REFERENCES

1. M. F. ARGUS and F. E. RAY, *Proc. Am. Ass. Cancer Res.* **2**, 92 (1956).
2. A. W. BREIDENBACH and M. F. ARGUS, *Q. J. Fla. Acad. Sci.* **19**, 68 (1956).
3. M. E. URQUHART, *Br. J. Cancer* **9**, 611 (1955).
4. E. K. WEISBURGER and J. H. WEISBURGER, *Adv. Cancer Res.* **5**, 331 (1958).
5. E. C. MILLER, J. A. MILLER and M. ENOMOTO, *Cancer Res.* **24**, 2018 (1964).
6. J. A. MILLER, J. W. CRAMER and E. C. MILLER, *Cancer Res.* **20**, 950 (1960).
7. M. KIESE, G. RENNER and I. WIEDEMANN, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **252**, 418 (1966).
8. M. KIESE and G. RENNER, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **252**, 480 (1966).
9. C. C. IRVING, *Fedn Proc.* **24**, 152 (1965).
10. E. C. MILLER and J. A. MILLER, *Pharmac. Rev.* **18**, 805 (1966).
11. M. KIESE, *Pharmac. Rev.* in press.
12. R. VON JAGOW, M. KIESE and G. RENNER, *Biochem. Pharmac.* In press.
13. C. C. IRVING, *Cancer Res.* **22**, 867 (1962).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1885–1888. Pergamon Press Ltd., Printed in Great Britain.

The effect of pH on glycogenolysis in turtle heart*

(*Received* 10 *March* 1966; *accepted* 6 *July* 1966)

CATECHOLAMINES, and several other agents, are known to enhance the activity of the phosphorylase enzyme system, which initiates the metabolic breakdown of glycogen.[1] One of the main reactions—the activation of phosphorylase *b* to the more active phosphorylase *a* by the phosphorylase *b* kinase—was reported to be affected also by hydrogen ion concentration. At low pH, e.g. 6·2, the substrate affinity of the phosphorylase *b* kinase is much less than at higher pH, such as 8·2.[2] Most of the data on the pH effect were obtained under aerobic conditions. It was decided, therefore, to investigate the effect of pH on the glycogenolytic response during anoxia, as well as its possible modification by exogenous glucose. Isolated turtle hearts were chosen as the experimental preparation, since they can be used under aerobic and anerobic conditions for prolonged periods of time. The production of lactic acid, the terminal metabolite in the anaerobic glycogenolytic scheme, was selected as the measure of glycogenolytic activity.

METHODS

Isolated turtle hearts of either sex of the species *Chrysemis picta* and *Pseudymus elegans* were used in the study. The experiments were carried out partly during the summer months (July to September) and partly in winter (December). The original procedure for removal and preparation of heart and perfusion as described by Hardman *et al.*[3] was followed. A constant rate of 24 beats per minute was maintained by suprathreshold stimuli originating from an A.E.L. model 104A stimulator and transmitted with the help of silver electrodes which were attached to the base of the ventricle. The borate–acetate salt solution of Mines,[4] adjusted to the desired pH, was used in all but one series. In order to test whether the presence of acetate in the buffer can alter the rate of lactate release, a series of aerobic hearts was run with the Tris buffer. The perfusing fluid for the anaerobic hearts contained 1×10^{-3} M sodium cyanide; glucose, when included, was used in a final concentration of 0·1%. The hearts were perfused at each of three pH values for at least 30 min; a 2-min perfusate sample was then collected and the pH altered to the next level. Lactic acid in the perfusate was determined by the Barker-Summerson method.[5] Most samples were also analyzed by a gas chromatographic method.[6]

* This work was supported in part by United States Public Health Service Grant HE-08311.

RESULTS

The aerobic turtle hearts, perfused with the acetate–borate buffer at pH 8·5, produced about 21 μg lactic acid/min of perfusion (Table 1); the corresponding value for the hearts in Tris buffer was 22 μg/min, suggesting that inclusion of acetate in the borate buffer did not affect the rate of glycogenolysis. The reduction in pH in the perfusing fluid significantly depressed the lactic acid output of the aerobic hearts, regardless of the buffer used.

