Comparison of Two Perfusates in Ammonia Metabolism of Isolated Rat Livers (34002)

ANTHONY J. BARAK[1] AND HARRIET C. BECKENHAUER
(Introduced by H. P. Jacobi)

Medical Research Laboratory, Veterans Administration Hospital, Omaha, Nebraska 68105

Two perfusate media have been generally used to maintain viability of livers in studies of isolated perfused rat livers. These are diluted rat blood and a semisynthetic medium proposed by Schimassek (1). Schimassek (2) has shown that the synthetic medium is comparable to rat blood in promoting oxygen consumption in perfused rat livers and it has been used successfully by other research teams (3, 4).

Other workers, however, have found that semisynthetic perfusates are not as desirable as rat blood in certain research studies. Ferris (5) studying the conversion of ^{14}C-labeled acetate to ^{14}C-labeled cholesterol by the isolated perfused liver reported that the accumulation of cholesterol in the synthetic perfusate of Schimassek was only 10% of that produced when diluted rat blood was used. Likewise, Roheim, *et al.* (6) studying the release of lipid from fatty livers reported that considerably less lipid was released when a type of semisynthetic perfusate was used than was released by the use of rat blood.

Since ammonia metabolism is a primary field of interest in our laboratory, the present study was conducted to compare the semisynthetic medium to diluted rat blood in the metabolism of ammonia in both the preformed state as well as that arising from the metabolism of an amino acid.

Method and Materials. Sprague-Dawley rats fed a commercial diet (Rockland rat pellets) were used in this study. The liver perfusion procedures used were essentially those of Miller *et al* (7). All perfusions were carried out for 4-hr periods either with freshly drawn heparinized rat blood diluted 1:1 with sterile Tyrode solution or with the synthetic medium described by Schimassek (1). A volume of 190 ml was used in all perfusions and 2-ml aliquots were drawn for a basal sample and every hour during the experiments for blood ammonia and urea nitrogen determinations. Perfusate withdrawn for serial analyses was replaced with Tyrode solution to keep the volume of perfusate constant throughout the experiment. Weights were obtained on livers before they were placed on the perfusion apparatus.

The operative procedure usually lasted a period of 20–25 min with the length of time that the liver was without an active blood flow lasting from 4 to 6 min. Blood flow, bile flow, and gross appearance of the liver were used as indices of viability. If the blood flow through the liver did not proceed to 20 ml/min within the first 0.5 hr of the experiment, the experiment was abandoned. Livers were covered with wet gauze to keep them moist during the experiments and if all lobes of a liver did not stay pink throughout the 4-hr period, the experiment was terminated. In the experiments bile flow varied from 2 to 3 ml for the period of the perfusion.

Ammonia metabolism studies were conducted on normal livers from donor rats that had been fasted for 16 hr. Rats sacrificed as blood donors were not fasted in order to keep the blood glucose levels comparable to the level of glucose in the semisynthetic medium. Control experiments in which no supplement was added to the perfusate were performed with each type of perfusate. All supplement infusions into the perfusion system were made instantaneously by adding the compounds in solution directly to the blood reservoir. The ammonia producing supplements used in this study were ammonium acetate and glycine. Ammonium acetate was added in the amount of 8 mmoles/kg of body weight of the liver donor rat. Glycine was added in the amount of 24 mmoles/kg of body weight.

[1] Also in Department of Biochemistry, University of Nebraska College of Medicine and The Creighton University, Omaha, Nebr.

TABLE I. Average Ammonia Nitrogen Removed in Perfusion (1-hr).

Perfusate	No. of expts.	Normal livers, NH_4 acetate supplement: Per gram of liver protein (μmoles/190 ml)	SD	Significance of difference from rat blood: t_{90}	p
Diluted rat blood	7	435	108		
Synthetic blood	9	424	62	1.75 <.80	Not sig.

These pharmacologic doses were employed even though they were considerably higher than physiologic levels because the experiments were designed to be loading tests for the liver utilizing different sources for ammonia. The doses used here were chosen because they were found in a previous study (8) to be nontoxic to the intact dog.

