We wish to report the use of partition columns[5] in the isolation of sheep hormone preparations of high biological activities (200 to 400 u./mg.) *without prior peptic digestion.*

Three to seven mg. of material purified by the oxycellulose method[2b] and containing 30 to 40 u./mg. were placed on a column 1.2 cm. in diameter containing 12 g. of kieselguhr[6] and 10 ml. of 0.2 *N* HCl saturated with isobutyric acid[7] as the stationary phase. The moving phase was composed of isobutyric acid saturated with 0.2 *N* HCl. The column was run at a constant temperature of 24° and at a flow rate not greater than 6 ml. per hour. After the fraction containing the biological activity had emerged, the remainder of the material was eluted with 6 *N* HCl.

The results of a typical experiment in which material containing 0.820 mg. of nitrogen was placed on the column are shown in Fig. 1 (open circles). The amount of substance in each tube was determined by the method of Lowry, *et al.*[8] As judged by this colorimetric procedure, the starting material was recovered completely from the column. Fraction I (11% of the nitrogen and 5% of the activity) started to emerge from the column after the first 17 ml. and was contained in the following 14 ml. The material in the next 17 ml., Fraction II, contained 10% of the nitrogen and 95% of the activity. The material eluted by 6 *N* HCl, Fraction III, contained 74% of the nitrogen and no activity.

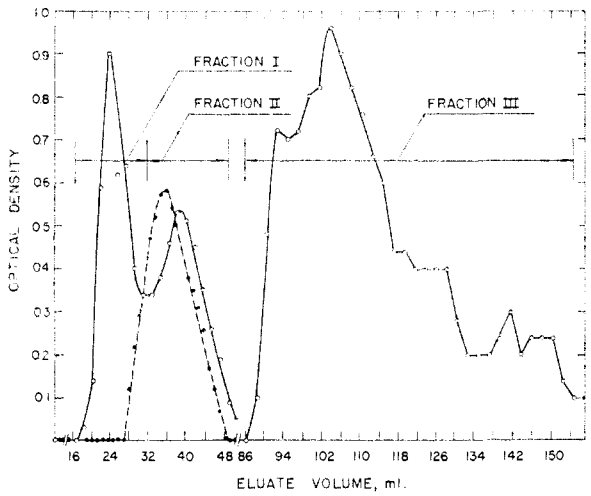

Fig. 1.—Chromatogram of oxycellulose purified sheep ACTH preparation (open circles); rechromatogram of Fraction II (closed circles).

In many experiments on different preparations of starting material similar results were obtained. The material in Fraction II had approximately the same *R* value[5a] (0.60 to 0.70) from experiment to experiment and represented on a nitrogen basis an 8- to 12-fold purification of the starting material. This purification was also reflected in the biological assays in which starting materials with assay values of 30 to 40 u./mg. gave Fractions II with 200 to 400μ./mg.[9] When 1.1 mg. of the solids of Fraction II were rechromatogramed, Fig. 1 (solid circles), a symmetrical peak containing virtually all of the material (96%) emerged close to the expected position (*R* value of 0.72). Thus the material comprising Fraction II showed no gross inhomogeneity by the criterion of partition chromatography which was applied.

The authors are greatly indebted to Professor C. H. Li for the starting materials and for furnishing the facilities for the biological assays reported in this paper.

(9) Based on weight as determined by the colorimetric procedure.[8]

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INCORPORATION OF LABELED CARBON DIOXIDE INTO PYRUVATE AND α-KETOGLUTARATE[1]

Sir:

The pyruvic oxidase of pigeon breast muscle[2] catalyzes the incorporation of $C^{14}O_2$ into pyruvate (Table I). No addition of cofactors is required for this reaction but with added cocarboxylase the rate of incorporation is increased 3 to 4 fold. However, without added Mg^{++} or cocarboxylase there is no detectable decarboxylation of pyruvate to acetoin. Similarly the enzymatic oxidation of pyruvate by ferricyanide requires Mg^{++} and cocarboxylase.[3] Thus the only activity exhibited by pyruvic oxidase without any additions is the incorporation of CO_2, presumably by an exchange reaction as follows

$$\text{Pyruvate} \rightleftarrows (\text{acetaldehyde-enzyme}) + CO_2$$

The only known cofactor present in pyruvic oxidase is protogen or thioctic acid[3] which according to Gunsalus, *et al.*,[4] is part of a more complex coenzyme tentatively identified by Reed, *et al.*,[5] as lipoicyl-thiamine pyrophosphate.