TABLE 1. A COMPARISON OF BUFFER SYSTEMS UPON THE EFFECT OF pH ON LACTIC ACID OUTPUT IN PERFUSED AEROBIC TURTLE HEARTS*

	pH 8·5 (μg/min)		pH 7·5 (μg/min)		pH 6·5 (μg/min)
Acetate–borate buffer, N = 6	$\bar{x} = 20{\cdot}9$	S.E. = 1·29† $t = 6{\cdot}57$ $P < 0{\cdot}01$	$\bar{x} = 9{\cdot}1$	S.E. = 0·70 $t = 5{\cdot}58$ $P < 0{\cdot}01$	$\bar{x} = 5{\cdot}2$
Tris buffer N = 3	$\bar{x} = 21{\cdot}9$	S.E. = 1·59 $t = 8{\cdot}06$ $P < 0{\cdot}05$	$\bar{x} = 9{\cdot}1$	S.E. = 1·28 $t = 4{\cdot}54$ $P < 0{\cdot}05$	$\bar{x} = 3{\cdot}2$

* Summer hearts, *Chrysemis picta*.
† Statistical analyses by paired differences.

The hearts run in the two anaerobic summer series were initially tested under aerobic conditions at pH 8·5, in the acetate–borate buffer; their average lactate production—17 μg and 18 μ/min—was similar to that obtained in the previous aerobic summer series (Fig. 1). Inclusion of 0·1% dextrose

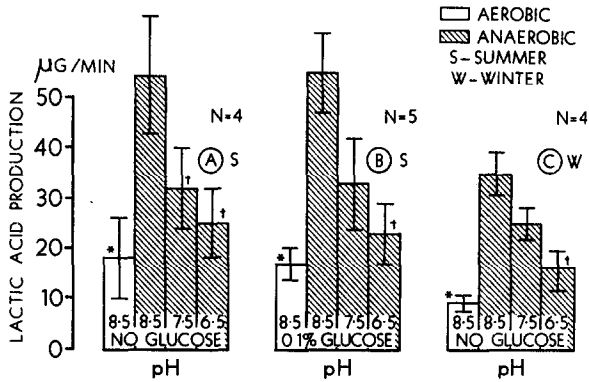

FIG. 1. Effect of pH on lactic acid output in anaerobic turtle hearts. Series A and B run with *Chrysemis picta*, series C with *Pseudymus elegans*. The vertical bars represent standard error. * $P < 0{\cdot}01$, † $P < 0{\cdot}05$; paired comparison against pH 8·5, anaerobic.

in the perfusing fluid appeared to be without any marked effect on the total lactic acid output. When the hearts were perfused with the pH 8·5 buffer containing NaCN, the lactic acid production increased to 55 μg/min. As in the aerobic hearts, reduction in buffer pH was followed by a significant decrease ($P < 0{\cdot}05$, paired differences) in lactate production: an average of 32 μg/min was measured at pH 7·5 and 25 μg/min at pH 6·5. A similar downward trend in lactic acid output reaching statistical significance at pH 6·5 ($P < 0{\cdot}05$) was observed in the summer hearts perfused with buffer plus dextrose, suggesting again that inclusion of the sugar in the buffer did not modify the metabolic response.

Although the winter hearts of the species *P. elegans* produced in general less lactate than the summer hearts of *C. picta*, the changes in lactate output paralleled those seen in the summer series (Fig. 1). There was again a significant increase after the transition from aerobic to anaerobic metabolism, and then gradual decrease with the decreasing pH of the buffer ($P < 0.05$ at pH 6·5).