Blood ammonia determinations were carried out by the method of Humoller *et al.* (9) and blood urea nitrogen was determined by the urease-indophenol method of Searcy *et al.* (10). Liver protein content was measured by the method of Lowry *et al.* (11). In the experiments where ammonium acetate was used as a supplement, the data were expressed in terms of micromoles of ammonia nitrogen removed from the perfusate in 1-hr or urea nitrogen produced in 4-hr/g of liver protein. The results of ammonia nitrogen removal from the perfusate were reported on a 1-hr basis because very little added ammonium ion remains in the perfusate following this time period. Efficiences of livers were compared on this hourly basis and in the amount of urea nitrogen produced in 4 hr. In the experiments in which glycine was used as a supplement, efficiencies of livers were compared on the basis of ammonia formed by oxidative deamination and urea nitrogen produced by the Krebs–Henseleit cycle during a 4-hr period. In all cases the basal levels of ammonia and urea were determined and were considered in the computation of the results.

Results. One means of testing isolated perfused livers in their efficiency to metabolize ammonia is to add preformed ammonium ion to the perfusate and measure the ammonia nitrogen removal during the first hour of perfusion. Table I shows that normal isolated perfused livers are capable of removing the same amount of ammonia per gram of liver protein in the presence of either diluted rat blood or Schimassek's synthetic blood.

Removal of ammonia from the circulating blood in itself is not a complete means of studying ammonia metabolism. Consequently, urea nitrogen production was studied to give a more complete picture of efficiency. Table II shows that some urea is produced in 4 hr of perfusion with both diluted rat blood and synthetic blood when no supplement is added to these perfusing media. When the

TABLE II. Average Urea Nitrogen Production in Perfusion (4-hr) of Normal Livers.

Perfusate	No. of expts.	Per gram of liver protein (μmoles/190 ml)	SD	Significance of difference from rat blood: t_{90}	p
		No supplement			
Diluted rat blood	4	423	103		
Synthetic blood	5	306	89	1.86 <.10	Sig.
		NH_4 acetate supplement			
Diluted rat blood	7	1296	240		
Synthetic blood	4	1231	128	1.81 <.60	Not sig.

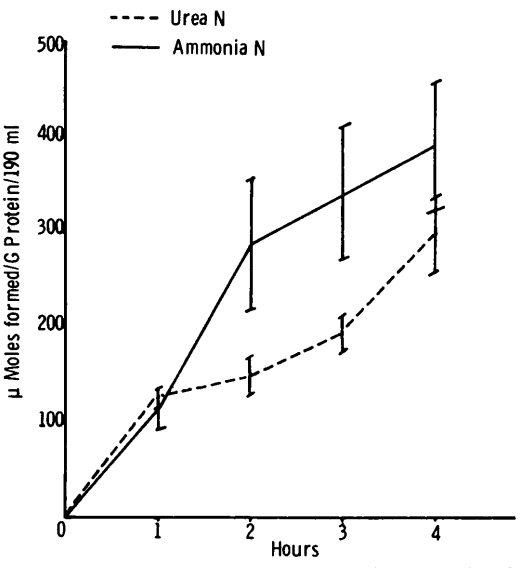

FIG. 1. Glycine metabolism in rat livers perfused with synthetic blood.

livers are subjected to an increased load of preformed ammonium ion, as shown in Table II, livers perfused with the synthetic medium are as efficient in urea production as when diluted rat blood is used.

Somewhat different results were obtained when the amino acid glycine was added to the perfusates of normal livers. These results are depicted in Fig. 1 where each curve represents the average of three experiments in which glycine was added in the amount of 24 mmoles/kg of body weight in 190 ml of perfusate. In the figures the ranges represent 1 standard deviation. From Fig. 1 it is obvious that when semisynthetic blood was used to maintain livers, added glycine generated increasing amounts of blood ammonia as measured by the method described.

Figure 2 demonstrates that amino acid metabolism was quite different in perfused livers when diluted rat blood was used. In the figure each curve represents the average of five experiments in which glycine was added in the same amount as added for experiments in Fig. 1. Here urea production was greatly enhanced following the administration of glycine to this perfusing medium. Ammonia was kept low even though oxidative deamination with rat blood may have occurred to a greater extent than with synthetic blood.

