

value. The commercial tablets<sup>4</sup> disintegrate in the gastrointestinal tract liberating drug in fine particle size, and the results (7) are distinctly different.

**Correlation Between Initial Lowering of Blood Sugar Level or Amount of Tolbutamide in Circulation and *In Vitro* Dissolution Rate of the Preparations.**—The biological response obtained in these experiments is related to the physical chemical property of dissolution rate of the preparations. This may be seen from an examination of Fig. 3 where extent of blood sugar lowering after 1 hour is compared to the *in vitro* dissolution rates of the disks that served as dosage forms in the experiments. The *in vitro* dissolution rates used in the plot were from determinations in the nearly neutral mediums (Table IV). A generally similar relationship holds if the rates in acidic mediums are used in the plot. Comparison of data in Table IV to corresponding data in Tables I, II, and III also indicates the dependency of extent of carboxy-tolbutamide excretion or blood sugar level lowering, respectively, on dissolution rate.

### SUMMARY

A study was made of the influence of the rate of absorption of tolbutamide on the initial depression of blood sugar levels in normal adult humans. Initial depression in blood sugar level is related to the absorption rate (increasing with increasing rate of absorption), amount of tolbutamide in the body in the time taken for com-

parison (increased with increased amounts of tolbutamide in the body), and to the *in vitro* dissolution rate of the various salts of tolbutamide used in the tests. Ultimately, differences in either depression of blood sugar level or amount of tolbutamide in the body during the times at which the comparisons were made was dependent on the *in vitro* dissolution rate of the preparations which surely determined *in vivo* absorption rate in this *in vivo*, solution rate-limited absorption.

### APPENDIX

**Experimental Design Used in the Blood Sugar Lowering Tests.**—A pool of 35 normal human subjects were used. Subjects were assigned at random to one of three treatments, namely; T, one of the test preparations in the form of cylindrical disks; O, a compressed tablet of tolbutamide; P, a placebo tablet. Nine subjects (39% of the 23 receiving the test preparations) were administered all four of the test preparations. The schedule of treatments is shown in Table VIII.

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<sup>4</sup> Orinase tablets, The Upjohn Co.

## Separation of Amino Acids on Ion Exchange Resin Papers

### Dependence of $R_f$ Values on pH and Ionic Strength

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Fifteen amino acids have been chromatographed on paper impregnated with an ion exchange resin of the sulfonic acid type. The  $R_f$  values have been determined at various pH and ionic strengths of the buffer eluant. By means of several chromatograms of appropriate pH values and ionic strengths, most amino acids can be separated and identified in about 2 hours. The ion exchange resin papers can be regenerated and used again.

**D**URING our studies of the amino acid composition of opium (1-3), various methods of separation and quantitative determination of amino acids have been investigated. Two-

dimensional paper chromatography is satisfactory for most qualitative work, whereas the ion exchange procedure of Moore and Stein (4-6) is often preferred for quantitative analysis because of its accuracy and reproducibility.

In recent years papers impregnated with ion exchange resins have become available. These combine many of the advantages of paper chroma-

Received April 10, 1961, from the University of California Medical Center, School of Pharmacy, San Francisco.

Accepted for publication July 6, 1961.  
This work was supported by a research grant (M-3487) from the National Institutes of Health, Bethesda, Md.

tography with those of the ion exchange method, and several investigators have reported on the application of such papers to the separation of amino acids. Lautsch, Manecke, and Broser (7), as well as Tuckerman, Osteryoung, and Nachod (8), separated certain amino acids on carboxylated ion exchange paper. Myhre and Smith (9), using an aqueous-organic solvent, obtained results that were quite different from those which one would expect on the basis of ion exchange phenomena alone. Partition effects apparently modified the migration rates. Tuckerman (10) separated arginine, histidine, and lysine in casein hydrolysates by means of paper impregnated with a sulfonated ion exchange resin. This method was later used for quantitative determinations by Roberts and Kolor (11).

In the column chromatographic method, all the major amino acids are separated by increasing the pH, the ionic strength, and the temperature during the elution. This paper reports the effect of pH and ionic strength on the separation of amino acids on ion exchange paper.

