TABLE I.
Perfusion Rates Through Portal Circulation of Isolated Livers of Rats and Guinea Pigs after Intraportal Administration of Salmine Sulfate at Various Dose Levels.

Animal	No. of animals	Dose of salmine sulfate mg/100 g	Perfusion flow rate—cc/min Perfusion pressure: 2 cm	4 cm	8 cm	16 cm	32 cm	42 cm
Rat	4	Control	1	5	11	25	50	50
	3	10	0	0	1	4	26	39
	2	20	0	0	0	1	15	27
	2	50	0	0	0	0	0	0
Guinea Pig	4	Control	4	8	20	34	51	62
	3	10	0	1	9	17	45	70
	2	20	0	1	5	17	33	60
	2	50	0	0	4	17	39	51

considered in conjunction with our previous observations, they are consistent with the hypothesis that embolus formation plays a role in the toxicity of intravenously administered salmine sulfate. No studies have been made to evaluate the importance of direct histotoxic effects.

Conclusion. 1. Vascular occlusion, as shown by perfusion studies, occurred in the livers of rats and guinea pigs receiving lethal doses of salmine sulfate. 2. This effect was of greater magnitude in the rat than in the guinea pig.

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Metabolism of Glycolic and Glyoxylic Acids.*

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In the metabolism of both glycine and fatty acids it has been proposed that 2 carbon acids are formed which in turn take part in the formation of glucose or the acetone bodies. The purpose of this communication is to present further information on the metabolism of 2 compounds, glycolic acid ($HOCH_2$—COOH) and glyoxylic acid (O:HC—COOH) either of which might be involved in the above transformations.

Methods. Three types of experiments were performed. (1) Liver glycogen formation studies were done by administering the test substances by stomach tube to adult male rats which had been previously fasted for 48 hours. Glycogen was determined by the method of Good, Kramer and Somogyi.[1] (2) Urine acetone body excretion studies were made by administering the test substance by stomach tube over a 4-day period to fasting adult male rats which previous to fasting had been maintained on a high fat-low protein diet. Daily urine collections were made from individual rats and acetone bodies were determined by the method of Van Slyke.[2] (3) Blood acetone bodies were studied in rats which were taken from the stock diet and immediately fed the test substances by stomach tube. The rats were anesthetized with amytal and bled from the large abdominal vessels and blood acetone bodies were determined by the method of Barnes and Wick.[3]

Results. Liver glycogen after administering either glycolic acid or sodium glycolate, the

* Aided by a grant from the Rockefeller Foundation.

1 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

2 Van Slyke, D. D., *J. Biol. Chem.*, 1917, **32**, 455.

3 Barnes, R. H., and Wick, A. N., *J. Biol. Chem.*, 1939, **131**, 413.

TABLE I.
Liver Glycogen at Various Time Intervals after Feeding Glycolic Acid to Fasting Male Rats.

Substance fed*	Time, hr	No. of rats	Body wt, g	Liver wt, g	Liver glycogen, %
		Experiment I.			
	0	2	97	3.61	0.23
1M glycolic acid	4	2	105	3.68	0.29
" " "	8	1	100	3.36	0.97
" " "	12	1	93	3.00	0.02
" " "	16	1	95	3.38	0.57
" " "	22	1	103	3.60	0.94
		Experiment II.			
	0	2	114	4.19	0.18
1M sodium glycolate	6	2	113	3.84	0.15
" " bicarbonate	6	2	113	3.95	0.09
" " glycolate	12	2	114	3.95	0.18
" " bicarbonate	12	2	114	4.23	0.51
" " glycolate	20	1	115	4.45	0.82
" " bicarbonate	20	1	112	4.13	0.39

*In Experiment I: Male rats were fasted for 48 hours and then fed by stomach tube 1 cc of 1M glycolic acid per square decimeter body surface. The rats were then killed at the stated intervals.

In Experiment II: Male rats were fasted for 48 hours and then fed by stomach tube 1 cc of 1M sodium salts of either carbonic acid or glycolic acid per square decimeter body surface. Four hours later they received by stomach tube 1 cc of 0.5 M sodium salts of the 2 acids respectively per square decimeter body surface.

Body surface was calculated by the formula of Carman and Mitchell.[12]

TABLE II.
Urine Acetone Bodies After Administering Glycolic Acid to Fasting Adult Male Rats.