TABLE I

INCORPORATION OF LABELED CARBON DIOXIDE INTO PYRUVATE

The components of the system were pyruvate (35 μmoles), $KHC^{14}O_3$ (2.1×10^5 cts./min.) and pyruvic oxidase (250 units) in a total volume of 1.3 ml. at *p*H 7.0; incubated in nitrogen at 37°.

Time of incubation, minutes	Cts./min./μ mole
0	0
30	20
60	63
120	150

A similar equilibration of $C^{14}O_2$ with α-ketoglutarate is catalyzed by the α-ketoglutaric oxidase

(5) (a) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941); (b) A. J. P. Martin and R. R. Porter, *ibid.*, **49**, 215 (1952).

(6) Hyflo Super-cel, Johns-Manville Company.

(7) F. H. Carpenter, G. P. Hess and C. H. Li, *J. Biol. Chem.*, **197**, 7 (1952).

(8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. S. Randall, *ibid.*, **193**, 265 (1951).

(1) Supported by a grant from the American Heart Association.

(2) V. Jagannathan and R. S. Schweet, *J. Biol. Chem.*, **196**, 551 (1952).

(3) R. S. Schweet and K. Cheslock, *ibid.*, in press.

(4) I. C. Gunsalus, L. Stuglia and D. J. O'Kane, *ibid.*, **194**, 859 (1952).

(5) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 3964 (1952).

of pig heart[6] (Table II). Cysteine used in this experiment was later found to be unnecessary. This enzyme contains bound protogen and cocarboxylase. Addition of cocarboxylase, or of diphosphopyridine nucleotide (DPN), coenzyme A (CoA) and cysteine did not change the rate of incorporation. C^{14} labeled formate and succinate were not incorporated with or without CoA and DPN.

TABLE II

INCORPORATION OF LABELED CARBON DIOXIDE INTO α-KETOGLUTARATE

The components of the system were α-ketoglutarate (10 μmoles), cysteine (10 μmoles), $NaHC^{14}O_3$ (1.8×10^5 cts./min.) and α-ketoglutaric oxidase in a total volume of 0.5 ml. at *p*H 7.0; incubated at 37° for 15 min.

Oxidase units	Cts./min./μ mole
5	15
13	40
25	86
50	198
75	256
75 (boiled)	0

The keto acids were purified for counting by (a) partition chromatography of the 2,4-dinitrophenylhydrazones on silica gel column[7] and (b) recrystallization of the 2,4-dinitrophenylhydrazones (with carrier) to constant specific activity. Both methods showed the same specific activity.

(6) D. R. Sanadi and J. W. Littlefield, XIIth International Congress of Pure and Applied Chemistry, New York, Sept., 1951; *J. Biol. Chem.*, in press.

(7) D. O. Brummond, "The oxidation of organic acids by mitochondria from plants," M.S. Thesis, University of Wisconsin, 1952.

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THE CONFIGURATION OF UNDISSOCIATED SULFAMIC ACID

Sir:

In a recent paper on the ionization constant of sulfamic acid,[1] the statement is made that "Although the acid occurs in its crystals as a dipolar ion, $^+NH_3SO_3^-$, the un-ionized acid in aqueous solution is largely in the form of the neutral molecule, NH_2SO_3H." As a basis for this statement, reference is made to a paper by Baumgarten.[2] This same concept of the configuration of undissociated sulfamic acid in aqueous solution is expressed by Kanda and King,[3] apparently on the same basis.

