oxidized, this interference with streptomycin activity gradually decreases as the broth stands. What may be called the baseline of interference of the broth, which is reached after some 40 days of standing in one thioglycollate medium we have used, is similar to the interference caused by the same medium containing sodium glycollate instead of sodium thioglycollate.

It is interesting that the action of streptothricin is affected to a similar or perhaps even greater extent in thioglycollate broth. The minimum inhibiting concentration of a streptothricin preparation (320 units/mg) which was tested in thioglycollate broth No. 1 (7 days after preparation) was 2.65 units/ml as compared to 0.053 units/ml in 0.75% tryptone broth—a ratio of 50:1.

It perhaps should be pointed out that the concentration of sodium thioglycollate in the media tested was 0.5 mg per ml, while the concentrations said by Denkewalter, *et al.*[3] to cause no appreciable destruction of streptomycin ranged from 0.50 to 3.0 mg per ml. Hence remarkable interference with streptomycin activity may occur without destruction of the streptomycin. Since thioglycollates reduce the oxidation reduction potential of a culture medium, could this interference indicate that streptomycin interferes more with aerobic than anaerobic metabolism?

Summary. Under standard test conditions, raising the concentration of tryptone from 0.50% to 1.0%, in a medium containing only tryptone and water, raised the minimum inhibiting concentration of streptomycin from *0.036* units/ml to *0.084* units/ml. The further addition of glucose to the medium increased the minimum inhibiting concentration to a lesser, but nevertheless, definite degree.

The minimum inhibiting concentration of streptomycin in an enriched broth containing sodium glycollate was about 10 times greater than that in 0.75% tryptone. The substitution of sodium thioglycollate for sodium glycollate further greatly increased the MIC. This additional interference by sodium thioglycollate was observed to decrease as the broth aged (and hence as the thioglycollate was oxidized). Since thioglycollate *per se* is said to cause no significant destruction of streptomycin, it is proposed that its interfering action may be due to its role in reducing the oxidation-reduction potential of the medium and that perhaps streptomycin interferes more with the aerobic than anaerobic metabolism of the test organism.

15282

Metabolism of Glycine by the Completely Isolated Mammalian Heart Investigated with Carboxyl-Labeled Glycine.*

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When acetate containing heavy carbon, C^{13}, in the carboxyl position is administered to the completely isolated, working, mammalian heart, the isotope appears in the respiratory CO_2 rapidly and in large amounts, indicating that the 2-carbon chain is readily broken and converted to CO_2, in amounts approximating 20-30% of the total CO_2 output of the heart.[1] In the present experiments, glycine labeled with an excess of C^{13} in the carboxyl carbon, was injected into the blood perfusing the

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[1] Lorber, V., Lifson, N., Wood, H. G., and Barcroft, J., *Am. J. Physiol.*, 1946, **145**, 557.

TABLE I.
Isotopic Content of Respiratory CO_2 Following Administration of Labeled Glycine to the Isolated Cat Heart.

Exp. No.	Mg of glycine given*	CO_2 collection period in min	mM of carbon collected as respiratory CO_2	% C^{13} in the respiratory CO_2†
1	50	15	0.38	1.07‡
		15	0.53	1.09
		15	0.65	1.10
		17	0.46	1.07
		15	**0.41**	—
		30	1.17	1.10
2	100	30	1.70	1.11‡
		30	1.68	1.07
		30	1.52	1.12
		27	0.70	1.13
3	125	30	1.46	1.08‡
		30	1.42	1.09
		30	1.31	1.12
		34	1.13	1.11

* The glycine was given at the beginning of the second collection period in each experiment.

† $\%\ C^{13} = \dfrac{\text{Moles } C^{13}}{\text{Moles } C^{12} + \text{Moles } C^{13}} \times 100$

‡ Control value prior to injection of glycine. Normal C^{13} content of C from mineral sources is 1.09%.

isolated cat heart in order to determine whether this compound, the amino derivative of acetic acid, is similarly metabolized by cardiac muscle.

Methods. The experiments were performed on cat hearts, of 6 to 7 g weight, isolated in a manner previously described.[2] Respiratory carbon dioxide was collected by passing the ventilating gas through absorption bottles containing carbonate-free sodium hydroxide. Total circulating blood volume was 50 to 60 cc.

