ANESTHESIOLOGY

The Journal of

THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

Volume 17 MAY, 1956 Number 3

# THE EFFECT OF HYPOTHERMIA ON MORPHINE METABOLISM IN AN ISOLATED PERFUSED LIVER

RICHARD A. RINK, M.D., IRVING GRAY, Ph.D.,
ROLAND R. RUECKERT, B.S., AND HARVEY C. SLOCUM, M.D.

PERFUSION of the isolated liver has been used by a number of investigators, particularly in the past five years (1–5). Prudden and his coworkers (6) have used this method in the study of the effects of insulin and growth hormone on liver metabolism. Miller *et al.* reported in 1954 that perfusion of the intact, isolated liver with oxygenated blood permits the liver to synthesize plasma proteins, to separate them from its own tissue at need, and to contribute them to the circulating plasma in a manner closely approximating that seen in intact normal animals (7). They also reported that the perfused liver repeats its action quantitatively when a second dose of substrate is given, even after having been perfused for four hours.

With the advent of surgery under hypothermic conditions, it was felt that the liver perfusion method for the study of metabolism and detoxification would yield valuable information for this clinical problem. The present study utilized this technique as an *in vitro* method of observing the effect of hypothermia on liver respiration, metabolism and its ability to conjugate morphine and thiopental. In addition to the usual methods for measuring carbon dioxide production and the rate of drug detoxification, the apparatus has been so designed as to allow the measurement of oxygen consumption.

## DESCRIPTION OF APPARATUS

The liver perfusion apparatus described in this report measures volumetrically the oxygen uptake of the perfused organ by recirculating the gas mixture in a closed system. It is a modification of perfusion equipment previously described (5, 7). The apparatus is enclosed

Accepted for publication January 27, 1956. Dr. Rink and Dr. Slocum are members of the Anesthesia and Operative Service, Walter Reed Army Hospital, and Dr. Gray and Mr. Rueckert are in the Department of Biochemistry, Army Medical Service Graduate School of the Walter Reed Army Medical Center, Washington, D. C.

in a thermoregulated cabinet.* The system consists essentially of three components: A pump which maintains blood flow; a "lung" which ventilates the blood, and an organ which utilizes this blood (fig. 1).

The circuit of the perfusion fluid is conveniently traced beginning with the reservoir flask from which the perfusate is lifted through a filter to remove any clots that may form during the experiment. The pump, a Brewer pipetting machine with variable speed and stroke, activates a finger-stall pump. Unidirectional flow is obtained by attach-

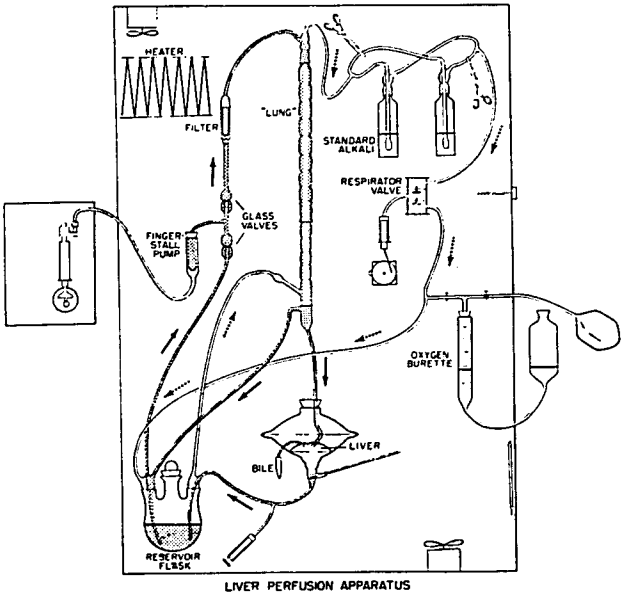


FIG. 1. Liver perfusion apparatus.

ing glass perfusion valves to the finger-stall pump. From this point blood passes to the top of a condenser column that functions as an artificial lung. The blood is spilled from the top of the "lung" as a thin, falling film passing countercurrent to a stream of oxygen which oxygenates the blood and sweeps it free of its load of carbon dioxide. The "arterial" blood is collected in a small reservoir at the base of the "lung" where two outlets are provided, one channeling blood to the

* Fabricated by General Service Division, Carpenter Shop, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

portal vein cannula, and the other serving as an overflow returning surplus blood to the reservoir flask. This overflow outlet is suspended 20 cm. above the liver, thereby providing a constant perfusion pressure. Oxygenated blood, entering the portal vein, perfuses the liver. Hepatic vein outflow enters a thermometer well, thus affording an opportunity to observe the temperature of blood leaving the liver. Flow continues from the well through a flowmeter † and into the reservoir flask.