## EXPERIMENTAL

**Materials.**—Amberlite SA-2 ion exchange paper was used. This is prepared from alphacellulose pulp and finely ground ion exchange resin of the sulfonic acid type (Amberlite IR-120). Citrate buffer solutions were prepared in the pH range of 2.2 to 6.5 with sodium ion concentrations ranging from 0.1 to 0.8 *N*. The buffer solutions described by Moore, Spackman, and Stein (6) were used as starting points and adjustments made as needed to include a wider range of pH and ionic strength.

**Procedure.**—Sheets of ion exchange papers were cut into sections, 22 inches long and 9 inches wide. The lower edge was cut in a saw-tooth fashion so as to facilitate the flow of liquid off the paper. The papers were placed in a chromatographic cabinet and washed overnight with a buffer solution of the same pH and ionic strength to be used for elution. The buffer was then replaced with water to rinse out the excess of salts, and finally the papers were suspended in air until they were almost dry. Five microliters of 0.02 *M* solutions of the various amino acids were applied as small round spots four inches from the upper edge of the papers and about one inch apart. The papers were again placed in the chromatographic cabinet and developed with appropriate buffer solutions. The descending method was used for this as well as for the previous activation and washing. After about 2 hours, when the solvent front had progressed about 15 inches beyond the point of sample application, the papers were removed from the cabinet, air-dried, and sprayed with a 0.5% solution of ninhydrin in ethanol. The papers were left for 1 hour at room temperature and then heated at 100° for 10 minutes. The  $R_f$  values were calculated on the basis of six to eight chromatograms for each amino acid. At pH values where the neutral and acidic amino acids travelled closely

behind the liquid front, the spots tended to become diffuse and poorly defined.

The ion exchange papers could be used again several times. The amino acid spots and excess of ninhydrin were removed with alcoholic hydrochloric acid (about 1 *N* hydrochloric acid in ethanol). The papers were washed with water and treated with buffer as described above. Care must be exercised in handling the wet papers as they tear very easily.

## RESULTS AND DISCUSSION

The  $R_f$  values obtained for 15 amino acids at various pH values and ionic strengths are given in Table I. The effect of pH is also illustrated in Fig. 1, while Figs. 2 to 5 show the effect of the ionic strength. It is apparent that both factors, pH and ionic strength, have a pronounced effect on the rate of migration of the amino acids. No complete separation of all amino acids can be achieved in one single run. However, by running several chromatograms of appropriate pH and ionic strengths, most amino acids can be separated and identified in a relatively short time.

For a preliminary, exploratory examination of an amino acid mixture of unknown composition, a buffer solution of relatively low pH and concentration will yield much information. At a pH of 3.5 and a sodium concentration of 0.2 *N*, a reasonably good separation of the majority of common amino acids may be accomplished (*cf.* Fig. 1). If several acidic and neutral amino acids are present, these are best separated at a still lower pH value, for example 2.2, and at sodium concentrations of 0.6 and 0.8 *N* (Fig. 2). Basic amino acids are easily separated in the pH range of 5 to 6 (Figs. 1 and 5).

The  $R_f$  value of  $\gamma$ -aminobutyric acid is very much affected by pH between 4.5 and 6.5, while phenylalanine and tyrosine show greater response to pH

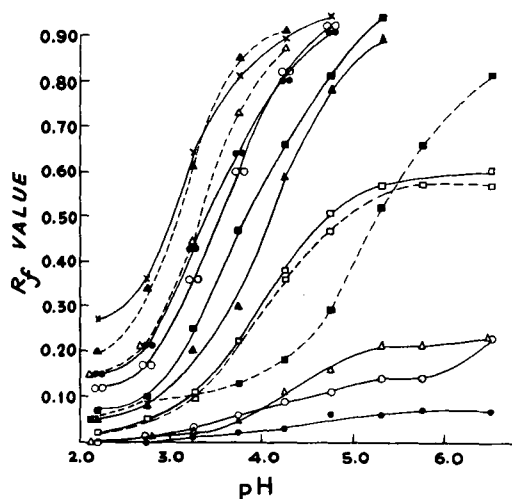


Fig. 1.—Influence of pH on the  $R_f$  values of arginine (●-●-), histidine (○-○-), lysine (△-△-), tyrosine (□-□-), phenylalanine (◻-◻-), leucine (▲-▲-), isoleucine (■-■-),  $\gamma$ -aminobutyric acid (◼-◼-), valine (○-○-), alanine (●-●-), glutamic acid (△-△-), aspartic acid (▲-▲-), and threonine (×-×-).