Total respiratory carbon dioxide was determined by analysis of the absorption alkali by the Van Slyke manometric method. The C^{13} content of the carbon dioxide for each collection period was measured in the mass spectrometer.[3]

After isolation of the heart was completed, a preliminary control period of 15 to 30 minutes was permitted to elapse, during which time the respiratory carbon dioxide was collected. Following this period, 50 to 125 mg of labelled glycine, dissolved in 0.6 ml of Ringer solution, was injected into the blood, and the carbon dioxide collection was continued, fresh absorption bottles being introduced at 15 to 30 minute intervals. The glycine used was synthesized[4] with 7.59% C^{13} in the carboxyl position.

Results. The results are presented in Table I. A small increase in the % C^{13} in the respiratory CO_2 above the initial control values is noted in each experiment. The irregularities occurring in Exps. 1 and 2, *i.e.*, the low value found in the fourth collection period in 1, and the high value found in the control period in 2, cannot be accounted for, but difficulties in the operation of the mass spectrometer occurring at the time may have been in part responsible. Because of these irrational values, the figures are to be regarded with some reserve. Although if taken at face value the results indicate the breakdown of minute amounts of glycine (about 1 mg) by the heart, the important conclusion to be derived from the data is that the degradation of glycine, under the conditions of our experiments, does not serve as an appreciable source of energy for the heart muscle. This is contrary to the

[2] Lorber, V., *Am. Heart J.*, 1942, **23**, 37.

[3] Nier, A. O., *Rev. Scient. Instruments*, 1940, **2**, 212.

[4] Olsen, N. S., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, 1943, **148**, 611.

expectation that the high concentrations of glycine maintained, and the low blood carbohydrate values obtaining after 1½ to 2 hours (glucose, 14 mg% at the close of Exp. 2) in the usual experiment of this type, should have favored the metabolism of glycine. Again, with the present results taken at face value, the glycine may be calculated to have yielded only around 0.5% of the total CO_2 output of the heart (on the assumption that both carbons of the molecule have gone to CO_2) as compared with values of 20-30% from acetate,[1] administered in concentrations of the same order of magnitude as those of glycine.

Summary. Glycine, labeled with heavy carbon in the carboxyl position, was administered to the completely isolated, working, cat heart. Isotopic analysis of the collected respiratory CO_2 indicated that if splitting of the 2-carbon chain of glycine with conversion of the carbon to CO_2 was accomplished by the heart muscle, it occurred only to a slight extent.

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15283 P

Blood and Tissue Chemical Studies in Fowl.*

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Normal blood and tissue levels of the usual organic constituents with especial reference to total cholesterol and cholesterol esters and other lipids, were determined as a basis for projected fat metabolism studies. Domestic fowl was the chosen experimental animal because of the known high normal cholesterol production, especially of certified 260 to 320 eggs per year R.O.P. sired hens. Laying hens, after the sixth month, have been found to have a subintimal atherosclerosis of the aorta and coronary arteries, quite similar microscopically to the human type, in half the specimens examined by Dauber.[1] As the age increases, the frequency of gross lesions has been noted to increase.

We have sacrificed 26 barred rocks fed on a standard growing mash (Purina) at ages from 11 weeks through 22 weeks and 26 hens of various standard breeds that had been on a standard laying mash, (Purina). Blood was collected from the severed neck vessels; part was oxalated and part was allowed to clot and the serum expressed. The aorta, heart, and in later studies, about 0.25 g of liver were removed. The gross adipose tissue was dissected off and approximately 0.25 g of cleaned aorta and lean heart muscle and liver were weighed accurately, minced carefully, extracted for 12 hours with 3 to 1 parts of alcohol and ether.

The blood serum was analyzed by Folin's microchemical methods for glucose;[2] non protein nitrogen;[3] and Leiboff and Kahn's method for urea nitrogen;[4] Bloor's original method for cholesterol[5] and Bloor and Knudson's digitonin method[6] for cholesterol esters;

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1 Dauber, D. V., *Arch. Path.*, 1944, **38**, 46.

2 Folin, O., *J. Biol. Chem.*, 1929, **82**, 92.

3 Folin and Wu, *J. Biol. Chem.*, 1919, **38**, 81.

4 Leiboff, S. L., and Kahn, B. S., *J. Biol. Chem.*, 1929, **83**, 347.

5 Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

6 Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, **27**, 107.