The ventilating mechanism is a closed circle absorption circuit system. Unidirectional gas flow is provided by a nonbreathing type valve, the center chamber of which is attached to an activating pump, the "respiratory muscle" of the system. The pump ‡ is simply a 50 ml. syringe attached to a 60 r.p.m. motor by an eccentric wheel. From the respiratory valve, gas flows through the reservoir flask carrying away any $CO_2$ that may have accumulated. Next, the gas enters the base of the condenser column (lung) just above the blood overflow opening. The oxygen-rich mixture passes up the column, oxygenating the thin film of perfusate falling down the internal surface of the "lung" and sweeping it free of its $CO_2$. The gas now passes through the $CO_2$ absorber and the circuit is completed by returning the gas to the respirator valve. By inserting a T-tube between the respirator valve and the base of the "lung," a graduated oxygen burette with leveling bulb is connected to the system.

Since all parts of the gas system are relatively rigid (except the water level in the oxygen burette and the blood level in the reservoir flask, including the blood-filled tubing leading to the thermometer well) the oxygen consumption is read directly from the graduated burette, after restoring the initial manometric pressure (1 cm. $H_2O$) with leveling bulb. It is essential that the level of blood in the thermometer well be at its initial level each time an oxygen reading is made. Since changes in the volume of fluid within this rigid-walled system will be reflected in the oxygen measurements, approximate corrections must be made for the volume of all samples withdrawn or materials added to the system. Another correction factor is required when the standard alkali bottles are changed. The final volume of oxygen utilized is then corrected to standard temperature and pressure.

The liver preparation with portal vein and bile cannulas in place is supported on a wire-mesh stage wrapped with Cellophane to protect the liver. The stage and liver are enclosed between two glass desiccator lids. This minimizes the loss of $CO_2$ diffusing from the liver capsule and hepatic vein outflow, and maintains the liver in an environment of constant humidity. The opening in the upper lid contains a

† A T-tube is inserted in the line and a 100 ml. syringe is attached to the side arm. Flow rate is checked by drawing the venous outflow into the syringe while maintaining a constant blood level in the thermometer well.

‡ Fabricated by the Instrumentation Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

rubber stopper with glass tube connecting the "arterial" flow from the "lung" and the portal vein cannula, while the opening in the lower lid has a standard taper joint fitted to the thermometer well. The bile cannula is placed through a small opening drilled in the lower lid and a graduated tube is attached to collect bile.

## METHODS

A nonfasting rabbit of either sex was anesthetized with pentobarbital (30 mg. per Kg.) and heparinized. The common bile duct and portal vein were cannulated. The liver with attached cannulas was rapidly excised, weighed, and placed in the perfusion apparatus. The liver was without circulation for an interval of five to ten minutes. The perfusate used was heparinized, whole rabbit blood drawn from donor rabbits by cardiac puncture. The pooled donor blood (300 ml.) was diluted with normal saline (150 ml.) and a commercial 5 per cent protein hydrolysate solution in 5 per cent glucose (50 ml.). The final hematocrit was 20. Aureomycin, 25 mg., was added to the perfusate. The gas system was flushed with oxygen several times, and an equilibration period of ten to thirty minutes allowed for the gas mixture, perfusate, and liver before starting measured observations.

Oxygen uptake was determined volumetrically using the method described above. Carbon dioxide production was determined by titration of the standard alkali solution.

When the conjugation of morphine (or thiopental) was to be studied, 50 mg. of the drug was added to the reservoir flask and the hepatic vein samples were analyzed for free and bound morphine.

When the effect of temperature was to be studied, the temperature of the blood and cabinet for the first ninety minutes was 24 C.; the cabinet heater was then started and the temperature was raised to 37 C. for two hours. A second dose of 50 mg. of morphine was added to the reservoir and the hepatic vein samples were analyzed.

Free and bound morphine present was determined by the ultraviolet spectrophotometric method of Goldbaum (8).

In other experiments (to study the effect of thiopental on morphine conjugation), 50 mg. of the thiopental was added to the reservoir flask, "arterial" and "venous" samples being drawn and analyzed by the method of Jailer and Goldbaum (9). Morphine sulfate (50 mg.) was added to the perfusate at the same time as thiopental, and both "arterial" and "venous" samples followed for free morphine content.

A graduated test tube was attached to the bile cannula and the volume collected was recorded at hourly intervals.

