVERSITY
STANFORD, CALIFORNIA

BERNDT SJÖBERG⁸

ARNE FREDGA

CARL DJERASSI

RECEIVED AUGUST 17, 1959

PURIFICATION OF PARATHYROID HORMONE BY USE OF COUNTERCURRENT DISTRIBUTION

Sir:

The parathyroid hormone extracted from bovine glands with 70% phenol has been purified by countercurrent distribution in two solvent systems.

Crude gland powders were extracted with 70% aqueous phenol by a modification of the method of Aurbach.¹ After precipitation of the hormonal activity with ether, the active material was dissolved in 30% acetic acid. Precipitation of inactive protein was induced by the addition of 35% sodium chloride to a final salt concentration of 7.5%. The active material was then brought out of solution by precipitation with trichloroacetic acid at a final concentration of 7.5%. This TCA precipitate was dissolved in 0.1 M acetic acid, passed through a column of Dowex 2X8 acetate and then lyophilized. The resulting powder had a biological activity of 150-200 units/mg. dry weight when assayed for its calcium-mobilizing activity in parathyroidectomized rats.² This powder was further purified by use of countercurrent distribution. Two solvent systems were employed.

In a system of 6 parts of 6% acetic acid-1.0% NaCl and 4 parts of a 1:1 mixture of 1-butanol and 1-propanol (system 1) the material distributed as shown in Fig. 1. The substance having a K of 0.88 was the only hormonally active material and was found to have a potency of 2000-2500 units/mg.

The other system (system 2) was composed of 12 parts 0.1% acetic acid, 5 parts 1-butanol and 3.5 parts pyridine. After 200 transfers in this system the hormonal activity distributed with a K of 0.22. This was recovered, and rerun in the same

(1) G. D. Aurbach, *Arch. Biochem. Biophys.*, **80**, 466 (1959).

(2) P. L. Munson, *Ann. New York Acad. Sci.*, **60**, 776 (1955).

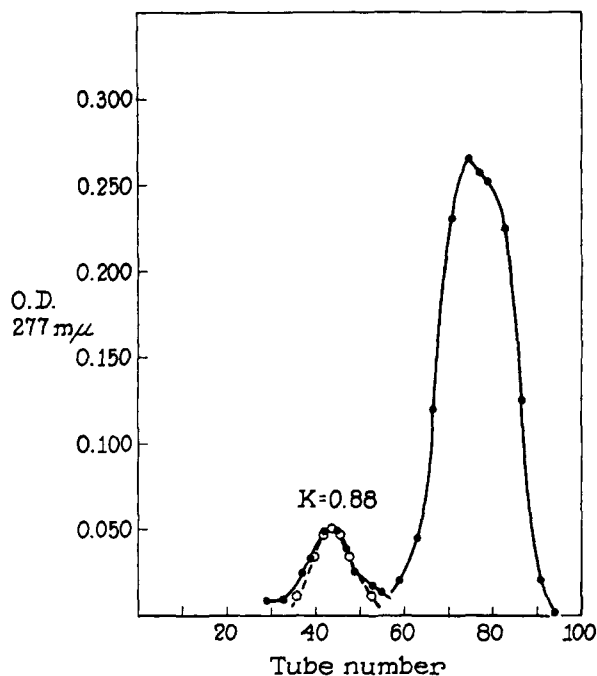


Fig. 1.—Countercurrent distribution pattern of parathyroid hormone after 100 transfers in system 1. The absorption at D_{277} ●—● and the theoretical distribution O—O are plotted.

system for 471 transfers. The pattern shown in Fig. 2 was obtained. Material from tubes 73-93 was recovered and found to have an activity of 2500-3000 units/mg. Because there is an error of approximately $\pm 25\%$ with this assay procedure, the biological activities of the two preparations were not significantly different.

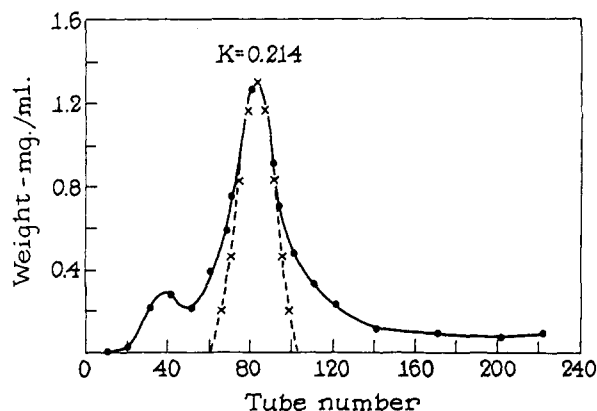


Fig. 2.—Countercurrent distribution pattern of parathyroid hormone after 471 transfers in system 2. The weight recovered ●—● and theoretical distribution X—X are plotted.

When the active substance from the pyridine system was distributed in system 1, it appeared as a single peak with a K of 0.86. The molecular weight estimated by both ultracentrifugation and dialysis was 7000 ± 1500 . In both studies the material behaved as a single component.

THE ROCKEFELLER INSTITUTE
NEW YORK 21, N. Y.

HOWARD RASMUSSEN
LYMAN C. CRAIG

RECEIVED JULY 29, 1959