the apparent diffusion coefficients, an increase in temperature causing an increase in the rate of diffusion. The diffusion coefficients have an order to around 10^{-9} cm.²/second. These values were slightly less than, but not too far from, the values observed with a group of weak organic acids reported previously (3). Table III and Fig. 6 reveal that the original concentration of solution will have a direct effect on the apparent diffusion coefficient at any particular temperature or, in this case, as the original concentration in the solution is increased, the apparent diffusion coefficient decreases. These results suggest that the larger number of sorbic acid molecules (at the higher concentration) will have a tendency to reduce the mobility of the molecules as they attempt to traverse the matrix of the plastic. Conversely, in a more dilute solution of sorbic acid, less of the sorbic acid molecules will enter the plastic and less hinderance to passage or diffusion will occur.

The activation energy (ΔE) further confirms the effect concentration of the solution has on diffusion—from a low value of 3.07 Kcal./mole for the highest concentration of sorbic acid studied to a value of 26.2 Kcal./mole for the lowest concentration employed.

SUMMARY

Sorption studies were conducted on sorbic acid at a number of concentrations and at several temperatures, nylon 66 being used as the substrate. From these experiments it was possible to evaluate a number of constants such as (*a*) saturation value of solute in plastic, (*b*) standard affinity of solute for nylon 66, (*c*) standard heat of sorption, (*d*) apparent diffusion coefficient of sorbic acid in nylon 66, and (*e*) activation energy of diffusion in the plastic. The value of approximately −10.0 Kcal./mole for $\Delta H°$ suggested that there was a possibility of a double hydrogen bond formation between the sorbic acid and a reactive site in the plastic. Some evidence during the experiments also suggested that a slight chemical reaction might be taking place between the sorbic acid and the nylon 66. This point must, however, be further investigated. Results of the diffusion experiments revealed that concentrations of the original solutions influenced the rate of diffusion: the higher the concentration the slower the rate.

REFERENCES

(1) Marcus, E., Kim, H. K., and Autian, J., This Journal, 48, 457(1959).
(2) Autian, J., and Shaikh, Z. I., *Drug. Std.*, 28, 103(1960).
(3) Kapadia, A. J., Guess, W. L., and Autian, J., This Journal, 53, 28(1964).
(4) Vickerstaff, T., "The Physical Chemistry of Dyeing," Interscience Publishers, Inc., New York, N. Y., 1954, pp. 100–102.
(5) Berthier, G., *J. Chim. Phys.*, 49, 527(1952).
(6) Crank, J., "The Mathematics of Diffusion," Oxford University Press, London, 1956, p. 55.
(7) Kapadia, A. J., Guess, W. L., and Autian, J., This Journal, 53, 720(1964).

Wurster Coated Aspirin II

An *In Vitro* and *In Vivo* Correlation of Rate from Sustained-Release Preparations

By JOHN H. WOOD and JOHN SYARTO

In vitro **and** ***in vivo*** **evaluations have been made of the salicylate release characteristics of aspirin coated by the Wurster process. The films used were of methyl and ethyl cellulose plasticized by glycerol. The** ***in vitro*** **release rates of such coatings were shown to be first order. The** ***in vivo*** **release may be best represented as first order. A mathematical correlation between** ***in vivo*** **and** ***in vitro*** **rates is possible, but this correlation is directly dependent on film composition. It is shown that the slower the release pattern, the less complete is the apparent plasma availability, due to incomplete physical release and insufficient time for complete biologic absorption.**

The last decade has seen an exponential increase in number and diversity of publications, both original and review, dealing with some aspect of the controlled rate of release of medicament following oral administration. These have covered both *in vivo* and *in vitro* evaluation and their possible correlations.

As part of our program of the study of dosage forms suitable for proprietary use, we were interested in release performance of crystalline drugs coated in the Wurster apparatus (1) to yield delayed release characteristics. The first paper of this series (2) describes the preparation of granules and their incorporation into a rapidly disintegrating tablet. The coatings were made of various ratios and amounts of ethyl and methylcellulose plasticized with glycerol.

