it might be concluded that there is a higher concentration of protein in the granules. As this result is opposed to that of Caspersson *et al.*, we tried to investigate the causes of this discrepancy. Caspersson and his colleagues used material treated by the freeze-drying method which, as is well known, does not denature many cellular proteins. In our sections prepared also by the same method, we found that, when glycerol was added, the granules could no longer be seen. This observation induced us to use only denatured material for the absorption studies, and to examine whether the difference between Caspersson's results and ours was due to the solubility of the granules, a supposition which was also supported by Bensley's results[5], who found that the zymogenic granules of the pancreas are soluble in glycerol.

Frozen and dried sections of mouse, rabbit and guinea pig pancreas were accordingly treated with glycerol and afterwards denatured with absolute alcohol and stained with iron hæmatoxylin. Other sections, untreated with glycerol, were used on controls. The results (Figs. 2 and 3) show that the glycerol dissolves the zymogenic granules.

From these experiments we conclude that in Caspersson's results the low curves of absorption are not due to the dilution of the secretion, as supposed by him and his collaborators, but to the solution of the granules through the glycerol treatment.

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[1] Caspersson, T., Landström, H., and Aquilonius, L., *Chromosome*, 2, 111 (1941).
[2] Gersh, I., and Baker, R. F., *J. Cell. Comp. Phys.*, 21, 213 (1943).
[3] De Robertis, E., Nuñez, I., and Del Conte, E., *Rev. Soc. Arg. Biol.*, in the press.
[4] Kunitz, M., *J. Gen. Phys.*, 24, 15 (1940).
[5] Bensley, S. H., *Anat. Rec.*, 72, 131 (1938).

Estimation of *l*(+)-Arginine in Protein Hydrolysates by the Use of *l*(+)-Arginine Decarboxylase

THE estimation of *l*(+)-lysine, *l*(−)-histidine, *l*(+)-ornithine, *l*(−)-tyrosine and *l*(+)-glutamic acid in protein hydrolysates by the use of specific preparations of the corresponding amino-acid decarboxylases has been described previously[1]. A coliform organism has now been isolated which is specific for the decarboxylation of *l*(+)-arginine and has been deposited in the National Collection of Type Cultures under the reference number 7020. The organism is grown for 20–24 hr. at 25° C. in tryptic digest of casein containing 2 per cent glucose and the crop then centrifuged down and washed once in distilled water. The washed organism is made up into a thick cream in water, poured into 5 vol. of ice-cold acetone and stirred vigorously until coagulation occurs. The coagulum is filtered off on a Büchner funnel, washed once with acetone and once with ether, dried under suction, powdered and finally dried overnight *in vacuo*. The acetone-powder so prepared can be used for the estimation of arginine without further purification and the dry powder retains its arginine decarboxylase activity for some weeks.

The action of the enzyme is to effect a quantitative decarboxylation of *l*(+)-arginine to agmatine with the liberation of carbon dioxide which is measured manometrically. For estimation purposes a suspension of the acetone-powder, approximately 20 mgm./ml., is made up in 0·2*M* citrate-phosphate buffer *p*H 5·2, and 0·5 ml. used for each test. The enzyme suspension is placed in the side-bulb of a Warburg manometer, the main cup of which contains 1·5 ml. of 0·2*M* citrate-phosphate buffer *p*H 5·2, and 1·0 ml. arginine solution or sample to be analysed; the manometers are shaken in a bath at 30° C., and the contents of the side-bulbs added after an equilibration period of 10 min. Using 1·0 ml. of *M*/60 arginine, a series of eleven tests gave mean gas output = 354 μl.; standard deviation 2·7; range 344–362. The output represents 95 per cent theory (= 373 μl.) and is constant at this value over the useful manometric range of 50–500 μl. If the retention of carbon dioxide is estimated by the 'acid-tip' method, then the output represents 98 per cent theory; but it has been found satisfactory in practice to use the straightforward gas evolution and calculate results on the basis of a 95 per cent gas output.