## RESULTS

A one-hour control period was observed in all experiments before either morphine or thiopental was added to the perfusate. Thus each liver served as its own control concerning the effect, if any, of the drugs on oxygen uptake and carbon dioxide production. With the concen-

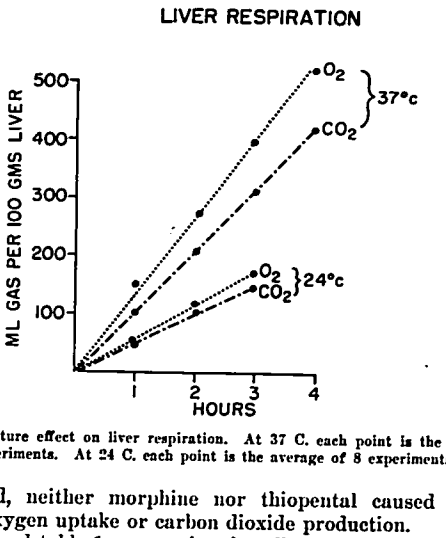


FIG. 2. Temperature effect on liver respiration. At 37 C. each point is the average of 13 experiments. At 24 C. each point is the average of 8 experiments.

trations used, neither morphine nor thiopental caused observable changes in oxygen uptake or carbon dioxide production.

Figure 2 and table 1 summarize the effect of temperature on liver respiration. The marked depression in respiration as a result of the decreased temperature is quite apparent. However, regardless of the temperature, the oxygen utilization and carbon dioxide production remains linear over the time followed.

There is a marked effect of hypothermia as well as of barbiturate on the ability of liver to detoxify morphine (fig. 3). The biologic half-life for the loss of free morphine from the plasma at 37 C. is 3.7 minutes; 94 minutes at 24 C.; and in the presence of Pentothal at 37 C.,

TABLE 1

EFFECT OF HYPOTHERMIA ON LIVER RESPIRATION

| Temperature \ Time after Perfusion Started, hrs. | Oxygen Consumption ml./hr./100 gm. | | | | Carbon Dioxide Production ml./hr./100 gm. | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 37 C. | 162± 4 | 140±11 | 133±9 | 131±10 | 111±8 | 113±8 | 120±10 | 110±8 |
| 24 C. | 68±10 | 63± 7 | 55±7 | 47± 8 | 59±7 | 54±6 | 44± 2 | 39±3 |

All values ± standard deviation of the mean.

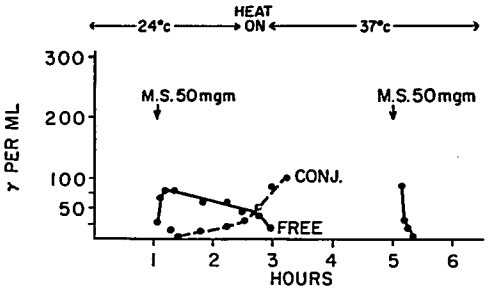


FIG. 3. Temperature effect on morphine metabolism. At 24 C. and 37 C. 15 mg. of morphine (M.S.) was added to the perfusate at the times indicated by arrows. Conj. = conjugated morphine.

14.7 minutes. Table 2 summarizes these figures and, in addition, gives the biologic half-life of thiopental.

The level of free morphine rises extremely rapidly in the hepatic outflow at 37 C. (fig. 5). At 24 C. there is a very definite delay in the time required to reach the maximum conjugation (fig. 4). This may be the result of the change in flow of the perfusion fluid which decreased by about 25 per cent, but with a large variation, when the temperature was changed to 24 C.

There was a three to six minute delay in the appearance of the bound morphine, suggesting that morphine is held briefly by the hepatic cells while being conjugated. Further support of this possibility was observed by the rise in level of bound morphine in the hepatic vein blood for ten to fifteen minutes after all free morphine had been removed from the "arterial" blood.

The rate of bile formation at 37 C. was two to four times that observed at 24 C.

These observed metabolic effects may well be the result of the inability of the liver to form the glucuronide of morphine (10). It has

TABLE 2

EFFECT OF HYPOTHERMIA ON THE RQ OF THE ISOLATED PERFUSED LIVER

| | RQ | | | |
|---|---|---|---|---|
| Temperature \ Time after Perfusion Started, hrs. | 1 | 2 | 3 | 4 |
| 37 C. | 0.70±.04 | 0.83±.04* | 0.89±.09 | 0.84±.08 |
| 24 C. | 0.85±.09* | 0.87±.12 | 0.80±.10 | 0.85±.15 |

All values ± standard deviation of mean.
* $P < 0.025$ when compared to 1 hr. RQ at 37 C.

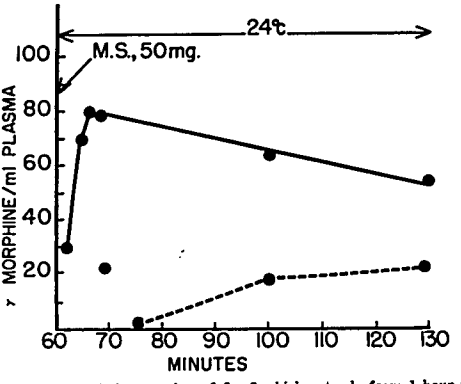


FIG. 4. Enlargement of that portion of fig. 3 which extends from 1 hour to 2 hours and 10 minutes. A complete run at 24 C. Dashed line indicates the concentration of conjugated morphine.

recently been demonstrated (11, 12) that the glucuronide is formed through the formation of uridine diphosphate glucose. In view of the markedly reduced respiration of the liver as a result of the hypothermia, it is not unexpected that this mechanism is slowed down with a corresponding decrease in the binding of morphine. Furthermore, in light of the reported fact that thiopental uncouples oxidative phosphorylation (13, 14) and generally reduces cellular respiration, we have another mechanism for slowing the conjugation system of morphine.