Since it has been shown that stomach pH's may be quite variable (3) and since the normal stomach residence time and average emptying time may range up to several hours (4, 5), it is desirable for more uniform release between individuals

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TABLE I.—COMPOSITION AND CHARACTERISTICS OF TEST DELAYED-RELEASE ASPIRIN PRODUCTS USED IN THIS STUDY

	Code			
	134B	134C	152	138
Ratio ethyl to methylcellulose	75/25	25/75	82.5/17.5	100/0
Aspirin mesh size	−20	−40	−20+40	−20+40
Amount of coating (wt. %)	2.7%	4.8%	6%	6%
Tablet disintegration time, sec.	40–60	25–35	3	6
Aspirin content	5 gr.	5 gr.	5 gr.	5 gr.
Cornstarch	0.87 gr.	0.90 gr.	0.96 gr.	0.96 gr.
Talc	...	...	0.13 gr.	0.13 gr.

that the coatings not be significantly dependent upon ambient pH for their rate of release. This criterion is met by the coatings chosen here.

From a series of preparations of various *in vitro* release rates, it was desirable to establish the extent to which these could be differentiated *in vivo*. In this study acetylsalicylic acid was the drug of choice. Normally, salicylate would not be considered as a likely candidate for sustained release since its biologic half-life in a normal population is 6.2 hours by plasma studies (6) and 6 hours by urinary recovery (7). However, sustaining is practical and, in the case of the chronic arthritic under salicylate therapy, can be highly desirable for minimizing the excursions in plasma level (8) that are a necessary consequence of the usual periodic massive dosages. The hydrolysis *in vivo* of aspirin is very rapid so that the apparent plasma half-life is short (9). Thus, the permissible rate of release to maintain an aspirin level is limited by the residual free salicylate levels.

In vivo evaluation of aspirin release was, therefore, monitored in this report by the plasma salicylate levels rather than by urinary recovery.

EXPERIMENTAL

Preparation of Dosage Forms.—The preparation of the granules and tablets is described elsewhere (2). The specific composition of the test products is given in Table I. In each case the cellulose derivatives are plasticized with 17% glycerol.

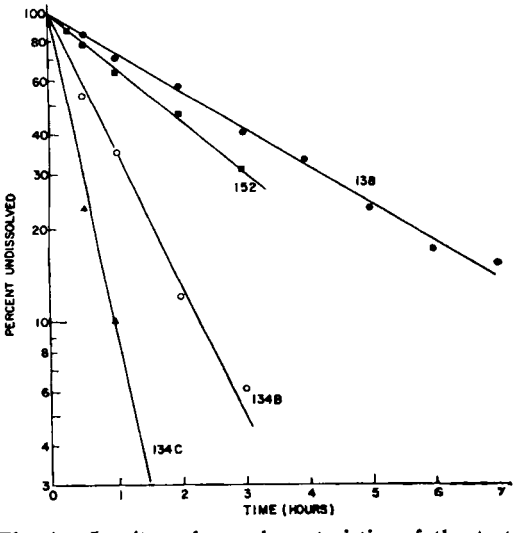


Fig. 1.—*In vitro* release characteristics of the test tablets.

In Vitro Evaluation.—Because tablet environmental conditions *in vivo* were felt to be variable and indeterminate, a test medium of pH 4 was arbitrarily chosen for use. Initially, McIlvaine standard buffer solution (12.29 ml. of 0.1 *M* citric acid plus 7.71 ml. of 0.2 *M* Na_2HPO_4) was used. Since the pH shifted, in test, from 4.00 to 3.80, the Na_2HPO_4 concentration was arbitrarily raised to 0.2125 *M* so that the pH shift was 4.05 to 3.95 during test. To 500 ml. of test solution in a 1-L. round-bottom flask thermostated at 37°, 15 gr. of coated aspirin product was added, either as granules or tablets. Stirring was with a 1/4-in. stainless steel rod, bent to a 1/2-in. loop off the axis of rotation, the end being on the axis. This was rotated at 420 r.p.m. using an Eberbach 7065 three-speed stirring motor.[1] This was a similar physical assembly to that which has been in use since 1957 and found valuable in study of normally fast-releasing tablets (10).

Aliquots were removed periodically and analyzed for salicylate following alkaline hydrolysis by reading on a Beckman DU at 298.5 mμ.

Release characteristics are shown graphically in Fig. 1. In Table II, replicability of the test procedure is demonstrated.

In Vivo Evaluation.—Plasma salicylate levels were determined as a function of time over a 24-hour period using the Brodie (11, 12) procedure after allowing the whole blood to stand for several hours to permit complete enzymatic hydrolysis of any aspirin (ASA) to the salicylate (SA)[2] (13). Thus, the procedure measures total plasma salicylate (and salicylurate, SUA, if any) present. It does not detect salicyl glucuronides (SG).