Discussion. As shown in a review by Meister (12) on the metabolism of the amino acids, glycine is a substance that is utilized in many different ways in the organism. Of the various reported metabolic pathways involving glycine, the three following mechanisms appear to be most pertinent to ammonia formation and ammonia detoxification in the liver: (a) As reported by Ratner *et al.* (13) glycine is deaminated to ammonia and glyoxylic acid by glycine oxidase. (b) Glycine has been shown by Meister (14) to be involved in a transamination reaction producing ornithine which in turn can function as an ammonia acceptor in the Krebs–Henseleit cycle. (c) As described by Cammarata and Cohen (15) glycine is transaminated to con-

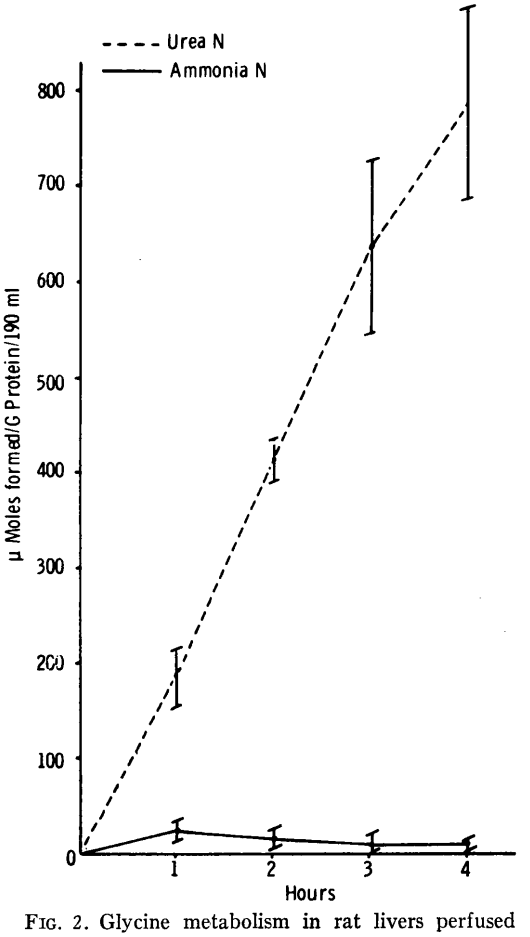

FIG. 2. Glycine metabolism in rat livers perfused with rat blood.

vert α-ketoglutarate to glyoxylate and glutamate. Even though the equilibrium of this reaction favors glycine formation, administered glycine may produce a feedback increasing levels of glutamate. The further transamination of the elevated glutamate would produce aspartate which may be utilized in urea production in the Krebs–Henseleit cycle.

Fahey (16) demonstrated that the infusion of glycine into the blood stream of humans caused a prompt and highly elevated blood ammonia level. Barak *et al* (8) showed that the same elvation occurs in dogs receiving intravenous administration of glycine. Apparently a portion of infused glycine is not utilized as such in the organism and is metabolized by way of oxidative deamination. The rapid formation of ammonia by this metabolic pathway suggested glycine as an excellent substrate to use in the study of metabolism of ammonia as it is derived from an amino acid source in contrast to the metabolism of preformed ammonia.

The data shown here indicate that preformed ammonium ions are easily accessible to perfused hepatic cells and are detoxified to the same extent by the isolated perfused rat liver either in the presence of rat blood or the artificial medium. The uptake of preformed ammonia and its eventual conversion to urea by the Krebs–Henseleit cycle occurs whether the liver is subjected to normal levels of ammonia or to an increased load of ammonia. It also demonstrates that the enzymes in the Krebs–Henseleit cycle of the perfused liver can increase their efficiency as the substrate level increases.

Administered glycine appears to undergo oxidative deamination by the isolated perfused liver regardless of the perfusion medium. The glycine just like preformed ammonia is accessible to perfused liver cells. Even though the glycine supplement exceeded the ammonia supplement by 3 times on a molar basis, the urea production with preformed ammonia proceeded at a greater rate than with the glycine in the presence of diluted rat blood.