DISCUSSION

The observed reduction of lactate output in aerobic turtle hearts by the decreasing pH is consistent with the findings of Krebs *et al.*[2] concerning the effect of pH on phosphorylase *b* kinase and thus indirectly on phosphorylase. Decreased lactate amounts in the perfusate could also reflect an increased lactate utilization by the aerobic heart for energy production; however, our previous results indicating that the inotropic activity did not change[7] or even may have decreased with lower pH, and the observation in the present study that a similar decrease occurred in anaerobic hearts, make this explanation unlikely.

The anaerobic turtle hearts which produced significantly more lactic acid at pH 8·5 than the aerobic hearts, also responded to the decreasing pH values with a reduction in lactate output. Since it is believed that the higher lactate production in anaerobic hearts is due to a stimulation of phosphorylase *b* kinase by anoxia,[8, 9] the observed reduction in lactate output suggests that the phosphorylase was the enzyme system inhibited by the low pH values. Additional factors possibly contributing to the observed differences in lactic acid output could be a change in availability of inorganic orthophosphate and adenosine diphosphate and an effect of pH on phosphofructokinase activity. It has been reported[10, 11] that phosphofructokinase occurs in active and inactive forms and that their interconversion is pH dependent. The available data are not sufficient to decide which proposed metabolic pathways might have participated in the final results and to what extent.

In spite of the inhibition by the high hydrogen ion concentrations, the production of lactic acid in anaerobic hearts at low pH was consistently higher than in the corresponding aerobic hearts. This might have been due to an increased activity of the unconverted phosphorylase *b* during anoxia. Such activation of phosphorylase *b* was suggested by the work of Morgan and Parmeggiani.[12]

In contrast to the findings in *anoxic rat* hearts,[13] addition of glucose to the perfusing medium did not increase lactate production in *anaerobic turtle* hearts. Even in anoxia, the turtle heart seems to utilize preferentially its considerable glycogen stores as an energy source and metabolizes exogenous glucose only after glycogen stores are depleted.[14] It is interesting, however, that the anaerobic turtle hearts did not utilize exogenous glucose when their glycogenolysis was markedly depressed by the high hydrogen ion concentration. One could assume, therefore, that the still functioning portion of the glycogenolytic pathway was able to supply a sufficient amount of energy for the heart, or that the lower pH in some way interfered with the uptake or utilization of the exogenous glucose.

The presence of lactic acid in the non-recirculated perfusates of aerobic hearts run at higher pH values was a constant finding. This is in variance with a report on the absence of lactate in *recirculated* perfusate.[15] This discrepancy can probably be explained by utilization of lactate from the recirculated fluid. In addition, the recirculated fluid contained plasma from heparinized turtles and therefore probably supplied appreciable amounts of unesterified fatty acids, which could serve as an alternative source of energy and thus reduce the rate of glycogenolysis and lactate production.

In conclusion, the data from the present study indicate that glycogenolysis in the isolated turtle heart is suppressed by increasing hydrogen ion concentration in both aerobic and anaerobic conditions; this depression is not prevented by inclusion of 0·1% dextrose in the perfusing solution. It would seem, therefore, that pH can be used—in addition to a number of other known pharmacological agents—to regulate the rate of glycogenolysis in aerobic and anaerobic isolated turtle hearts.

Acknowledgements—The assistance of Doctors Walter D. Meester and Jose Serrano is gratefully acknowledged.

Research Service,
Wood Veterans Administration Hospital;
and Department of Pharmacology,
Marquette University School of Medicine,
Milwaukee, Wis., U.S.A.