Administration was 20 gr. ASA at one time. As the control, buffered aspirin[3] was used to provide rapid complete absorption (13). The plasma levels obtained for the various dosage preparations tested are given in Table III.

It has recently been emphasized (14) that, because of the kinetics involved, a metabolite cannot *per se* be used directly to follow the rate of uptake of a parent drug. In the case of a sustained-release aspirin preparation it can be shown that plasma salicylate levels do meet the requirements of the simplified kinetic picture, where K_A and K_E are the apparent rate constants for absorption and elimination.

$$\text{Solid Drug} \xrightarrow{K_A} \text{Plasma Salicylate} \xrightarrow{K_E} \text{Organs of Elimination}$$

$$\text{Plasma Salicylate} \rightleftarrows \text{Apparent Volume of Distribution}$$

[1] Eberbach Corporation, P.O. Box 1024, Ann Arbor, Mich.

[2] We wish to acknowledge that these levels were obtained by Drs. W. D. Paul and J. I. Routh, School of Medicine, State University of Iowa, Iowa City.

[3] Marketed as Bufferin by the Bristol-Myers Co.

TABLE II.—REPLICABILITY OF *In Vitro* TEST PROCEDURE, PERCENTAGE DISSOLVED AS A FUNCTION OF TIME

Time	Sample 138 Run 1	Sample 138 Run 2	Sample 138 Run 3	Sample 152 Run 1	Sample 152 Run 2	Sample 152 Repeat Run 1	Sample 152 Repeat Run 2
5 min.	0	0	..	7	7	6	6
15	6	6	..	13	11	13	13
30	17	16	..	22	20	22	23
1 hr.	28	29	31	36	36	37	37
2	44	42	42	51	55	57	61
3	58	58	60	69	69	74	74
4	68	64	69	77	79	80	85

TABLE III.—SALICYLATE PLASMA LEVELS FOR INDIVIDUALS FOR THE VARIOUS FORMS AS FUNCTION OF TIME (20 GR. ASPIRIN ADMINISTERED)

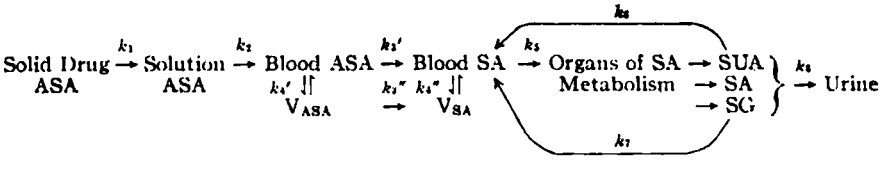
Sample	Subject[a]	Plasma Levels, mg./ml. 1/3 hr.	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	24 hr.
Buffered aspirin	1	87.0	120.0	125.0	108.0	69.3	36.5	0.0
	2	30.5	79.2	111.0	87.0	60.5	40.0	5.3
	3	20.7	64.2	103.4	104.4	64.4	38.2	0.4
	4	54.5	84.0	86.0	70.0	46.4	24.4	4.0
	5	105.7	112.2	104.7	94.7	58.2	31.7	0.6
134B	1	38.7	65.9	95.9	99.2	69.5	41.7	0.3
	2	3.0	8.0	44.7	77.2	59.7	37.1	2.0
	3	0.7	6.2	38.4	91.2	75.4	41.4	0.4
	4	9.3	17.3	58.0	62.5	40.4	23.4	0.3
134C	1	56.5	98.2	131.2	116.2	78.0	50.9	6.5
	2	6.3	20.3	55.2	81.7	56.7	36.7	1.7
	3	9.0	12.8	24.0	87.2	75.5	43.0	0.0
	4	34.0	57.5	59.0	45.4	21.3	8.0	1.0
138	2	6.0	13.3	25.3	36.4	35.0	29.0	9.3
	3	1.7	6.0	17.7	29.8	37.8	37.4	7.4
	4	2.0	10.3	19.4	24.8	27.0	23.4	1.3
	5	7.7	17.4	22.0	32.4	45.4	48.4	5.4
152	2	1.0	6.4	20.0	42.9	49.0	29.2	1.0
	3	2.6	11.3	25.0	49.3	57.5	53.5	5.3
	4	6.6	13.0	30.8	42.5	37.5	24.3	0.0
	5	20.0	36.8	60.0	65.0	64.0	45.0	2.0

[a] Subject 1, female, age 30, 110 lbs. Subject 2, male, age 48, 155 lbs. Subject 3, male, age 39, 150 lbs. Subject 4, male, age 32, 205 lbs. Subject 5, female, age 40, 139 lbs.

