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Research Articles

Influence of the Absorption Rate of Tolbutamide on the Rate of Decline of Blood Sugar Levels in Normal Humans

By EINO NELSON, E. L. KNOECHEL, W. E. HAMLIN, and J. G. WAGNER

The absorption of tolbutamide and several of its salts was studied by means of carboxytolbutamide excretion rate measurements following the oral ingestion of the drugs in nearly constant surface dosage forms by normal adult humans. Lowering of blood sugar levels produced in normal adult humans after the oral ingestion of the same preparations was also studied. Initial rate of decline of blood sugar level could be correlated with either the absorption rate of tolbutamide or the amount of tolbutamide in the body 1 hour after drug ingestion; increasing with increasing absorption rate or increase in amount of tolbutamide in the body. In most instances, increases in *in vitro* dissolution rate were reflected in increases in rate of decline of blood sugar level, absorption rates, or amounts of tolbutamide in the body at 1 hour.

THE RELATIONSHIP between the rate of absorption of the antidiabetic drug tolbutamide[1] and resulting depression in blood sugar level has not beeen previously studied. The availability of several salts of tolbutamide with different dissolution rates and, hence, different absorption rates, made possible the present study which was concerned with the nature of this relationship.

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Tolbutamide is 1-butyl-3-*p*-tolylsulfonylurea.

EXPERIMENTAL

Materials.—Pharmaceutical grade tolbutamide and its sodium salt were used.

The 2-amino-2-methyl-1-propanol salt of tolbuta-

mide was prepared in the following manner: 100 Gm. (0.37 mole) of tolbutamide was added to about 1200 ml. of deionized water, previously heated to 50°. To this slurry was added, in small portions, 34.8 Gm. (0.37 mole) of 2-amino-2-methyl-1-propanol (tech. grade). Stirring and heating were continued and a small additional amount of the amine (1.0 Gm.) was added to effect complete solution of the acid. The hot solution was filtered, placed in a lyophilizing flask, and frozen.

Following the removal of the solvent by lyophilization, the resulting light, fluffy, white solid was triturated with successive portions of ether in a porcelain mortar. The resulting slurries were filtered and the filter cake was washed with fresh portions of ether. The solid was dried for 48 hours *in vacuo;* yield, 131 Gm., m.p. 150.2–153.8°.

Anal.—Calcd. for $C_{16}H_{29}N_3O_4S$: C, 53.46; H, 8.13; N, 11.69; S, 8.92; equiv. wt., 359. Found: C, 53.77; H, 8.36; N, 11.52; S, 8.99; equiv. wt., 353; % H_2O (Karl Fischer), none.

The 1-amino-2-propanol salt of tolbutamide was prepared by the following procedure: to 1200 ml. of deionized water heated to 50° was added 100 Gm. (0.37 mole) of tolbutamide. To the resultant slurry 28 Gm. (0.37 mole) of 1-amino-2-propanol (tech. grade) was added in small portions with constant agitation. An additional amount (1 Gm.) of the amine was added to effect a complete solution. The hot solution was filtered, placed in a lyophilization bottle, and frozen.

The white, fluffy solid produced upon removal of the aqueous phase by lyophilization was triturated with small portions of ether in a porcelain mortar. Following filtration of the resulting slurries, the filter cake was washed with fresh portions of ether. The solid was dried *in vacuo* for 68 hours; yield, 90 Gm., m.p. 91.4–94.6°.

Anal.—Calcd. for $C_{15}H_{27}N_3O_4S$: C, 52.15; H, 7.88; N, 12.16; S, 9.28; equiv. wt., 345. Found: C, 52.17; H, 7.91; N, 11.75; S, 9.37; equiv. wt., 345; % H_2O (Karl Fischer), none.