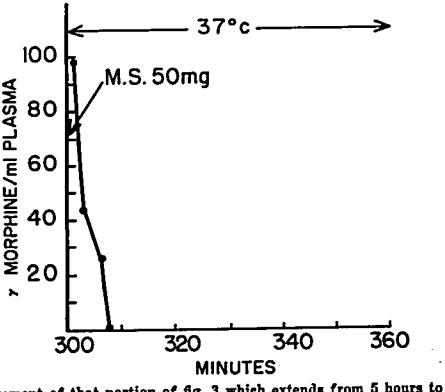


FIG. 5. Enlargement of that portion of fig. 3 which extends from 5 hours to 6 hours and with the temperature at 37 C.

TABLE 3

EFFECT OF HYPOTHERMIA ON DRUG DETOXIFICATION BY LIVER

| | No. of Experiments | | Half-Life (Minutes) | |
|---|---|---|---|---|
| | 37 C. | 24 C. | 37 C. | 24 C. |
| Morphine | 8 | 4 | 3.7 | 94[b] |
| Morphine (with Pentothal) | 4 | — | 14.7[a] | — |
| Pentothal | 5 | 2 | 46 | 530, 185 |

[a] $P < 0.05$ when compared to morphine alone.
[b] $P < 0.001$ when compared to morphine at 37 C.

## SUMMARY

A liver perfusion apparatus with closed gas system providing a means of measuring oxygen uptake volumetrically has been described. The effect of hypothermia on oxygen uptake, carbon dioxide production, morphine and thiopental detoxification, and bile formation in the isolated perfused rabbit liver was observed. The effect of thiopental on morphine conjugation was also observed. In addition, the effect of thiopental on morphine conjugation was followed.

It is believed that the alterations in metabolic activity observed *in vitro* with liver perfusion can be attributed to the effect on respiration and oxidative phosphorylation.

## REFERENCES

1. Brauer, R. W., Leong, G. F., and Pessotti, R. L.: Vasomotor Activity in Isolated Perfused Rat Liver, Am. J. Physiol. **174**: 304 (April) 1953.
2. Andrews, W. H.: Technique for Perfusion of Canine Liver, Ann. Trop. Med. **47**: 146 (March) 1953.
3. D'Silva, J. L., and Neil, M. W.: Potassium, Water and Glycogen Content of Perfused Rat Liver, J. Physiol. **124**: 515 (June) 1954.
4. Lundsgaard, E.: Observations on Factor Determining Metabolic Rate of Liver, Biochim. et biophys. acta **4**: 322 (Jan.) 1950.
5. Young, M. K., Jr., Prudden, J. F., and Stirman, J. A.: Perfusion Technique for Study of Liver Metabolism, J. Lab. and Clin. Med. **46**: 155 (July) 1955.
6. Prudden, John A.: Personal communication to the authors.
7. Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F.: Dominant Role of Liver in Plasma Protein Synthesis, J. Exper. Med. **94**: 431 (July) 1951.
8. Goldbaum, L. R., and Miller, S. A.: Determination of Barbiturates, Federation Proc. **13**: 358 (Oct.) 1954.
9. Jailer, J. W., and Goldbaum, L. R.: Studies on Plasma Concentration and Tissue Distribution of Sodium Pentothal (Sodium Ethyl (1-Methylbutyl) Thiobarbiturate), J. Lab. & Clin. Med. **31**: 1344 (Dec.) 1946.
10. Seibert, R. A., Williams, C. E., and Huggins, R. A.: Isolation and Identification of "Bound" Morphine, Science **120**: 222 (Aug.) 1954.
11. Smith, E. E. B., and Mills, G. T.: Uridine Nucleotide Compounds of Liver, Biochim. et biophys. acta **13**: 386 (March) 1954.
12. Mills, G. T., and Smith, E. E. B.: Enzymatic Conversion of Glycogen and Starch to Glucuronides, Federation Proc. **14**: 256 (March) 1955.
13. Brody, T. M., and Bain, J. A.: Barbiturates and Oxidative Phosphorylation, J. Pharmacol. & Exper. Therap. **110**: 148 (Aug.) 1954.
14. Persky, H., Goldstein, M. S., and Levine, R.: Enzymatic Mechanism of Barbiturate Action, J. Pharmacol. & Exper. Therap. **100**: 273 (July) 1950.