TABLE IV.—ACCUMULATED SALICYLATE DELIVERY TO THE PLASMA WITH TIME AS A PERCENTAGE FUNCTION OF THE TOTAL AMOUNT DELIVERED FROM A FAST-RELEASING TABLET

Sample	Subject	Percentage of Possible Total Delivery 1/3 hr.	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	24 hr.
134B	1	21	37	58	72	89	93	90
	2	3	7	42	82	89	86	80
	3	1	5	30	73	91	87	80
	4	7	14	48	62	74	77	77
	Av.	8	16	44	72	86	86	82
134C	1	27	48	69	75	92	97	100
	2	6	25	59	95	88	87	80
	3	6	9	18	69	88	86	73
	4	26	46	53	52	55	52	53
	Av.	16	32	50	73	81	81	77
138	2	5	11	22	36	46	52	54
	3	1	4	13	35	42	55	59
	4	2	8	17	26	40	59	59
	5	5	11	16	28	50	69	72
	Av.	4	8	17	31	44	59	61
152	2	1	5	17	41	61	57	51
	3	2	8	19	41	65	82	80
	4	5	11	27	44	59	63	61
	5	12	25	44	58	79	90	89
	Av.	5	12	27	46	66	73	70

A more realistic approximation of the various processes involved is given by



where V_{ASA} and V_{SA} are the apparent volumes of distribution of both ASA and SA, respectively.

It has been shown by Leonards (15, 16) that ASA is absorbed intact and very rapidly. Therefore, in a sustained-release preparation k_2 will be very much faster than k_1 and hence k_1 will be the rate determining step, equivalent to K_A of the simplified version. Although there is speculation concerning the site of ASA hydrolysis, the measured apparent *in vivo* blood half-life is appreciably less than 10 minutes and the half-life for k_4'' is of the same magnitude (6). Judging by Leonards' dog data (15), k_4' is of the same order as k_4''. Thus, it may be concluded that there is rapid hydrolysis and rapid dynamic equilibration of both species for their respective volumes of distribution. Both Leonards (15) and Smith (17) have reported that ASA yields slightly lower plasma levels than SA, presumably because of serum albumen binding of SA implying that the relative volumes of distribution are not greatly dissimilar. However, by 1 hour essentially no ASA remains in the plasma from a fast releasing form (15), and trivial percentages may be calculated to be present from a sustained form so that the consideration of an average volume of distribution V may be reasonably assumed.

Under dosage there is some tubular resorption of SUA and SG (18), totaling 1% at 2 hours and 6% at 24 hours of the total salicyl then present in plasma. Almost half of this is the SUA, which will be detected by the Brodie procedure. The correction then necessary to consider net k_5 equivalent to K_E of the simplified version is then negligible.

Thus, the Brodie procedure as used to measure the summation of drug and essentially all plasma metabolite gives a direct measure of total drug absorption and loss as required (14) for simplified kinetics. Internal self-consistency of the data thus obtained then becomes a verification of the assumptions.

From the plasma levels obtained from buffered aspirin, the apparent biologic half-life for each subject was determined. The range and average (6.1 hours for $t_{1/2}$), for the group was within that normally to be expected (6). The rate constants for elimination from the plasma were for subjects one to five 0.147, 0.099, 0.129, 0.150, and 0.145 hours^{-1}, respectively. Certain assumptions must then be made to calculate percentage recoverable from the delayed-release forms. First, for the time intervals used, plasma levels are in dynamic equilibrium with the tissues, and no appreciable quantity is irreversibly removed by a depot. For salicylates, this would appear to be the case (6). Second, it is assumed that first-order elimination rates are not dependent on dosage form. In this case, the kinetics are as already shown. The elimination is, at any time, proportional to the level present, and the total amount eliminated up to any time is readily obtained by mathematical or graphical integration. Thus, the amount absorbed to the plasma, x_t, at any time t is given by a summation of the amounts eliminated and the level C_t at that time. One of the possible forms of the equations representing this process is that given by Wagner, *et al.* (19), and was

$$X_t = V\left\{k\int_0^t C\,dt + C_t\right\} \qquad \text{(Eq. 1)}$$

where V is the effective equivalent distribution volume, and k is the salicylate elimination constant. For each individual the value of X_t may be calculated as a function of time in units of the individual V.