Dosage Forms.—The sodium, 2-amino-2-methyl-1-propanol, and 1-amino-2-propanol salts of tolbutamide, as well as tolbutamide free acid, were compressed into thin cylindrical disks at a pressure of about 40,000 p.s.i., using commercial tableting equipment. The disks were 1.28 cm. in diameter and their thickness varied from 0.168 cm. to 0.229 cm., depending on the form of tolbutamide used. Each disk contained 250 ± 10 mg. tolbutamide equivalent and had a surface area of 3.38 ± 0.09 cm.[2].

Test Subjects and Conduction of Tests.—Two separate test panels were used: one for the studies on the absorption and excretion rates of carboxytolbutamide[2] following the ingestion of 0.5 Gm. tolbutamide in the various forms, and the other for studying blood sugar level following ingestion of 1.0-Gm. doses of tolbutamide in the same forms. The test subjects for all experiments were normal adult humans. Drug was ingested on fasting stomachs in the morning and no food was taken until 1 hour had lapsed from the time of drug ingestion in the absorption experiments. In the blood sugar lowering tests the compounds were administered after overnight fasting and no food was taken during the active course of the experiments (10 hours).[3]

Analytical Methods.—Urine was assayed for carboxytolbutamide by a previously described method (1). Blood sugar determinations were made on blood obtained from the antecubital veins by means of an Autotechnicon. The analytical method was that of Hoffman (2).

In Vitro Dissolution Rate Studies.—Dissolution rates of each of the forms were determined in 0.1 *N* hydrochloric acid and 0.2 *M* tris(hydroxymethyl)-aminomethane (adjusted to pH 7.2 with hydrochloric acid) solutions at 37° using a method previously described to study dissolution rates of slightly soluble weak acids and their sodium salts (3).

RESULTS

Excretion Experiments.—Cumulative excretion of carboxytolbutamide following the ingestion of tolbutamide in the several hours is given in Table I.

Blood Sugar Level Studies.—Results of blood sugar tests in 23 normal subjects are shown in Tables II and III.

In Vitro Dissolution Rate Studies.—The dissolution rates *in vitro* determined by the method described are summarized in Table IV.

DISCUSSION

Treatment of Excretion Data.—Cumulative amounts of carboxytolbutamide excreted in 1, 2, 3, and 4 hours by each of the test subjects in each test were fitted to polynomials with powers of time of the form

$$Y = at + bt^2 + ct^3 + dt^4 \qquad \text{(Eq. 1)}$$

where Y was the amount excreted in mg. and t was time in hours. The coefficients of Eq. 1, for each of the tests, are listed in Table V.

It has been previously reported that absorption rate (A.R.) may be calculated from excretion measurements by the following expression (4)

$$\text{A.R.} = \frac{1}{f}\left(\frac{dAe}{dt} + \frac{1}{K}\frac{d^2Ae}{dt^2}\right) \qquad \text{(Eq. 2)}$$

In Eq. 2, dAe/dt is excretion rate, d^2Ae/dt^2 its derivative, K the rate constant for elimination of drug, and f the fraction of drug reaching circulation that is excreted unchanged. All quantities in Eq. 2 refer to unchanged drug. Tolbutamide is nearly quantitatively oxidized to carboxytolbutamide *in vivo* and this metabolite is quantitatively excreted in the urine about 12 times more rapidly than it is formed (5). Therefore, even though carboxytolbutamide excretion was followed, this very closely represented tolbutamide's oxidation and application of Eq. 2 to calculate absorption rate should not result in the introduction of any substantial error. In order to calculate absorption rate of tolbutamide by Eq. 2 the first and second derivatives of Eq. 1 were taken at 1.0, 1.5, and 2.0 hours. A mean value of K for each of the subjects used in the excretion experiments was available from other work (5), hence absorption rate could be calculated at the

[2] Carboxytolbutamide is *p*-[(butylcarbamoyl)sulfamoyl]-benzoic acid.

[3] The experimental design used in the blood sugar lowering tests is shown in the Appendix.

