1942

Tryptophane Metabolism X. The Effect Of Feeding 1(-)-, Dl-, And D(+)-Tryptophane, D(-)And Dl-(β-3-Indolelactic Acid,(β-3-Indolepyruvic Acid, And L(-)Kynurenine Upon The Storage Of Liver Glycogen And The Urinary Output Of Kynurenic Acid, Kynurenine, And Total Acetone Bodies

Raymond Borchers
State University of Iowa

Clarence P. Berg
State University of Iowa

Newton E. Whitman
State University of Iowa

J. Biol. Chem. 1942 145: 657-666.

TRYPTOPHANE METABOLISM

X. THE EFFECT OF FEEDING *l*(−)-, *dl*-, AND *d*(+)-TRYPTOPHANE, *d*(−)- AND *dl*-β-3-INDOLELACTIC ACID, β-3-INDOLEPYRUVIC ACID, AND *l*(−)-KYNURENINE UPON THE STORAGE OF LIVER GLYCOGEN AND THE URINARY OUTPUT OF KYNURENIC ACID, KYNURENINE, AND TOTAL ACETONE BODIES*

BY RAYMOND BORCHERS, CLARENCE P. BERG, AND NEWTON E. WHITMAN

(From the Biochemical Laboratory, State University of Iowa, Iowa City)

(Received for publication, August 6, 1942)

The amount of tryptophane required for maintenance and growth is small (2). When an excess is supplied to some animals, kynurenic acid is excreted (3) and, under certain conditions, kynurenine also (4). Although these are quantitatively the most important of the known metabolic products of tryptophane, the amounts excreted in extensive tests in the dog and rabbit have usually accounted for less than half, more often for less than a third, of the tryptophane administered (4–6).[1]

Whether tryptophane is glycogenic or ketogenic is not clear. A number of years ago Dakin (7) observed that the injection of 14.5 gm. of *l*(−)-tryptophane into the phlorhizinized dog yielded 2.7 gm. of extra glucose, 3 gm. of kynurenic acid, and a precipitate with mercuric sulfate equivalent to about 3 gm. of tryptophane. He considered the yield of glucose too small to warrant concluding that it was formed from tryptophane. Perfusion of a surviving liver with 1.5 gm. of *l*(−)-tryptophane yielded no acetoacetic acid or acetone.

The studies recorded in this communication were directed toward determining whether a change could be shown in acetone body output upon feeding tryptophane to the fasted, but otherwise normal, rat, or to the rat fed sodium butyrate, and whether such feeding would affect the storage of liver glycogen. We were also interested in determining how the *dl* and

* The experimental data in this paper are taken from a dissertation submitted by Raymond Borchers in May, 1942, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry in the Graduate College of the State University of Iowa. A preliminary report has been published (1).

Some of the procedures employed are based on data from a thesis submitted by Newton E. Whitman for the degree of Master of Science in August, 1939.

The assistance afforded by Grant 311 of the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, is gratefully acknowledged.

[1] Berg, C. P., and Rowe, V. K., unpublished data.

d(+) forms of tryptophane, indolepyruvic, *dl*- and *d*(−)-indolelactic acids, and *l*(−)-kynurenine would compare with *l*(−)-tryptophane in these respects and in their effect upon the excretion of kynurenine and kynurenic acid in this species.

EXPERIMENTAL

The compounds tested were prepared by methods previously employed in this laboratory. Total nitrogen was determined by a semimicro-

TABLE I

Physical Properties of Compounds Studied

Compound	Melting point		Total nitrogen		$[\alpha]_D^{25}$ found	$[\alpha]_D^{20}$ recorded
	Found (uncorrected)	Recorded	Found	Calculated		
	°C.	°C.	*per cent*	*per cent*	*degrees*	*degrees*
l(−)-Tryptophane (8)*	274–281	277 (8) 281–282 (9)	13.4–13.7	13.72	−32.0 to −33.5	−33.0 to −33.6 (10)
dl-Tryptophane (11)	280	282–283 (12)	13.6	13.72	0.0	
d(+)-Tryptophane (9)	280	281–282 (9)	13.6	13.72	+32.1	+32.45 (9)
dl-Indolelactic acid (12)	143	145 (12)	6.7	6.83	0.0	
d(−)-Indolelactic acid (12)	99	100–101 (12)	6.7	6.83	−5.3	−5.36 (12)
β-3-Indolepyruvic acid (13, 14)	205	211 (12)	6.9	6.89		
l(+)-Kynurenine sulfate† (4)‡	175 185	180 (4)‡	7.8 -8.1	8.38	+10.1§	+10.7 +10.03 (4)‡

* The references which follow the names of compounds are to methods of preparation.