The value of X at infinite time—here 8, 12, and 24 hours—for the fast-releasing tablet is then considered to represent complete biologic recovery for 20 gr. of aspirin. Thus

$$X_\infty = Vk\int_0^\infty C\,dt \qquad \text{(Eq. 2)}$$

Then, for each of the test drugs, X_t may be evaluated as a function of time for each person. The ratio X_t/X_∞ then constitutes the fraction absorbed at that time, and this fraction becomes independent of the distribution volumes. The individual percentages of theoretical maximum are tabulated in Table IV, and the averages shown graphically in Fig. 2.

DISCUSSION

The general validity of the use of Eq. 1 is demonstrated by the good plateauing of total delivery values occurring for the later time periods for all the drug forms as shown in Table IV.

Careful minor reassignment of half-lives of the individuals would prevent the slight apparent fall off in total recovery tabulated for 24 hours; but this violates the calculation criterion set in the beginning of this report—namely, that the fast-release form of aspirin would be used as the sole criterion against which to judge the delayed forms.

As first pointed out by Wiegand and Taylor (20), the *in vitro* release of many sustained preparations is exponential and usually displays first-order kinetics. This is clearly seen in Fig. 1 for the dosages used in this study and was found for all preparations tested (2). It is surprising that no induction period appears to exist, since some initial limited destruction of the film might reasonably be expected. Any major cracking of the film is evidenced by rapid initial release, *i.e.*, a curve showing two release rates with time.

Plots of the cumulative *in vivo* release by both zero and first order gave somewhat better fits for the first order as shown in Fig. 2.

The literature contains frequent reference (21)

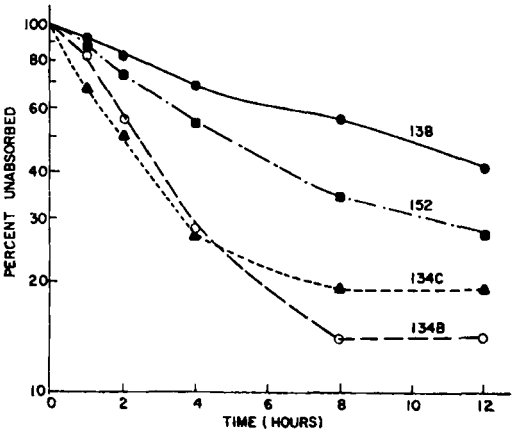


Fig. 2.—*In vivo* absorption characteristics of test tablets.

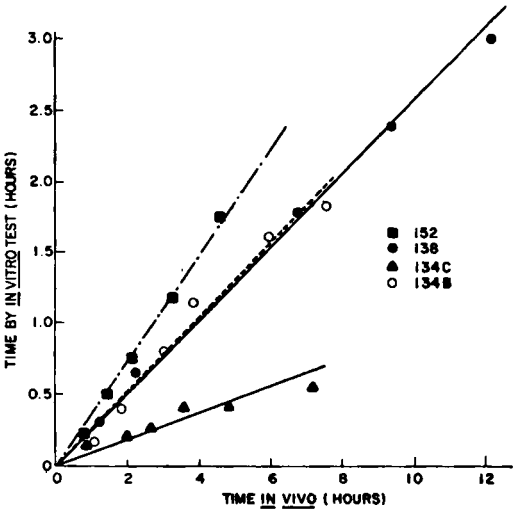


Fig. 3.—Correlation between *in vivo* absorption and *in vitro* release rates for corresponding fractions of total salicylate considered.

to the fact that no *in vitro* test can *per se* directly predict precise *in vivo* release. It was therefore of interest to determine for this study the extent to which a correlation might exist. Figure 3 was constructed by taking corresponding times for *in vivo* and *in vitro* release of the same fraction of the total salicylate used. Since both the *in vitro* test and the *in vivo* release could be represented by first-order plots, the lines of the correlation should be expected to be linear. This was so. It is immediately apparent that two lines are coincident, a third is close, and the fourth is somewhat divergent. The grouping of upper three lines represent the coatings of 75 to 100% ethylcellulose of various thicknesses, and there is no obvious correlation existing within this ranking. Probably the best average of the three would be most valid. The *in vivo* release between four individuals is so different that a larger panel would probably facilitate more rigid correlation. The obviously divergent line is for the 25% ethylcellulose.