Substance fed	No. of rats	Body wt g	Body surface sq cm	Urine acetone bodies per sq.dm. body surface per day (calculated as acetone) 1st mg	2nd mg	3rd mg	4th day mg
		Experiment III.*					
0.5M sodium bicarbonate	4	205	395	1.3	6.3	10.5	5.6
0.5M sodium glycolate	4	203	391	1.0	6.2	14.0	8.7
		Experiment IV.†					
0.75M sodium bicarbonate	5	242	441	2.5	8.2	15.3	12.3
0.75M sodium glycolate	5	241	438	4.8	4.9	19.7	10.2

*High fat-low protein diet containing 4% yeast was fed for 14 days before experimental fasting period was begun. 1.0 cc of either 0.5M sodium bicarbonate or sodium glycolate per sq.dm. body surface was fed by stomach tube twice daily.

†High fat-low protein diet containing the known required synthetic B vitamins, but without choline was fed for 10 days prior to the experimental fasting period. 1.0 cc of either 0.75M sodium bicarbonate or sodium glycolate per sq.dm. body surface was fed by stomach tube twice daily.

latter controlled with sodium bicarbonate, was followed for 20 hours. The results which are presented in Table I show that under the conditions of the experiment, glycolic acid is not converted into glycogen. Fed under similar conditions the same dose of glycine results in a marked liver glycogen increase. Therefore, the dose of glycolic acid employed here was adequate. A similar experiment was attempted with glyoxylic acid, but this compound was found to be too toxic in the amounts necessary for this study.

Upon administering glycolic acid urine acetone body excretion shows a negative response (Table II). If the glycolic acid had been ketogenic it would have been observed in these experiments for in the same concentration acetic acid is ketogenic.[4]

In Table III are presented data on the ketogenic activity of both glycolic and gly-

[12] Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1926, **76**, 380.

[4] MacKay, E. M., Barnes, R. H., Carne, H. O., and Wick, A. N., *J. Biol. Chem.*, 1940, **135**, 157.

TABLE III.
Blood Acetone Bodies After Administering Either Glycolic Acid or Glyoxylic Acid to Fed Adult Male Rats.*

Exp. No.	Substance fed	No. of rats	Body wt, g	Time, hr	Blood acetone bodies calculated as acetone mg%
		Glycolic Acid Experiments.			
5		3	211	0	0.11
5	1M sodium bicarbonate	3	216	6	0.00
5	1M sodium glycolate	3	208	6	0.00
5	1M sodium bicarbonate	3	208	12	1.00
5	1M sodium glycolate	3	208	12	0.35
6	Controls	4	233	†	0.71
6	0.5M glycolic acid	4	227	†	0.42
		Glyoxylic Acid Experiments.			
7		2	228	0	0.48‡
7	0.5M sodium bicarbonate	3	233	6	0.43
7	0.5M sodium glyoxylate	3	232	6	1.25
7	0.5M sodium bicarbonate	3	243	12	0.80
7	0.5M sodium glyoxylate	3	237	12	1.50
8	Controls	3	275	†	1.26
8	0.5M sodium glyoxylate	3	300	†	3.43

*In experiments 5 and 7, 1 cc of the test solution per square decimeter body surface was administered by stomach tube. After the stated time intervals the rats were killed and blood acetone body determinations were made. All experiments included in this table were performed on rats taken directly from a stock diet.

†In experiments 6 and 8, 5.0 cc of the test solutions were administered by stomach tube; 4 hours later a second dose of 3.0 cc was given. Three hours after the second dose the animals were killed and blood acetone body determinations were made.

‡Not included in this average was one rat having a fasting blood acetone body concentration of 2.30 mg per 100 cc. This single value was higher than that of any other animal in experiment 7.

oxylic acids in fed animals. Glycolic acid gave no evidence of acetone body formation while glyoxylic acid resulted in a rise in blood acetone bodies in every instance. Control determinations showed that glyoxylic acid *per se* did not react with the Deniges reagent.

Discussion. Both of these substances have been considered as intermediates in the metabolism of the amino acid glycine. For instance Griffith[5] has found that the retardation of growth that is observed when large quantities of benzoic acid are fed is overcome by feeding glycolic acid. Although it is now established that the administration of glycine causes the deposition of glycogen in the fasted animal,[6] Greenwald[7] was unable to increase the urinary glucose of a phlorhizinized dog with glycolic acid, and the present investigation shows a lack of glycogenic effect of the acid in the fasted rat.