† In the free form, kynurenine is levorotatory ((4) and foot-note 1).

‡ See foot-note 1.

§ The concentration was 1.0 gm. per 100 cc. of solution in water; in all other instances the concentrations were 0.5 gm. per 100 cc. of aqueous solution.

Kjeldahl procedure. Optical rotations were read in a Schmidt-Haensch polariscope with an electric sodium lamp. Pertinent analytical data and comparisons with the literature are summarized in Table I.

Kynurenic acid, which is produced from several of these compounds, separates from the urine upon strong acidification. Kynurenine, tryptophane, and indole derivatives form sparingly soluble complexes with the mercuric sulfate reagent used in the Van Slyke procedure for determining total acetone bodies (16). Hence the following routines were devised to

determine kynurenic acid, "kynurenine," and total acetone bodies in each 24 hour sample of urine.

Kynurenic acid was precipitated by acidifying the urine to Congo red with sulfuric acid. After 24 hours in the refrigerator the precipitate was separated by centrifugation and washed by suspending it in 10 cc. of 5 per cent (by volume) sulfuric acid and recentrifuging. The supernatant fluid and washings were decanted into a 100 cc. volumetric flask. The kynurenic acid was redissolved, reprecipitated, and washed with water-saturated butyl alcohol to remove extraneous indole derivatives, essentially as directed elsewhere (6). The supernatant urine and washings were diluted to volume with water and reserved for estimating "kynurenine" and total acetone bodies.

"*Kynurenine*" refers collectively to all substances precipitable as the mercuric sulfate complex from urines previously freed of kynurenic acid, acetone, and acetoacetic acid; for convenience in making comparisons, the precipitates were calculated as kynurenine. For the determination, 20 cc. of the reserved supernatant urine and washings were mixed with 1 cc. of 50 per cent (by volume) sulfuric acid and boiled to expel the preformed acetone and the acetone produced by the decomposition of the acetoacetic acid. The solution was cooled, adjusted to 10 cc., and mixed with 20 cc. of 10 per cent mercuric sulfate in 5 per cent (by volume) sulfuric acid solution. After 48 hours, the precipitate which formed was filtered off on tared, sintered glass crucibles, washed with 10 cc. of 5 per cent sulfuric acid, then with 10 cc. of water, dried at 110°, and weighed. Similar precipitation of known weights of kynurenine with mercuric sulfate indicated that 1 mg. of the complex was equivalent to approximately 0.23 mg. of kynurenine. This factor was used in calculating "kynurenine."

Total acetone bodies were determined on 50 cc. of the reserved supernatant urine and washings, clarified as directed by Van Slyke (16) and made up to a volume of 250 cc. for filtration. 25 cc. of the filtrate were mixed, in a 500 cc. distilling flask, with 5 cc. of 17 N sulfuric acid and 100 cc. of water. The distilling flask was connected, through ground glass joints, to a condenser and a delivery tube extending nearly to the bottom of a 500 cc. Erlenmeyer flask which contained a mixture of 5 cc. of 17 N sulfuric acid and 17.5 cc. of 10 per cent mercuric sulfate in 4 N sulfuric acid solution. Preformed acetone and acetone produced by the decomposition of acetoacetic acid were distilled over until the volume of the solution in the distilling flask had been reduced by half. The residue was then cooled, 17.5 cc. of the 10 per cent mercuric sulfate reagent were added, and the mixture was set aside for 48 hours to allow the "kynurenine" to precipitate. The precipitate was filtered off and washed with 5 to 10 cc. of water. The filtrate and washings, which still contained β-hydroxybutyric acid, were

added to the distillate collected previously. Total acetone bodies were determined on the mixture by heating it to boiling, adding 5 cc. of 5 per cent potassium dichromate solution, and refluxing for 1½ hours to oxidize the β-hydroxybutyric acid to acetone and to precipitate the acetone as the mercuric sulfate complex. Total acetone bodies were computed with the usual assumptions that 75 per cent was excreted as β-hydroxybutyric acid and that, upon oxidation, this product yielded 75 per cent of the acetone to which it was equivalent (16).