Nitrogen from oxidative deamination was not metabolized as was preformed ammonium ion in the synthetic perfusate. Figure 1 shows that even though blood ammonia nitrogen was made available by the glycine no more urea was produced by the livers than was produced when no supplement was added to the synthetic medium. Although the glycine nitrogen supplement again exceeded the ammonia supplementation, the urea production with the preformed ammonia was highly significant. One would expect that ammonia obtained from oxidative deamination would be detoxified in the same manner as preformed ammonia but this did not occur. At the present time there is no simple explanation for these results.

Explanations are more easily reached for results obtained when only glycine supplementation was used with each of these two perfusates. Equal results with ammonia supplements using both perfusates may be due to the fact that the ammonia was in the preformed state. However, when glycine is the additive the amino nitrogen can produce urea by two separate pathways. One, by way of oxidative deamination and metabolism through the Krebs–Henseleit cycle. Secondly, by conversion to aspartate which contributes a nitrogen to urea production also in the Krebs–Henseleit cycle. Different results with the two perfusates may have been due to the fact that the semisynthetic medium is deficient in essential enzyme cofactors. One of these cofactors is FAD which is required for glycine oxidase activity in the liver. Others would be those cofactors affecting the rate of transamination reactions needed to convert glycine to aspartate. The artificial medium also would lack such compounds as arginine or ornithine which are known to increase the capacity of the urea cycle.

The above observations illustrate that semisynthetic perfusates may or may not produce the same results as freshly drawn blood in liver perfusion experiments. They suggest that the investigator should first compare a synthetic perfusate with blood from an animal species before launching into a liver perfusion study with that particular animal species.

Summary. A comparison of diluted rat blood and a semisynthetic perfusate was made with the use of isolated perfused rat livers. This was effected by determining the efficiency of ammonia metabolism in these media. Both perfusates allow a high degree of conversion of preformed ammonia to urea. Diluted rat blood, however, was far more efficient in the metabolism of nitrogen derived from oxidative deamination of glycine. A suggestion is made to investigators that synthetic perfusates be compared to freshly drawn blood within the proposed study parameter before designing research projects involving liver perfusion.

1. Schimassek, H., Biochem. Z. **336**, 460 (1963).

2. Schimassek, H., Life Sci. **11**, 629 (1962).

3. Matschinsky, F., Meyer, V., and Wieland, O., Biochem. Z. **333** 48 (1960).

4. Eymer, K. P., Kopetz, K., Schwarz, K., and Weinges, K. F., Klin. Wochschr. **40**, 434 (1962).

5. Ferris, R. M., Ph.D. thesis, University of Nebraska, 1967.

6. Roheim, P. S., Miller, L., and Eder, H. A., J. Biol. Chem. **240**, 2994 (1965).

7. Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F., J. Exptl. Med. **94**, 431 (1951).

8. Barak, A. J., Humoller, F. L., Mahler, D. J., and Holthaus, J. M., Gastroenterology **43**, 35 (1962).

9. Humoller, F. L., Barak, A. J., and Holthaus, J. M., Clin. Chem. **10**, 589 (1964).

10. Searcy, R. L., Gough, G. S., Kocotyer, J. L., and Berquist, L. M., Am. J. Med. Technol. **27**, 255 (1961).

11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, J., J. Biol. Chem. **193**, 265 (1951).

12. Meister, A., "Biochemistry of the amino acids," p. 278. Academic Press, New York (1957).

13. Ratner, S., Nocita, V., and Green, D. E., J. Biol. Chem. **152**, 119 (1944).

14. Meister, A., J. Biol. Chem. **206**, 587 (1954).

15. Cammarata, P. S. and Cohen, P. P., J. Biol. Chem. **187**, 439 (1950).

16. Fahey, J. L., J. Clin. Invest. **36**, 1647 (1957).

Rceived Mar. 20, 1969. P.S.E.B.M., 1969, Vol. 131.