TABLE I.—CUMULATIVE MG. CARBOXYTOLBUTAMIDE EXCRETED FOLLOWING INGESTION OF TOLBUTAMIDE IN SEVERAL FORMS

Time, hr.	Subject E	Subject I	Subject B	Subject C	Time, hr.	Subject E	Subject I	Subject B	Subject C
	1-Amino-2-propanol Salt					Sodium Salt			
1	25	5	23	21	1	28	9	18	27
2	82	34	80	65	2	73	40	67	78
3	127	73	138	112	3	122	81	122	123
4	173	113	187	190	4	163	119	166	164
6	241	176	270	278	6	236	174	243	220
8	298	232	320	338	8	278	227	307	277
10	343	281	362	387	10	328	273	340	316
12	374	320	395	432	12	364	312	370	345
24	468	467	534	547	24	437	433	490	444
48	541	535	586	567	48	514	502	559	490
Theory[a]	555	555	555	555	Theory[a]	537	545	545	555
	2-Amino-2-methyl-1-propanol Salt					Free Acid			
1	7	4	12	2	1	6	3	11	1
2	34	14	30	9	2	10	5	12	2
3	68	29	57	36	3	19	8	16	4
4	107	49	93	75	4	30	13	19	9
6	190	91	173	163	6	47	25	26	21
8	243	129	235	232	8	61	37	46	33
10	289	167	281	292	10	71	49	66	46
12	334	203	326	344	12	84	59	98	54
24	434	375	481	502	24	124	105	180	93
48	489	480	565	602	48	219	182	216	124
Theory[a]	547	555	555	555	Theory[a]	569	569	569	569

[a] 1.11 × dose of tolbutamide taken.

TABLE II.—AVERAGE DIFFERENCES IN BLOOD SUGAR, IN MG. %, IN 0 TO 1-HOUR AND 0 TO 2-HOUR PERIODS FOLLOWING ORAL ADMINISTRATION OF THE EQUIVALENT OF 1 GM. OF TOLBUTAMIDE IN THE FORM OF DISKS[a]

Form of Tolbutamide	Difference in Blood Sugar Level, mg. %: 0–1 hr. Av.	0–1 hr. S.E.	0–1 hr. 95% Confidence Interval of Av.	0–2 hr. Av.	0–2 hr. S.E.	0–2 hr. 95% Confidence Interval of Av.
1-Amino-2-propanol salt	27.2	1.8	24.0–31.4	17.0	1.0	14.9–19.1
Sodium salt	19.1	2.3	14.3–23.9	23.7	1.8	20.0–27.4
2-Amino-2-methyl-1-propanol salt	16.0	1.5	12.9–19.1	20.0	1.2	17.5–22.5
Tolbutamide (acid)	5.2	1.3	2.5– 7.9	7.0	1.1	4.7– 9.3

[a] 23 Normal subjects.

TABLE III.—ADJUSTED[a] AVERAGE BLOOD SUGARS IN MG. % AND AS PER CENT OF PLACEBO GROUP AVERAGE AT EACH HOUR[b]

Form of Tolbutamide	Hours After Administration[c]: 0.5	1	2	4	6	8	10
1-Amino-2-propanol salt	...	53.0	63.4	67.3	72.4	73.7	67.3
	...	68.6	83.2	92.8	96.1	99.6	99.0
Sodium salt	62.4	57.6	68.3	70.9	70.6	70.3	70.0
	76.4	73.9	84.7	90.2	93.6	93.2	97.3
2-Amino-2-methyl-1-propanol salt	...	70.1	66.2	69.2	70.0	68.8	66.5
	...	83.2	82.2	85.6	88.8	91.5	91.5
Tolbutamide (acid)	...	82.0	79.8	78.7	77.1	74.2	72.1
	...	97.7	97.4	97.0	97.8	99.7	101.7

[a] Adjusted for difference in initial blood sugar level by covariance analysis. The zero-hour blood sugar level was used as a covariate. [b] 23 Normal subjects for disks and 5 normal subjects for placebo. [c] Top row, average blood sugars in mg. %; bottom row, per cent of placebo group average.

indicated times for each of the preparations. The calculated rates are listed in Table VI in terms of tolbutamide equivalent (1 Gm. tolbutamide = 1.11 Gm. carboxytolbutamide). The value of K for subjects E, I, B, and C in Tables I, V, VI, and VII were, respectively, 0.139, 0.105, 0.133, and 0.124 hours^{-1}.

