Anaerobic Loss of Endogenous Glycogen in Rat Heart Slices. (22693)

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In the course of a series of comparative studies it was found that rapid depletion of the endogenous glycogen of rat heart occurred *in vitro* under anaerobic conditions. Attempts to demonstrate an accumulation of lactic acid under these conditions were unsuccessful and lactic acid was actually found to disappear slowly during anaerobic incubations. A summary of typical data appear in Table I. No significant concentration of preformed or 7 minute hydrolyzable reducing substances, pyruvate, acetoin, citrate, volatile acids or triose phosphates was found in any time period. Small concentrations of fructose and high concentrations of hexosemonophosphate were demonstrated in all time periods. Very high concentrations of 5 hour hydrolyzable reducing substances of undetermined origin were found in all time periods but wide variations between individual experiments were also found: 898 μg/100 mg (542-1131) at zero time, 288 (107-392) at 10 minutes, and 760 (733-851) at 30 minutes. On the basis of these results the following compounds are thought to be excluded as end products of the observed glycogen breakdown in heart slices: glucose, hexose phosphates and diphosphate, fructose, glyceraldehyde phosphate and dihydroxyacetone phosphate, pyruvate and lactate, acetoin, acetate and citrate.

An attempt was made to evaluate this unusual finding in the whole animal. To simulate the anaerobic *in vitro* incubations the heart was allowed to remain within the chest for varying times after the rat was beheaded. Under these conditions the endogenous glycogen of the heart fell rapidly but there was a concurrent accumulation of lactic acid, in agreement with the usual glycolytic theory (Table II).

The "extra" lactate formed in this system probably represents that formed in the terminal metabolism from the various glycolytic intermediates.

Discussion. A survey of the literature failed to reveal a comparable study. In a series of reports in 1932-35, Haarman indicated that glycogen added to heart *brei* was destroyed but that lactic acid was not formed (15,16). From our experiences with attempting to prepare a heart brei it seems that Haarman's work should be considered a study of a variety of isolated soluble enzyme systems from heart. Barron, *et al.*(17) have shown that anaerobic glycolysis in rat heart slices which have been thoroughly oxygenated before incubation leads to an accumulation of lactic acid as indicated by chemical analysis and CO_2 production from the buffer. This apparent contradiction of our results is probably the result of different pre-treatments of the slices. In the present study the slices were collected in nitrogen-CO_2-gassed buffer rather than in oxygen-gassed buffer. The demonstration that hexosemonophosphate accumulates in the skeletal muscle of rats killed by decapitation or similar means(18), led us to believe that hexosemonophosphate was the accumulated end product of the observed glycogen breakdown. Determination of the hexosemonophosphate content of the heart slices at the various time periods showed a high concentration but no accumulation of this intermediate. The possibility that the failure of lactic acid to accumulate may reflect the operation of an alternate pathway of carbohydrate metabolism, such as those known for hexose(19) and pyruvate(20), has not been investigated. The report(17) that lactic acid accumulates as expected when the tissue has been thoroughly oxygenated or, as here, when incubation of the tissue takes place *in situ*, leaves some question as to the physiological significance of such a pathway.

Experimental. Five- to six-month-old Supplee white rats of both sexes were used. All preparations were carried out at 0-3° unless otherwise indicated.

In vitro incubations: The rats were beheaded and the heart removed immediately

TABLE I. Disappearance of Glycogen and Lactic Acid *In Vitro.*

Sample	Elapsed time, min.	Glycogen, μg/100 mg*	Lactic acid, μg/100 mg	CO_2, μl/flask
Pre-equilibration	0	273 (8)	75 (8)	
Post-equilibration	10	138 (4)	57 (4)	
Post-incubation†	40	20 (4)	46 (4)	0 (24)

* Wet wt.

† Incubations at 25° or 38°. Data typical of either temperature. Numbers in parentheses are No. of separate analyses or observations.

to an appropriately gassed buffer solution (pH 7.4 Krebs Ringer bicarbonate or phosphate (for acetate study) buffers(9)). After about 10 seconds the heart was removed, the auricles and major vessels trimmed off, and 0.5 mm slices prepared with a Stadie slicer (10). The first slice was discarded and the others placed in a fresh gassed buffer solution. The slices were blotted dry on filter paper and 100-300 mg (depending on the experiment) transferred to 3 ml of gassed buffer in standard Warburg vessels. The vessels were equilibrated at 25°C or 38°C under flowing nitrogen-CO_2 (95%-5%) or nitrogen (acetate study) for 10 minutes and then incubated 30 minutes by the standard Warburg technic. The reaction was stopped by the addition of 0.3 ml 35% trichloroacetic acid (TCA) or 0.3 ml 24 *N* H_2SO_4 (acetate study). The flask contents were then homogenized, centrifuged (except acetate samples) and the supernatant analyzed.