Under these experimental conditions, decarboxylation is specific for *l*(+)-arginine and there is no enzyme blank to be taken into account. The accompanying table shows results obtained by this method for the arginine content of various protein hydrolysates and catholytes prepared as previously described[1,2]. For comparison, values are given which were determined on the same solutions by Dr. Macpherson using a colorimetric method[2]. All manometric determinations were carried out in triplicate and very good agreement is found between the gas volumes measured. The gas evolution is complete in most cases within 20–30 min. of tipping the contents of the side-bulb into the reaction chamber.

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[1] Gale, E. F., *Biochem. J.*, 39, 46 (1945).
[2] Macpherson, H. T. (to be published).

l(+)-ARGININE CONTENTS OF PROTEIN HYDROLYSATES DETERMINED BY THE USE OF *l*(+)-ARGININE DECARBOXYLASE.

Protein	Total vol.	Total N	Sample	CO_2 liberated	Mean CO_2 output	Corr. CO_2 output	Total Arginine-N	Arginine-N as % total N	
								Found	Colorimetric method[2]
	(ml.)	(mgm.)	(ml.)	(μl.)	(μl.)	(μl.)	(mgm.)		
Horse hæmoglobin hydrolysate	50	182	1·0	93 98 96 52	96	101	12·6	6·92	6·95
Lactoglobulin hydrolysate	100	235	1·0	52 53 74	52·5	55	13·7	5·82	5·75
Edestin catholyte	100	70·6	1·0	75 76 204	75	79	19·7	27·9	28·7
Insulin catholyte	100	211	4–1*	206 205 251	205	216	13·45	6·36	6·35
Casein hydrolysate	200	416	4–1*	250 251	251	265	33·0	7·91	7·73

4–1* indicates 4 ml. evaporated down to 1·0 ml. as sample.

Acid Hydrolysis of Sulphapyridines

SULPHAPYRIDINE (*M* and *B* 693) is readily hydrolysed by dilute mineral acids[1]. Many isomerides of sulphapyridine have been made and it has been found that the three isomeric derivatives from 3-aminopyridine (3-(*o*-, *m*- and *p*-aminobenzenesulphonamido)pyridine) are stable to hot mineral acids. All but one of the derivatives of 2- and 4-aminopyridine which were tested were found to be more or less easily broken down to the corresponding aminopyridine and an aminobenzene sulphonic acid as is shown below:

Conditions, sulphonamide refluxed for 30 min. with 15 per cent aqueous hydrochloric acid.

2-(*o*-aminobenzenesulphonamido)pyridine	35 per cent unchanged
2-(*p*-aminobenzenesulphonamido)pyridine	Complete hydrolysis.
4-(*p*-aminobenzenesulphonamido)pyridine	Complete hydrolysis.

The rather surprising exception is 2-(*m*-aminobenzenesulphonamido)-pyridine, which is quite unchanged after the above treatment. Derivatives of sulphapyridine substituted in the pyridine ring by carboxy (3 position), sulphonamido (5) or iodine (5), or substituted in the benzene ring by chlorine (3-chloro-4-aminobenzene sulphonyl-) or methyl (3-methyl-4-aminobenzene sulphonyl-2-aminopyridine) are all completely broken down by the above treatment.

In all cases fission takes place thus:

$$R.SO_2NH.R' + H_2O \rightarrow R.SO_3H \quad NH_2.R'$$

(*R*, benzenoid; *R'*, pyridine residue).

2-(*p*-aminobenzenesulphonamido)quinoline is also completely broken down similarly, although experiments with weaker acid concentrations show it to be rather more stable than sulphapyridine.

It was at one time considered that readiness of fission might be related to chemotherapeutic activity and that sulphanilic acid might be the real agent causing the activity of most sulphonamides, including sulphanilamide itself. However, the activity of sulphathiazole, which is relatively stable to acid hydrolysis, rather weakens this hypothesis. 2-Benzenesulphonamidopyridine is quite stable to hot mineral acids and sulphadiazine is known to be broken down by this reagent in much the same way as is sulphapyridine.

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[1] Phillips, *J. Chem. Soc.*, 9 (1941).