JOSEPH J. BARBORIAK
HAROLD F. HARDMAN

REFERENCES

1. E. W. SUTHERLAND and T. W. RALL, *Pharmac. Rev.* **12**, 265 (1960).
2. E. G. KREBS, D. S. LOVE, G. E. BRATVOLD, U. A. TRAYSER, W. L. MEYER and E. H. FISCHER, *Biochemistry* **3**, 1022 (1964).
3. H. F. HARDMAN, J. I. MOORE and B. U. B. LUM, *J. Pharmac. exp. Ther.* **126**, 136 (1959).
4. G. R. MINES, *J. Physiol., Lond.* **46**, 188 (1913).
5. S. B. BARKER and W. H. SUMMERSON, *J. biol. Chem.* **138**, 535 (1941).
6. N. E. HOFFMAN, J. J. BARBORIAK and H. F. HARDMAN, *Analyt. Biochem.* **9**, 175 (1964).
7. H. F. HARDMAN and R. C. REYNOLDS, *J. Pharmac. exp. Ther.* **149**, 219 (1965).
8. W. H. DANFORTH, E. HELMREICH and C. F. CORI, *Proc. natn. Acad. Sci., U.S.A.* **48**, 1191 (1962).
9. M. CORNBLATH, P. J. RANDLE, A. PARMEGGIANI and H. E. MORGAN, *J. biol. Chem.* **238**, 1592 (1963).
10. T. E. MANSOUR, *J. biol. Chem.* **240**, 2165 (1965).
11. W. H. DANFORTH, in *Control of Energy Metabolism* (Eds. B. CHANCE, R. W. ESTABROOK and J. R. WILLIAMSON), p. 287, Academic Press, New York (1965).
12. H. E. MORGAN and A. PARMEGGIANI, in *Control of Glycogen Metabolism* (Eds. W. J. WHEELAN and M. D. CAMERON), p. 254. Little, Brown, Boston (1964).
13. H. E. MORGAN, J. R. NEELY, R. E. WOOD, C. LIEBECQ, H. LIEBERMEISTER and C. R. PARK, *Fedn Proc.* **24**, 1040 (1965).
14. R. B. REEVES, *Am. J. Physiol.* **205**, 23 (1963).
15. R. B. REEVES, *Am. J. Physiol.* **205**, 17 (1963).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1888–1890. Pergamon Press Ltd., Printed in Great Britain.

Effect of cinnamic acid on potassium stimulated respiration in rat brain cortex slices

(*Received* 27 *May* 1966; *accepted* 30 *June* 1966)

IT IS well established that most of the energy requirements of brain are satisfied by respiration and glycolysis.[1] Glucose is the preferred substrate for brain respiration in comparison to the other substrates which, though utilized, have been shown to sustain less stable respiration.[2] Changes in potassium ion concentration have a profound effect on the intermediary metabolism of brain cortex slices. Increase in the concentration of potassium ions has been shown to result in a simultaneous increase in the oxygen uptake by respiring cerebral cortex slices.[3, 4] Similar effects were observed by applying electrical impulses.[5] On the other hand, similar stimulation of the respiratory activity of rat brain cortex slices by thiosalicylic acid was found to be accompanied by a decrease in the production of lactic acid[6] and thus differed from that produced by potassium chloride where increase in lactic acid paralleled enhanced oxygen uptake and substrate utilization.[7] In the present study evidence has been provided that cinnamic acid, shown to interfere in cellular metabolism,[8] caused supression of the potassium activated respiratory carbon dioxide with little or no inhibitory effects on the unstimulated processes during oxidation of glucose and L-glutamate by rat brain cortex slices.

All the experiments were carried out on brain cortex slices from rats of either sex, weighing about 200–250 g. The rats were killed by decapitation. The brains were removed and placed in ice cold phosphate–Ringer solution containing NaCl 128 mM, KCl 5 mM, $CaCl_2$ 0·6 mM, $MgSO_4$ 1·3 mM; sodium phosphate buffer at pH 7·4, 10 mM. The substrates used were either glucose (10 mM) or L-glutamate (10 mM). Rat brain cortex slices were cut with a Stadie–Riggs tissue slicer and were weighed immediately. The dry weight of the slices was calculated by a factor determined by drying a known quantity of wet tissue to constant weight at 110°. The slices were incubated in a conventional Warburg Manometric apparatus at 37° in Krebs–Ringer phosphate solution using oxygen as the gas phase. The vessels were gassed for 5 min and were equilibrated for further 10 min. The central well contained 0·2 ml of 20% KOH. Potassium chloride and cinnamic acid were present in the side arm and were tipped in the main vessel after thermal equilibration.