In situ incubations: Rats were beheaded and the heart removed from the chest at the indicated time to gassed buffer solution. The washed heart was cut in half and one-half homogenized in a fresh buffer solution containing TCA. Lactate analyses were performed on the supernatant while glycogen was determined on the remaining half heart.

The following analytical methods were employed: glycogen(11), lactate(12), glucose and reducing substances(13,14,15), fructose (16), hexosemonophosphate(17), pyruvate (18), acetate(19), acetoin(20), triosephosphates(21) and citrate(22).

Summary. An anomalous situation has been encountered in a study of the endogenous carbohydrate metabolism of heart. It is believed that this anomaly, the failure of lactic acid to accumulate during anaerobic glycogen breakdown, may reflect the operation of an alternate pathway.

TABLE II. Changes in Glycogen and Lactic Acid *In Situ.*

Time after beheading, min.	Glycogen, μg/100 mg	Lactic acid, μg/100 mg	% recovery (glycogen as lactate)
0	249	84	
2	196	175	
5	196	184	
10	46	306	
30	39	335	119.8%

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Effect of N-Allylnormorphine Upon Massive Doses of Narcotic Drugs. (22694)

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Although N-allylnormorphine (nalorphine) is an effective antagonist against a wide spectrum of pharmacological actions of opiates (1,2), and has proven to be clinically useful in cases of narcotic overdosage(3-6), Koppanyi and Karczmar(7) did not succeed in saving mice from lethal doses of morphine by single injections of nalorphine. On the other hand, Unna(8) reported that nalorphine in divided doses reduced mortality in mice receiving 800 mg/kg of norphine, and Gruber (9) showed that even a single dose of the antagonist was protective against morphine sulfate up to 800 mg/kg if the ratio of the two drugs was properly chosen.

The present experiments demonstrate a difference in the protective effect of nalorphine against lethal doses of various opiates and a species difference in the responses.

Experimental. Preliminary experiments were performed in rabbits receiving meperidine hydrochloride by slow intravenous injection. The dose of meperidine used was 25 mg/kg, except in two rabbits in which severe convulsions forced discontinuance of the injection at 15 and 20 mg kg, respectively. The results (Groups I-III, Table I) show that nalorphine, administered subcutaneously 30 minutes before the opiate, was in no case successful in preventing convulsions. In each case where death occurred, respirations ceased at about the same time convulsions set in. Respirations were maintained, however, in the meperidine-injected rabbits pre-treated with 10 mg/kg of nalorphine (Group III). In these, the convulsant action of the opiate persisted for 4 to 10 minutes, and the animals then recovered. Two animals (Group IV) receiving the anticonvulsant agent, phenobarbital, in addition to nalorphine exhibited neither convulsions nor grossly visible respiratory depression when 25 mg/kg of meperidine was injected.

These preliminary observations in rabbits suggested that nalorphine antagonized the respiratory depressant effect of the opiate without markedly influencing the convulsant activity. In Group III, sufficient nalorphine was administered so that respiration did not stop and the animals survived, but the convulsions were as severe as in the previous groups. It appeared, then, that convulsant activity might be one effect of opiates that nalorphine would not antagonize. In order to test this further, experiments were conducted in mice and rats.

For these experiments, male IS32 mice of about 20 g body weight and male Holtzman rats of about 130 g were employed. Table II

TABLE I. Effect of N-Allylnormorphine upon Convulsant and Lethal Effects of Intravenous Meperidine in Rabbits.

Group	Antidote	No. of animals	No. convulsing	No. surviving
I	None	2	2	0
II	Nalorphine, 1 mg/kg	2	2	0
III	Nalorphine, 10 mg/kg	4	4	4
IV	Nalorphine, 10 mg /kg + phenobarbital, 100 mg/kg	2	0	2