When the mean values of absorption rate, following ingestion of the sodium or 1-amino-2-propanol salts of tolbutamide were plotted *vs.* time on semilogarithmic graph paper, the line was linear indicating that absorption was by an apparent first-order process. This is shown in Fig. 1. With the 2-amino-2-methyl-1-propanol salt, the absorption

rate was nearly constant in the 2-hour period where the calculations were made. The differences in the nature of absorption processes was not unexpected. Both the sodium and 1-amino-2-propanol salts of tolbutamide dissolve rapidly in mediums possessing hydrogen ion concentrations in the physiological range (Table IV). The 2-amino-2-methyl-1-propanol salt of tolbutamide and tolbutamide free acid dissolve substantially slower and, since the dosage forms could be expected to have presented a nearly constant surface to dissolution fluids *in vivo* due to their geometry, this dissolution process was apparently rate limiting in absorption in the time period under consideration.

The excretion rate of carboxytolbutamide is very nearly directly proportional to the amount of tolbutamide in the body (5). That is,

$$dTe/dt \cong KT \qquad \text{(Eq. 3)}$$

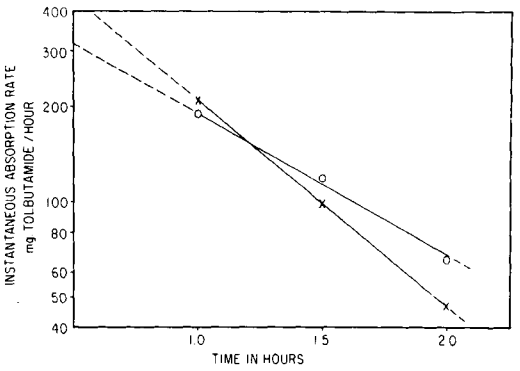

Fig. 1.—First-order plots of absorption rate. ×, 1-Amino-2-propanol salt of tolbutamide; ○, sodium salt of tolbutamide.

Table IV.—*In Vitro* Dissolution Rate of Tolbutamide in Several Forms

Form of Tolbutamide	Rate, mg./cm.²/hr. 0.1 *N* HCl	Rate, mg./cm.²/hr. 0.2 *M* Tris-(hydroxymethyl)-aminomethane[a]
1-Amino-2-propanol salt	264 (207)[b]	358 (280)[b]
Sodium salt	1156 (1069)	938 (868)
2-Amino-2-methyl-1-propanol salt	0.37 (0.28)	19 (14)
Tolbutamide (acid)	0.21 (0.21)	3.1 (3.1)

[a] Adjusted to pH 7.2 with hydrochloric acid. [b] Rate expressed in terms of tolbutamide equivalent.

where dTe/dt is the excretion rate of carboxytolbutamide, K the rate constant for the *in vivo* oxidation of tolbutamide in hours^{-1}, and T is the amount of tolbutamide in the body at any time. The value of T for each of the subjects in each of the tests was calculated at 1 hour and these are listed in Table VII in terms of tolbutamide equivalent. This was done by using the first derivative of Eq. 1.