These observations did not include a study of glycogen formation from glyoxylic acid, but this transformation would appear most unlikely as this substance was shown to be ketogenic. Furthermore MacKay (personal communication) has found that glycine administration under the same conditions as were employed in our experiments does not result in any rise in blood acetone bodies. It would appear that neither glycolic nor glyoxylic acids are glycogen formers and yet these two compounds are the usual "text-book" representations of the intermediates formed in the conversion of glycine to glucose.

The observation that acetic acid causes an increased production of acetoacetic acid in the perfused liver led Toenniessen and Brinkmann[8] to propose the hypothesis that acetic acid is split from fatty acids by β-oxidation and that this acetic acid then condenses with a similar molecule to form acetoacetic acid. MacKay and his coworkers have given additional evidence for an increased production of

[5] Griffith, W. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 216.

[6] MacKay, E. M., Wick, A. N., and Carne, H. O., *J. Biol. Chem.*, 1940, **132**, 613.

[7] Greenwald, I., *J. Biol. Chem.*, 1918, **35**, 461.

[8] Toenniessen, E., and Brinkman, E., *J. Physiol. Chem.*, 1938, **252**, 169.

acetone bodies in fasted rats upon feeding acetic acid.[4] By the use of heavy carbon labeling of acetic acid final proof of its conversion into the acetone bodies was established by Swendseid *et al.*[9] Although acetic acid is ketogenic in the fasting animal it has been found by MacKay (personal communication) and also in this laboratory that it will not form acetone bodies in the fed animal. MacKay, Wick and Barnum[10,11] in studying acetone body formation from the odd carbon-fatty acids, and short chain even numbered carbon fatty acids have found that if the chain contains 4 to 10 carbons, acetone bodies are formed even in the presence of normal liver glycogen. This gives ample proof that ketogenesis can, under certain conditions, go on in the fed animal. Furthermore, the fact that valeric acid forms acetone bodies under such conditions gives additional evidence that the acetone body formation takes place through a 2-carbon compound condensation.

There are 4 possible 2-carbon acids that may be formed by β-oxidation of a fatty acid. These are acetic, glycolic, glyoxylic, and oxalic acids. In order to fulfil the qualifications of the modified β-oxidation hypothesis, the 2-carbon acid must be capable of acetone body formation in the fed animal. Experiments which have been performed in this laboratory have shown that oxalic acid is too toxic for a study of acetone body formation. Neither acetic nor glycolic acids will form acetone bodies in the fed animal which leaves glyoxylic as the only possible intermediate of this type. It is possible that a preliminary reduction or some other reaction of the fatty acid prior to its cleavage might take place, and in such an event other intermediates might exist. However, as a working hypothesis it is proposed that in the catabolism of fatty acids glyoxylic is formed and under the proper conditions this compound is converted into the acetone bodies by the liver.

Summary. It has been shown that in the fasted rat glycolic acid does not form glycogen nor does it form acetone bodies in the fed or fasted rat. Glyoxylic acid causes the formation of acetone bodies in the fed rat. From this evidence it has been concluded that neither glycolic nor glyoxylic acids are intermediates in the transformation of glycine to glucose in the animal body.

9 Swendseid, M. E., Barnes, R. H., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, 1942, **142**, 47.

10 MacKay, E. M., Wick, A. N., and Barnum, C. P., *J. Biol. Chem.*, 1940, **135**, 183.

11 MacKay, E. M., Wick, A. N., and Barnum, C. P., *J. Biol. Chem.*, 1940, **136**, 503.

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Correlation of Extent of Tuberculosis with Amount of Polysaccharide in the Serum.*

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An intensive study has been made in recent years of tuberculous sera, with the objective of detecting the presence of antigen, antibody or any substance which may be characteristic of the disease. The primary approach has been based upon observation of the electrophoretic diagrams of sera by means of the Tiselius technic.

It was shown[1] that during the development of tuberculosis in the rabbit the beta globulin fraction increased in proportion to the extent of the disease. In the human being, on the other hand, the consistent change[1] was a progressive increase in the gamma globulin. Increases in alpha and beta globulin fractions

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1 Seibert, F. B., and Nelson, J. W., *Am. Rev. Tuberc.*, 1942, **47**, 66.