Analysis of urines containing acetone bodies alone gave essentially the same results with the modified procedure as with the usual routine. Addition of kynurenic acid and tryptophane, indolelactic acid, indolepyruvic acid, or kynurenine to such urines showed no appreciable interference.

The female rats used in the acetone body studies weighed 160 to 190 gm. each. They were housed in individual metabolism cages allowing collection of urine under oil and were fasted for 24 hours preceding each test period. Regenerated cellulose and water were available continuously. The compound tested was fed by stomach tube as a suspension in gum tragacanth, in doses of 2 cc. every 12 hours. Each dose contained 0.3 gm. of tryptophane (or its molecular equivalent of whatever other substance was tested), 0.0267 gm. of gum tragacanth, and 0.3 gm. of sodium butyrate or its equivalent of sodium chloride. The test material was dissolved in water containing sodium hydroxide equivalent to the ultimate sodium butyrate or sodium chloride content required. The powdered gum tragacanth was stirred in, and ample time was allowed for the mixture to become homogeneous. Butyric acid or hydrochloric acid exactly equivalent to the sodium hydroxide was then added, followed by enough water to dilute to appropriate volume. This yielded a smooth, permanent suspension which passed readily through the catheter used as a stomach tube.

Some of the compounds were fed for 3 days, some for only 2. Kynurenine was available as the sulfate; when this was used, enough additional sodium hydroxide was added to effect the liberation of the kynurenine. Control animals received suspensions prepared in the same way and containing equal concentrations of all of the components except the test material. The fasting was continued for 36 hours after the final feeding. The cages were rinsed and the urine was drawn off every 24 hours. Usually two tests were made on each animal. In the first, half of the rats served as the experimental subjects, the rest as controls; in the second, the regimens were reversed. When the same rats were used in successive tests, periods of at least 10 days on a stock diet of Purina dog chow intervened.

The experimental data are presented in condensed form in Table II. Equivalent weights of *l*(−)-tryptophane and *l*(−)-kynurenine produced approximately equal outputs of kynurenic acid. Indolepyruvic acid

yielde ı less, *dl*-tryptophane only a small amount, and the indolelactic acids and *d*(+)-tryptophane little or none.

TABLE II

Average Total Urinary Output of Kynurenic Acid, "Kynurenine," and Acetone Bodies in Female Rats Fed Tryptophane and Related Compounds, with or without Sodium Butyrate, after Preliminary 24 Hour Fast

	Days of feeding†	Kynurenic acid	"Kynurenine"	Acetone bodies calculated as acetone	Substances fed in gum tragacanth suspension‡
		mg.	*mg.*	*gm. per sq.m.*	
8	3	106	111	0.58	*l*(−)-Tryptophane + NaCl
8	3	14	27	0.35	NaCl
8	3	49	473	0.45	*dl*-Tryptophane + NaCl
	3	35	43	0.34	NaCl
	3	49	660	0.62	*d*(+)-Tryptophane + NaCl
4	3	76	87	0.18	NaCl
2	3			0.10	Ammonium chloride
2	3			0.13	None
24	3	166	175	1.30	*l*(−)-Tryptophane + Na butyrate
24	3	37	34	4.35	Na butyrate
8	3	39	297§	0.92	*dl*-Tryptophane + Na butyrate
8	3	35	41	4.96	Na butyrate
6	3	29	589§	1.36	*d*(+)-Tryptophane + Na butyrate
6	3	31	87	4.71	Na butyrate
10	3			2.81	NH_4Cl + Na butyrate
10	3			4.80	Na butyrate
3	2	22	724§	2.29	*dl*-Indolelactic acid + Na butyrate
3	2	24	81	4.01	Na butyrate
4	2	26	797§	2.26	*d*(−)-Indolelactic acid + Na butyrate
4	2	88	262	0.91	Indolepyruvic acid + Na butyrate
4	2	104	113	0.68	*l*(−)-Kynurenine + Na butyrate
12	2	19	49	3.98	Na butyrate

* The rats weighed 160 to 190 gm. each. Meeh's formula was used in calculating surface area.

† An extra day of fasting was allowed to insure collection of all of the urine voided during the metabolism period.