As may be seen from a comparison of Figs. 1 and 2, the reason for this duality of correlation lines lies in the rapid *in vitro* release from the 25% ethyl-75% methylcellulose film which was not paralleled *in vivo*. Thus, it is apparent that *in vitro* testing may be used in this case to choose modifications of release rate patterns from high content ethylcellulose films by one correlation line. However, it is inferred that intermediate ratios of ethyl to methylcellulose between the limits studied here would display intermediate release rate correlations. Considering the relative similarities of these two cellulose derivatives, this is a striking confirmation of the earlier literature that *in vitro* data alone cannot predict *in vivo* performance.

One other observation should be noted. Since the integrated plasma levels may be considered a measure of the completeness of delivery of a drug, these levels clearly demonstrate that there is a price for delayed action—namely, incomplete recovery. In Table IV, it will be noted that for the fastest acting preparations, 134B and 134C, the release of salicylate to the plasma essentially ends at 8 hours, and that about 15 and 20%, respectively, are not apparently available for release. In the slowest releasing preparation, 138, all subjects show uptake to the 12-hour period. It is suggested, since so much aspirin is still unaccounted for, that this may signal the end of the period of ready biologic adsorption.

Thus, for release to occur over an 8-hour period, there is some loss in yield to the plasma. This loss increases with the slowing of the release rate.

It is then evident that integrated plasma levels can serve as a very convenient method for the study of drug release characteristics, both for the establishment of rates of absorption and for completeness of recovery.

As a result of the incomplete plasma recovery from delayed release and the long salicylate half-life in plasma, at no point does the sustained form provide superior plasma levels to those obtained from a fast-releasing tablet. However, where the elimination rate has been accelerated, as for arthritics under salicylate therapy (6), it is apparent that prolonged levels may be attained by suitable choice of dosage release rate.

SUMMARY

For aspirin coated with films of ethylcellulose-methylcellulose plasticized with glycerol, *in vivo-in vitro* correlations of release rates have been made. High ratios of ethyl to methylcellulose would appear to have one correlation curve. Variations in ratio to higher methylcellulose content are reflected in individual divergent correlations.

In vitro release rates from the film coatings used obeyed first-order kinetics.

The plasma absorption rate in this study would appear to closely obey first-order kinetics. The slower the sustained release, the less complete is the absorption recovery of the drug, probably due to incomplete release in all preparations and insufficient time for biologic absorption in the slower releasing preparations.

REFERENCES

(1) Wurster, D. E., This Journal, 49, 82(1960).
(2) Coletta, V., and Rubin, H., This Journal, 53, 953 (1964).
(3) Kuna, S., *Arch. Intern. Pharmacodyn.*, to be published.
(4) Blythe, R. H., Grass, G. M., and Mac Donnell, D. R., *Am. J. Pharm.*, 131, 206(1959).
(5) Nelson, E., *Clin. Pharmacol. Therap.*, 4, 283(1963).
(6) Wood, J. H., unpublished work.
(7) Levy, G., and Sahli, B. A., This Journal, 51, 58 (1962).
(8) Wiegand, R. G., Buddenhagen, J. D., and Endicott, C. J., *ibid.*, 52, 268(1963).
(9) Cotty, V. F., Zurzola, F., Beesley, T., and Rodgers, A., to be published.
(10) Wood, J. H., Lieberman, S. V., *et al.*, unpublished work.
(11) Brodie, B. B., Udenfriend, S., and Coburn, A. F., *J. Pharmacol. Exptl. Therap.*, 80, 114(1944).
(12) Routh, J. I., "Standard Methods of Clinical Chemistry," Vol. 3, Reiner, M., ed., Academic Press, New York, N. Y., 1959, p. 200.
(13) Truitt, E. B., Jr., and Morgan, A. M., *Arch. Intern. Pharmacodyn.*, 135, 105(1962).
(14) Wagner, J. G., and Nelson, E., This Journal, 52, 610 (1963).
(15) Leonards, J. R., *Proc. Soc. Exptl. Biol. Med.*, 110, 304 (1962).
(16) Leonards, J. R., *Clin. Pharmacol. Therap.*, 4, 476 (1963).
(17) Smith, P. K., *Ann. N. Y. Acad. Sci.*, 86, 38(1960).
(18) Schacter, D., and Manis, J. G., *J. Clin. Invest.*, 37, 800 (1958).
(19) Wagner, J. G., Carpenter, O. S., and Collins, E. J., *J. Pharmacol. Exptl. Therap.*, 129, 101(1960).
(20) Wiegand, R. G., and Taylor, J. D., *Drug Std.*, 27, 165(1959); *ibid.*, 28, 31(1960).
(21) Lazarus, J., and Cooper, J., *J. Pharm. Pharmacol.*, 11, 257(1959).