Correlation Between Absorption Rate or Body Level of Tolbutamide and Initial Lowering of Blood Sugar Level.—The rate of lowering of blood sugar level was examined in respect to two parameters concerned with tolbutamide absorption. One of these was the rate of initial depression of blood sugar level compared to the half-life for absorption. This

Table V.—Coefficients of the Polynomial
$Y = at + bt^2 + ct^3 + dt^4$

Subject	a	b	c	d	Subject	a	b	c	d
	1-Amino-2-propanol Salt of Tolbutamide					Sodium Salt of Tolbutamide			
E	−19.9	64.1	−21.6	2.38	E	14.9	15.4	−2.42	0.024
I	−12.9	21.3	−3.58	0.021	I	−5.75	16.5	−1.75	−0.042
B	−10.7	44.0	−11.2	0.096	B	−7.83	31.7	−6.17	0.33
C	−9.17	43.5	−15.3	2.00	C	−3.00	41.7	−13.0	1.33
	2-Amino-2-methyl-1-propanol Salt of Tolbutamide					Tolbutamide (Acid)			
E	−10.08	21.5	−4.92	0.458	E	11.8	−9.08	3.67	−0.42
I	0.417	3.96	−0.417	0.042	I	4.42	−1.96	0.58	−0.42
B	10.7	0.125	1.25	−0.125	B	24.6	−19.3	6.42	−0.71
C	10.2	−15.5	8.25	−0.958	C	1.08	−0.04	−0.08	0.04

Table VI.—Absorption Rate of Tolbutamide at 1, 1.5, and 2 Hours after Ingestion of Several Forms[a]

Subject	1.0 hr.	1.5 hr.	2.0 hr.	Subject	1.0 hr.	1.5 hr.	2.0 hr.
	1-Amino-2-propanol Salt of Tolbutamide				Sodium Salt of Tolbutamide		
E	216	48	0	E	139	104	67
I	211	156	110	I	202	161	116
B	250	133	48	B	231	153	83
C	167	59	31	C	191	62	0
Mean	211	99	47	Mean	191	120	66
	2-Amino-2-methyl-1-propanol Salt of Tolbutamide				Tolbutamide (Acid)		
E	137	93	63	E	0	25	42
I	55	52	52	I	0	3	9
B	53	69	79	B	0	1	21
C	49	127	81	C	0	3	7
Mean	74	85	69	Mean		8	20

[a] Rate expressed in mg./hr. tolbutamide equivalent.

TABLE VII.—EXCRETION RATE IN MG./HR. TOLBUTAMIDE EQUIVALENT AND AMOUNT OF TOLBUTAMIDE IN MG. IN THE BODY 1 HOUR AFTER ORAL INGESTION OF 500 MG. TOLBUTAMIDE IN SEVERAL FORMS

Subject	Rate	Amount	Subject	Rate	Amount
1-Amino-2-propanol Salt of Tolbutamide			Sodium Salt of Tolbutamide		
E	46.1	333	E	33.7	244
I	17.1	163	I	19.1	181
B	41.2	309	B	33.3	250
C	24.6	280	C	40.7	328
Mean		271	Mean		251
2-Amino-2-methyl-1-propanol Salt of Tolbutamide			Tolbutamide (Acid)		
E	17.5	126	E	2.6	19
I	6.3	59	I	1.8	17
B	12.3	92	B	2.1	16
C	0.1	1	C	0.8	6
Mean		70	Mean		14

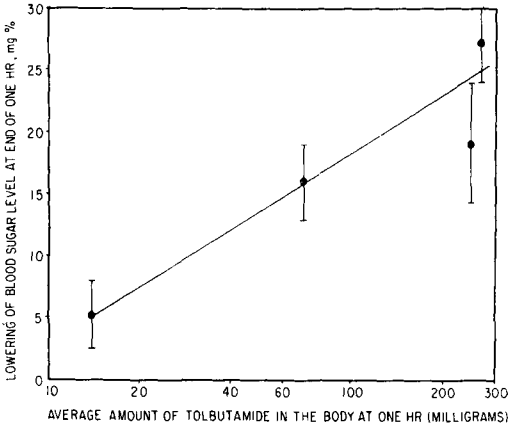

Fig. 2.—A plot of the lowering of blood sugar level at end of 1 hour against the average amount of tolbutamide in the body at 1 hour (on a logarithmic scale). Vertical bars mark off 95% confidence intervals of average blood lowering values.

comparison was only applicable in the case of the sodium and the 1-amino-2-propanol salts of tolbutamide since it was only with these materials that an apparent first-order absorption resulted (Fig. 1). From Table II the average rate of lowering of blood sugar level during the first hour was 27.2 and 19.1 mg. %/hr. for the 1-amino-2-propanol and sodium salt of tolbutamide, respectively. From Fig. 1, the half-lives for absorption were 0.46 and 0.68 hours, respectively; indicating the dependency of blood sugar level lowering on absorption rate.