‡ Per day each rat received 0.053 gm. of gum tragacanth and 0.6 gm. of sodium butyrate or its equivalent of sodium chloride. In addition, each experimental animal was fed 0.6 gm. of tryptophane or its equivalent of other test material.

§ These precipitates responded to the glyoxylic acid test. See the text.

Since the composition of the "kynurenine" precipitates was obscure, these were analyzed for total nitrogen. Estimation of amino nitrogen failed to give reproducible results and was abandoned, as a differential test, in favor of the Shaw and McFarlane quantitative adaptation of the glyoxylic acid

color reaction (17) for tryptophane. Mercuric sulfate precipitates obtained from pure solutions of tryptophane, kynurenine, and indolelactic acid showed nitrogen contents of 2.79, 2.70, and 1.23 per cent, respectively. Similar precipitates isolated after *l*(−)-, *dl*-, and *d*(+)-tryptophane and *l*(−)-kynurenine were fed contained 2.4 to 2.9 per cent of nitrogen; those obtained after indolepyruvic acid and *d*(−)-indolelactic acid were fed contained 1.9 and 1.4 per cent, respectively. Previous tests of the Shaw and McFarlane procedure had shown that kynurenine, indolepyruvic acid, and indole do not respond typically, but that skatole and indolepropionic acid produce colors qualitatively similar to the color formed with tryptophane.[2] The latter was found to be true also of indolelactic acid. Application of the method to pooled mercuric sulfate precipitates formed in the urines voided after *l*(−)-tryptophane, *l*(−)-kynurenine, or indolepyruvic acid was fed yielded no color; the "kynurenine" excreted after *dl*-tryptophane feeding produced a color equivalent to a content of 10 per cent of tryptophane; after *d*(+)-tryptophane feeding, to 33 per cent. The color produced by similar precipitates after *d*(−)-indolelactic acid feeding was compared with that developed in an indolelactic acid standard; apparently 88 per cent of the "kynurenine" was in this form. Unfortunately similar quantitative tests were not made after *dl*-indolelactic acid was fed. The colorimetric assays and the nitrogen data suggest that the substance precipitated from the urines of rats fed *l*(−)-tryptophane was chiefly kynurenine, that some kynurenine was probably produced from indolepyruvic acid, but that none was formed from *d*(−)-indolelactic acid. Subtraction of kynurenine equivalent to the estimated tryptophane content from the total "kynurenine" precipitated after *dl*- and *d*(+)-tryptophane were fed leaves a larger balance of "kynurenine" in each instance than was obtained after *l*(−)-tryptophane was fed. The precipitates probably contained *d*(+)-kynurenine which is formed from *d*(+)-tryptophane (15),[1] but cannot be converted into kynurenic acid (18), and hence must either be excreted as such or undergo further metabolism by some other route. Products resulting from intestinal putrefaction and subsequent detoxication may have been present, but probably in no greater amount than after *l*(−)-tryptophane was fed.

The average total acetone body excretion was slightly greater in the fasted rats fed tryptophane and sodium chloride than in their controls, but in rats fed sodium butyrate to augment the ketonuria, supplementation with tryptophane decreased the output markedly. Analysis showed that the acetone body precipitates from the urines of rats fed tryptophane contained an appreciable amount of nitrogen (an average of 0.084 per cent, as

[2] Buck. D. M. and Berg, C. P., unpublished data.

compared with 0.003 per cent for controls). Coprecipitation of nitrogenous products was therefore probably at least partly responsible for the slightly greater average weight of the mercuric sulfate-acetone complex produced in the urines of fasted rats fed tryptophane and sodium chloride than in the urines of their controls. On the other hand, the actual decrease in output of acetone bodies in the rats fed the compounds with sodium butyrate was probably slightly larger than the tabulated data indicate.