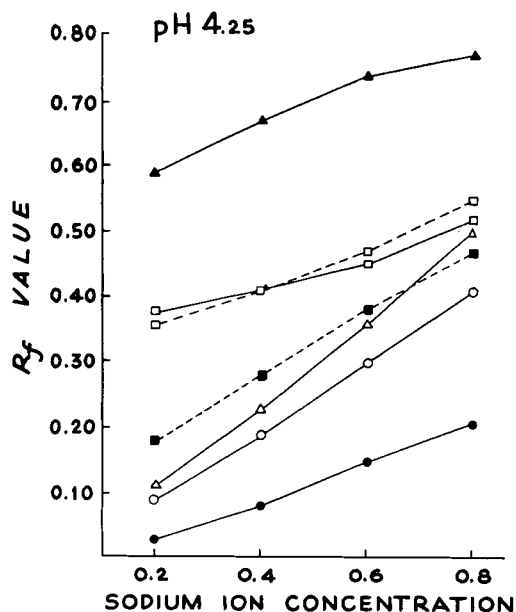


Fig. 4.—Influence of ionic strength of the  $R_f$  values of arginine (—●—●—), histidine (—○—○—), lysine (—△—△—),  $\gamma$ -aminobutyric acid (—■—■—), tyrosine (—□—□—), phenylalanine (—□—□—), and leucine (—▲—▲—).

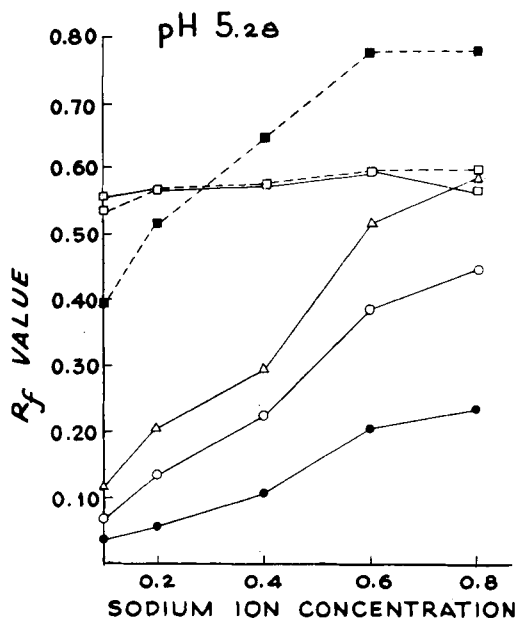


Fig. 5.—Influence of ionic strength of the  $R_f$  values of arginine (—●—●—), histidine (—○—○—), lysine (—△—△—),  $\gamma$ -aminobutyric acid (—■—■—), tyrosine (—□—□—), and phenylalanine (—□—□—).

changes at lower pH values. Thus, the curves for these amino acids in Fig. 1 intersect at about pH 3.25 and again at about pH 5.5.

Certain amino acids, such as leucine and isoleucine, which are difficult to separate by conventional paper chromatography show good separation on ion exchange papers, particularly at a high ionic strength in the pH range of 2.2 to 3.25. Phenylalanine and tyrosine, on the other hand, are more difficult to separate on ion exchange paper than on ordinary filter paper. Although they are affected to a somewhat different extent by changes in pH and ionic strength, it is difficult to obtain a clear-cut separation of the two amino acids in a mixture. The temperature, undoubtedly, plays an important role in ion exchange separations of amino acids. Thus, in column chromatography, tyrosine, phenylalanine,  $\beta$ -aminoisobutyric acid, and glucosamine appear together in the same fractions when eluted at 30° (12). When the temperature is raised to 50°, all four compounds are nicely separated (5,

6, 12). It is probable that similar improvements may be obtained by increasing the temperature in ion exchange paper chromatography.

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