In order to include all four test preparations in a correlation, lowering of blood sugar level at the end of the first hour of experiments is compared to the logarithm of the calculated amount of tolbutamide in the body at the same time on Fig. 2. The tendency for linearity indicates that a diminishing rate in blood sugar lowering is obtained with higher levels of tolbutamide. Examination of data on extent of initial lowering of blood sugar level as a function of dose of tolbutamide published by West and Johnson (6) indicates that the same general relationship between extent of lowering and dose holds.

Results obtained with the disks of tolbutamide (free acid) in these studies should not be taken as indicative of results which would be obtained with commercial tablets. In these studies the surface area of the tolbutamide was restricted to a very low

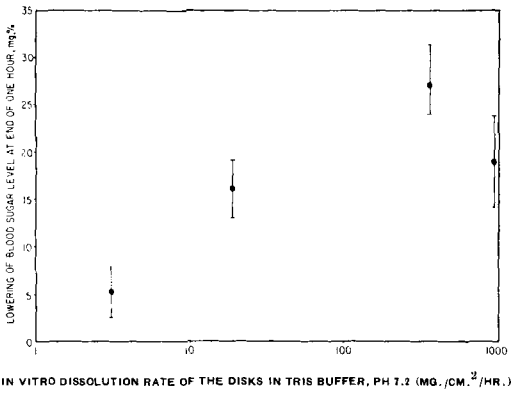

Fig. 3.—A scatter plot of lowering of blood sugar level at end of 1 hour in human subjects and *in vitro* dissolution rate of the tolbutamide dosage form in tris buffer, pH 7.2 (logarithmic scale).

TABLE VIII.—SCHEDULE OF TREATMENTS ASSIGNED TO POOL OF SUBJECTS AT RANDOM IN THE BLOOD SUGAR LOWERING TESTS

Subject No.	DAY 1	DAY 5	DAY 8	DAY 12
1	T[a]	O	T	O
2	O[b]	T	T	T
3	T	T	T	T
4	T	T	T	T
5	T	T	T	T
6	T	P	T	P
7	O	T	T	T
8	P[c]	T	T	T
9	T	O	P	P
10	T	T	O	T
11	T	O	P	T
12	T	T	T	T
13	T	T	O	T
14	T	T	T	T
15	O	P	T	O
16	T	T	T	T
17	T	O	T	P
18	T	P	T	T
19	T	..	..	..
20	T	P	O	P
21	T	T	T	T
22	T	T	P	T
23	T	T	T	O
24	O	T	P	P
25	P	T	T	T
26	T	T	T	T
27	P	P	..	..
28	P	O	T	T
29	T	T	O	T
30	T	T	O	O
31	P	T	T	T
32	O	T	T	T
33	T	T	T	T
34	..	T	T	O
35	..	..	P	T
Totals	23 T 5 P 5 O	23 T 5 P 5 O	23 T 5 P 5 O	23 T 5 P 5 O

[a] On day 1, T = free acid; on day 5, T = 2-amino-2-methyl-1-propanol salt; on day 8, T = sodium salt; on day 12, T = 1-amino-2-propanol salt. [b] O, a compressed tablet of tolbutamide. [c] P, a placebo.

value. The commercial tablets[4] 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 carboxytolbutamide 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 comparison (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.

[4] Orinase tablets, The Upjohn Co.

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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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.

DURING 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-

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