TABLE III

Deposition of Liver Glycogen in Rats Fed Tryptophane and Related Compounds after 48 Hour Fast

Substance fed*	Additional fasting or feeding period	No. of rats†	Minimum	Maximum	Average
	hrs.		*per cent*	*per cent*	*per cent*
l(−)-Tryptophane + NaCl	4	6	0.24	0.46	0.36
	8	6	0.11	0.67	0.30
	12	6	0.15	0.52	0.33
	20	4	0.20	0.46	0.35
dl-Tryptophane + NaCl	4	4	0.11	0.71	0.30
	12	4	0.11	0.45	0.23
d(+)-Tryptophane + NaCl	8	4	0.11	0.40	0.26
dl-Indolelactic acid + NaCl	8	4	0.15	0.74	0.41
Indolepyruvic acid + NaCl	8	4	0.18	0.60	0.36
l(−)-Kynurenine + NaCl	8	4	0.03	0.11	0.07
NaCl	4	6	0.12	0.56	0.26
	8	8	0.11	0.97	0.49
	12	8	0.37	0.77	0.49
	20	2	0.12	0.42	0.27

* Each rat received 0.026 gm. of gum tragacanth and 0.16 gm. of NaCl every 4 hours; each experimental animal was fed simultaneously also 0.3 gm. of tryptophane or its equivalent of other test material.

† The rats weighed 100 to 125 gm. each and were evenly divided as to sex.

Edson has observed that ammonium chloride stimulates the production of acetoacetic acid in liver slices from the well fed rat, but is without effect on liver slices from the fasted animal (19). It is of interest to note that administration of ammonium chloride to our rats in amounts molecularly equivalent to the tryptophane and other test substances had little effect in the animals which were simply fasted, but reduced appreciably the total acetone body output in the rats fed sodium butyrate. Correlation of this reduction with the greater reduction observed when tryptophane, indolepyruvic acid, or kynurenine was fed is complicated by uncertainty as to the relative rates of metabolism of these substances and the metabolic

paths followed. The lowering produced by the ingestion of indolepyruvic acid and of kynurenine was proportional to that produced by tryptophane in the same period; the reduction by the indolelactic acids was much smaller. The data obtained by Edson seem to show that *l*(−)-tryptophane lowers acetoacetic acid production in liver slices from fasted rats.

Substances which decrease the output of acetone bodies usually produce glycogen. To determine whether this was true of indolepyruvic acid, *l*(−)-kynurenine, and the isomers of tryptophane, these substances and *dl*-indolelactic acid were fed to rats subsequently examined for liver glycogen. Equal numbers of males and females, 100 to 125 gm. in weight, were removed from a stock diet of Purina dog chow and fasted for 48 hours before the compounds were fed. During the fasting and feeding periods they had continuous access to water and regenerated cellulose. Each substance tested was fed in a gum tragacanth suspension prepared with sodium hydroxide and hydrochloric acid as described in the acetone body studies. The experimental animals received 0.3 gm. of tryptophane or its equivalent every 4 hours; the controls were given all of the components of the suspension except the substance under investigation.

Groups of animals were killed for analysis by a blow on the head 4, 8, 12, or 20 hours after the initial feeding or the 48 hour fast. Their livers were removed at once and analyzed in their entirety for glycogen by the Good, Kramer, and Somogyi procedure (20); the glucose liberated by hydrolysis was determined by the Somogyi method (21).

The data are summarized in Table III. They do not favor the assumption that *l*(−)-, *dl*-, or *d*(+)-tryptophane, *l*(−)-kynurenine, *dl*-indolelactic acid, or indolepyruvic acid was glycogenic under the conditions employed. At present we have no explanation for this apparent discrepancy with data on other substances which similarly lower the acetone body output, but are also glycogenic.

SUMMARY

In rats fasted for 24 hours, then fed 0.6 gm. of *l*(−)-, *dl*-, or *d*(+)-tryptophane per day, the excretion of total acetone bodies appeared to be slightly greater than in control animals, but the small difference was probably due to contamination of the acetone-mercuric sulfate complex with traces of other compounds which contained nitrogen. In rats fed sodium butyrate to increase the ketonuria, similar administration of *l*(−)-, *dl*-, or *d*(+)-tryptophane reduced the acetone body output markedly. Proportionate decreases were produced by both *l*(−)-kynurenine and indolepyruvic acid, but the *d*(−)- and *dl*-indolelactic acids were much less effective. The feeding of molecularly equivalent amounts of ammonium chloride lowered the excretion of acetone bodies enough in the rats fed sodium butyrate to

suggest that production of ammonia and formation of urea from the other compounds tested may have been partly responsible for their effect in decreasing the acetone body output. However, since the decrease produced by indolepyruvic acid, which contains only indole nitrogen, was approximately equivalent to that induced by tryptophane, which contains both indole and amino nitrogen, and to the decrease induced by kynurenine, which contains two amino groups, other factors must also have been involved.

