The supernatants from the systems were also treated with norit to adsorb any ATP or ADP formed and the P_7* assayed from the thoroughly washed norit. With AMP as the sole acceptor system, ATP was formed and detected in the P_7 fraction (Table II). No ATP was formed in the presence of DNP. The amount of P_7 was reduced 50% in the presence of a complete acceptor system (AMP, hexokinase, and glucose). The lower value for P_7 in the complete system was due in part to the formation of glucose-6-phosphate, which was detected by Zwischenferment.

By the addition of ^{32}P labeled orthophosphate to the reaction mixture, the incorporation of phosphate into the P_7 fraction eluted from norit could be demonstrated in the presence of AMP as acceptor system. In this system 9% of the total activity of the ^{32}P was incorporated into P_7 whereas less than 1% was incorporated in the presence of dinitrophenol.

We wish to thank Dr. J. H. HANKS for advice, Dr. M. L. KARNOVSKY for making available facilities of the Biophysical Laboratory, and Mrs. TOBEY CANINO, Miss MILDRED LANGE, and Mr. ROBERT THOMAS for technical assistance.

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Received March 8th, 1955

* P_7, orthophosphate liberated by 7 minute hydrolysis in *N* HCl at 100° after treatment with activated norit.

** This investigation was supported (in part) by a research grant (E-827) from the National Microbiological Institute of the National Institutes of Health, U.S. Public Health Service.

Paper chromatography of mixtures of amino acids containing glutamic or aspartic acid

In a recent paper BECK AND ÉBREY[1] record having observed "interesting phenomena inhibiting the exact qualitative and quantitative evaluation of amino acid chromatograms". The observation is that when certain mixtures of glycine and glutamic acid are subjected to paper chromatography, using phenol as the solvent, the glycine sweeps along an appreciable amount of the glutamic acid leading to incorrect quantitative results. With butanol-acetic acid as solvent three spots appear. They report that other pairs of amino acids behave similarly. Their explanation is that the "amino acids react with each other, depending upon the ratio of their concentration and medium, thus the chromatograms show not only the spots corresponding to the free amino acids, but also those of the products of the indicated reaction". If this explanation is correct then the interpretation of paper chromatograms would become extremely complicated, results of earlier workers might have to be reassessed and the usefulness of paper chromatography for the quantitative and qualitative analysis of amino acids would be considerably reduced.

From the information published we estimate that they are using amino acid concentrations of up to something like 0.05 molar. In an endeavour to repeat their work the following chromatograms were run in 80% aqueous phenol by the ascending technique:

0.0133 *M* glycine + 0.0125 *M* glutamic acid, 0.0667 *M* glycine + 0.0125 *M* glutamic acid and 0.0133 *M* glycine + 0.0625 *M* glutamic acid using 5 μl spots

(estimated to be half that used by BECK AND ÉBREY) and Whatman No. 1 filter paper. The R_F values of the spots as detected by ninhydrin are given in Table I, experiments 1, 2 and 3. The spot of R_F 0.40 is that given by glycine, while the remaining spots, which overlapped, are given by the glutamic acid. Of the glutamic acid spots in experiment 3 that of R_F 0.35 is by far the most intensely coloured. There was no difference between the intensities of the colour of the glycine spots in experiments 1 and 3 nor between the intensities of the colour of the glutamic acid spots (R_F 0.22) in experiments 1 and 2. The other results given in the table serve to explain the results given in the first three experiments. From the results of experiments 1 to 7 it is clearly seen that there is no interaction between the glycine and the glutamic acid as the spot occupying the position of serine (our R_F 0.35), claimed by BECK AND ÉBREY to be the reaction product, is found when glutamic acid is run alone. Experiments 8 to 15 indicate that the spot of R_F 0.22 observed in experiments 1 to 7 is given by glutamate (although the free base of glutamic acid gives the same R_F value) and the spot of R_F 0.27 is given

by the free acid of glutamic acid and by acid glutamate. These conclusions are supported by the work of LANDUA, FUERST AND AWAPARA[2]. The spot of R_F 0.35 is therefore not given by an ionic species of glutamic acid.

TABLE I

VARIATIONS IN R_F VALUES OF GLUTAMIC ACID WITH CHANGES IN CONCENTRATION AND pH

Experiment number	*Amino acid(s) dissolved in*	*Glutamic concentration* $M \times 10^{-2}$	*Glycine concentration* $M \times 10^{-2}$	R_F *of spots*
1	water	1.25	1.33	0.22, 0.27, 0.40
2		1.25	6.67	0.22, 0.27, 0.40
3		6.25	1.33	0.22, 0.27, 0.35, 0.40
4		0.25	0.266	0.22, 0.40
5		1.25	0.266	0.22, 0.27, 0.40
6		6.25		0.22, 0.27, 0.35
7		0.25		0.22
8	buffer pH 1	6.25		0.27, 0.35
9	buffer pH 3			0.22, 0.27, 0.35
10	buffer pH 7			0.22
11	buffer pH 12			0.22
12	buffer pH 1	0.25		0.27
13	buffer pH 3			0.27
14	buffer pH 7			0.22
15	buffer pH 12			0.22

Aspartic acid gives similar results to glutamic acid which indicates that the spot of R_F 0.35 is not due to the internal lactam of glutamic acid (*i.e.* the pyrrolidone carboxylic acid). This was confirmed by the failure to obtain a spot of R_F 0.35 after 0.01 *M* aqueous glutamic acid had been boiled for 5 hours. Similarly 0.0625 *M* glutamic acid in 6 *N* hydrochloric acid after being boiled for 5 hours still gave a spot of R_F 0.35 (see FOREMAN[3]).

It is clear from the experiments described above that the results obtained by BECK AND ÉBREY are caused by the high concentrations of glutamic acid used and that there is no interaction between the two amino acids. Two of the spots (R_F 0.22 and 0.27) given by high concentrations of glutamic acid are due to ionic species. The intensely coloured spot of R_F 0.35 does not represent an ionic species of glutamic acid neither does it represent the pyrrolidone carboxylic acid. An explanation for this spot is that there is interaction between the glutamic acid and the phenol. This interaction produces an essentially new compound possessing its own partition coefficient and moving independently. The interaction only occurs with high concentrations of glutamic acid and it only occurs with some solvents. Butanol-acetic acid gives results similar to those given by phenol but in aqueous acetone both high and low concentrations of glutamic acid give single spots of the same R_F value. Usually for paper chromatography the concentrations of glutamic acid used are such that this interaction with solvents does not occur. Full details of this work will be published later.

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Received March 11th, 1955

Infra-red dichroism of fibrous proteins

The dichroic ratio of the NH(ν) absorption band at 3300 cm^{-1} is surprisingly low for most naturally occurring fibrous proteins[1,2]. Similar measurements of our own show that feather keratin from goose quill rachis has a perpendicular ratio of about 1.8:1, while α-keratin from porcupine quill tip has a parallel ratio of 1.5:1. The particular specimens on which these measurements were made were shown by their X-ray diffraction photographs to have good orientation as regards their more crystalline regions. By contrast, a well oriented specimen of poly-benzyl-L-glutamate prepared from *m*-cresol has been shown to give a parallel ratio of 15:1[3]. It seems reasonable to suppose that