It is the purpose of this communication to point out (1) that Baumgarten's paper gives no evidence for this configuration (in fact, the statement made by Baumgarten which has been interpreted[4] as claiming the normal configuration for the undissociated molecules probably was not meant to imply this at all) and further, (2) that there appears to be no compelling reason to expect the undissociated molecules in aqueous solution to be other than the dipolar ion form, which clearly is the configuration in the crystals,[3] although it is not at present possible to determine unambiguously the configuration in solution.

In order to determine, in the absence of direct molecular structure evidence, which of the two configurations is correct, one can only attempt to decide which would be the weaker acid. Then, since the ion produced by either configuration is the same, the addition of hydrogen ions to this sulfamate ion to form the undissociated molecules must necessarily form this more weakly acidic configuration.

Unfortunately, it does not seem possible to calculate expected acid strengths for either configuration with sufficient accuracy to permit an unequivocal answer. Using the semi-empirical method of Branch and Calvin,[5] which is known to be not too satisfactory for acids of this strength, one may estimate for the dipolar ion form $pK(^+NH_3SO_3^-) = pK(NH_4^+) + \log 4/3 + \{pK(HOSO_3^-) - pK(HOH) + \log 2 \times 4\} = 9.3 + 0.1 + \{1.7 - 16.0 + 0.09\} = -4.0$, or from the inductive constants given by Branch and Calvin, $pK(^+NH_3SO_3^-) = pK(NH_4) - I_s - 2I_+ - (3/2.8)I_0 - (3/2.8)I_- + \log 4/3 = 9.3 - 3.4 - 2(12.3) - (3/2.8)(4) - (3/2.8)(-12.3) + 0.1 = -9.7$. Similarly for the neutral molecule form $pK(NH_2SO_3H) = pK(H_2O) - I_s - 2I_+ - (2/2.8)I_0 - (2/2.8)I_- - (1/2.8)I_N + \log 3 = 16.0 - 3.4 - 2(12.3) - (2/2.8)(4) - (2/2.8)(-12.3) - (1/2.8)(1.3) + 0.5 = -6.2$ or $pK(NH_2SO_3H) = pK(HSO_4^-) + \log 3/4 + (1/2.8)\cdot I_0 + (1/2.8)I_- - (1/2.8)I_N = 1.7 - 0.1 + (1/2.8)(4) + (1/2.8)(-12.3) - (1/2.8)(1.3) = -1.9$. While these values appear to be slightly in favor of the neutral molecule form, it is obvious that the difference is not sufficient to make any real decision possible. The fact that the S–N bond distance in the sulfamate ion is 1.60 Å.,[6] indicating considerable double bond character, is in the direction to hinder the attachment of the proton to the nitrogen atom, again does not compel the assumption of the neutral molecule form for the acid.

Thus, since there seems to be no evidence indicating that the dipolar ion form is any less likely than the neutral form, and since it clearly exists in this form in the solid, it appears most reasonable to assume that the dipolar ion form is the configuration in aqueous solution, until more definite evidence is forthcoming. It should be pointed out that it is, of course, quite possible that both forms are present in equilibrium.

If this is the case, then the equilibrium with the intermediate, X, postulated by Maron and Berens[7] in their discussion of the kinetics of the hydrolysis of sulfamic acid, is merely the dissociation equilibrium. It is interesting to note that the values of ΔH and ΔS given by King and King[1] for the dissociation equilibrium, when extrapolated to 90°, are of the correct sign required for Maron and Berens' explanation of their data. This is certainly not any argument in favor of the dipolar ion form over the neutral molecule form, but does show that there is no difficulty in this interpreta-

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(4) *Cf. C. A.*, **23**, 5159 (1929).
(5) G. E. K. Branch and M. Calvin, "Theory of Organic Chemistry," Prentice–Hall, New York, N. Y., 1941, p. 201.
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(7) S. H. Maron and A. R. Berens, THIS JOURNAL, **72**, 3571 (1950).