In rats fasted for 48 hours, then fed 0.3 gm. of *l*(−)-, *dl*-, or *d*(+)-tryptophane (or its equivalent of *dl*-indolelactic acid, indolepyruvic acid, or *l*(−)-kynurenine) at 4 hour intervals for periods of 4, 8, 12, or 20 hours, no evidence for storage of liver glycogen was obtained. This would seem to indicate that the effect of these substances upon the acetone body output in the rats fed sodium butyrate cannot be ascribed to their conversion into glucose or a glycogenic intermediate, unless acetone body formation or excretion can be affected by amounts too small to induce glycogen storage.

The yields of kynurenic acid and kynurenine from *l*(−)-tryptophane were proportionately the same as from *l*(−)-kynurenine, but did not account for more than a fourth of the total ingested. Proportionate depressions in acetone body output indicate that the metabolism of *l*(−)-kynurenine is not limited to its conversion to kynurenic acid; quite likely *l*(−)-kynurenine may be an intermediate also in the metabolism of the large portion of exogenous tryptophane which is not eliminated as kynurenic acid. Little kynurenic acid was produced from *dl*-tryptophane and apparently none from *d*(+)-tryptophane; the mercuric sulfate precipitates obtained after these were fed contained appreciable amounts of tryptophane, but probably consisted chiefly of *d*(+)-kynurenine. A little over half of the *d*(−)-indolelactic acid fed was excreted; the accompanying decrease in acetone body output suggests that the rest was metabolized, but the data do not warrant concluding that either kynurenic acid or appreciable kynurenine was produced. Indolepyruvic acid was converted to kynurenic acid, apparently more readily than previously noted in the rabbit, though less readily than *l*(−)-tryptophane; the high nitrogen content of the mercuric sulfate precipitate and its negative response to the test for tryptophane suggest that it was composed in part of indolepyruvic acid and in part of kynurenine.

BIBLIOGRAPHY

1. Borchers, R., and Berg, C. P., *Federation Proc.*, **1**, pt. 2, 102 (1942).
2. Rose, W. C., *Science*, **86**, 298 (1937).
3. Gordon, W. G., Kaufman, R. E., and Jackson, R. W., *J. Biol. Chem.*, **113**, 125 (1936).

4. Kotake, Y., and Iwao, J., *Z. physiol. Chem.*, **195,** 139 (1931).
5. Correll, J. T., Berg, C. P., and Cowan, D. W., *J. Biol. Chem.*, **123,** 151 (1938).
6. Berg, C. P., *J. Biol. Chem.*, **91,** 513 (1931).
7. Dakin, H. D., *J. Biol. Chem.*, **14,** 321 (1913).
8. Cox, G. J., and King, H., in Clarke, H. T., *et al.*, Organic syntheses, New York, **10,** 100 (1930).
9. Berg, C. P., *J. Biol. Chem.*, **100,** 79 (1933).
10. Berg, C. P., and Potgieter, M., *J. Biol. Chem.*, **94,** 661 (1931–32).
11. du Vigneaud, V., and Sealock, R. R., *J. Biol. Chem.*, **96,** 511 (1932).
12. Bauguess, L. C., and Berg, C. P., *J. Biol. Chem.*, **104,** 675 (1934).
13. Boyd, W. J., and Robson, W., *Biochem. J.*, **29,** 555 (1935).
14. Berg, C. P., Rose, W. C., and Marvel, C. S., *J. Biol. Chem.*, **85,** 219 (1929–30).
15. Kotake, Y., and Ito, N., *J. Biochem.*, Japan, **25,** 71 (1937).
16. Van Slyke, D. D., *J. Biol. Chem.*, **32,** 455 (1917).
17. Shaw, J. L. D., and McFarlane, W. D., *J. Biol. Chem.*, **132,** 387 (1940).
18. Kotake, Y., and Ito, N., *J. Biochem.*, Japan, **26,** 161 (1937).
19. Edson, N. L., *Biochem. J.*, **29,** 2082, 2498 (1935).
20. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, **100,** 485 (1933).
21. Somogyi, M., *J. Biol. Chem.*, **70,** 599 (1926).