Summary. An agar gel electrophoretic method has been described for separation of both nonradioactive and tritiated thymine, thymidine and thymidine-5′-monophosphate. The results obtained by this technique were faster and simpler, but comparable to those of column chromatography of bone marrow fluids. The radioactive samples separated on a microscope coverglass were subsequently scanned using an automatic gas flow counter. This quantitative procedure was useful for the study of nucleosides and nucleotides separated by agar gel electrophoresis.

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Received July 14, 1966. P.S.E.B.M., 1966, v123.

Liver and Plasma Malic Dehydrogenase Activities in the Exercised Rat.* (31552)

J. R. BEATON†

Department of Physiology, University of Western Ontario, London, Ontario, Canada

Nikkila *et al*(1) have reported increased activity of serum malic dehydrogenase (MDH), and of other enzymes, as a consequence of exercise in untrained subjects and, to a lesser extent, in trained athletes Gardner *et al* (2) have reported similar observations with human subjects, noting a relationship of degree and duration of exercise (treadmill) with serum enzyme levels; training *per se* did not alter basal enzyme activities. More recently, we have reported (3) that liver and plasma MDH activities were increased in rats made to swim for 2 hours. Repeated daily exercise (training) caused an increased basal MDH activity in liver but not plasma; in the trained rat, a 2-hour swimming exercise did not elicit a further elevation of MDH activity in liver nor did it elicit an elevation in plasma. Neither acute nor repeated exercise caused any alteration in liver or plasma glutamic-pyruvate transaminase activities.

The lack of unanimity in observations from several laboratories on effects of exercise on enzyme activities indicates that several factors are involved in enzyme response. Critz(4) and Gardner *et al*(2) have provided evidence that the degree and duration of exercise are important in determining enzyme response. Another possible factor is the nature of the exercise; whereas some workers have used swimming, others have used treadmill running. A difficulty with swimming exercise is that it is not possible to quantitate. Further, at least on removal from the water, swimming could induce some degree of cold exposure which might alter enzyme activities. The present experiments were undertaken to investigate liver and plasma MDH in rats exercised on a treadmill to compare observations with those made using the swimming exercise(3).

Materials and methods. In these experiments, young male rats of the Wistar strain weighing 240-270 g were used. Prior to experimentation, the rats were housed in individual wire cages at an environmental temperature of 24 ± 1°C with laboratory chow and drinking water provided *ad libitum*. Exercise consisted of making the rats run on a circular treadmill at a speed of 1044 meters per hour; the exercise period consisted of 2 hours' continuous running and a total running

* This investigation was supported in part by a grant from Defence Research Board of Canada.

† Medical Research Associate of Medical Research Council of Canada.

TABLE I. Effect of Exercise (Treadmill, 2 Hours) on Plasma Malic Dehydrogenase Activity (MDH) in the Rat. Results expressed as mean ± S.E.M. for 6 rats.

Group	Plasma MDH, units/ml plasma
A Control	110 ± 12.5
B Acute exercise	452 ± 67.5
C Trained	218 ± 15.0
D Trained & exercise	249 ± 20.1
Probability, P	A *vs* B <.001. A *vs* C <.001.

distance of 2088 meters. During exercise, drinking water was freely available. Food was removed from non-exercised rats for 2 hours prior to sacrifice so that food intakes would be comparable with those of exercised animals. Following intraperitoneal injection of sodium pentobarbital, blood was removed by cardiac puncture, heparinized, and plasma was separated. A liver sample was taken and a 0.2% homogenate in cold phosphate buffer (pH 7.4) was prepared immediately. MDH activities were determined in plasma and liver by adaptation of the ultraviolet spectrophotometric method of Siegel and Bing(5).

In the first experiment, comparison was made of plasma MDH activities among: Group A, 6 non-exercised rats; Group B, 6 rats subjected to a single 2-hour treadmill exercise; Group C, 6 rats exercised for 1 hour on the treadmill on 11 occasions in 16 days but not on the day of killing (trained rats); Group D, 6 rats exercised as Group C but subjected to the 2-hour exercise on the day of killing (trained-exercised rats).

To investigate the possible effect on MDH activities of cold temperature as might be encountered in swimming exercise(3), an experiment was carried out in which 6 rats were wet with water to simulate the swimming condition and placed in a cold room at 6°C for 1 hour. Comparison was made of their liver and plasma MDH activities with those of 6 control animals maintained at 24 ± 1°C.

Results. As shown in Table I, acute exercise on the treadmill for 2 hours caused significant increase in plasma MDH activity compared with non-exercised control animals. Training resulted in a significant increase of basal plasma MDH activity. A 2-hour exercise period with trained animals did not cause a further significant elevation of plasma MDH activity.

It would appear from the data of Table II that cold exposure, under the conditions of this experiment, caused a small but significant increase of liver MDH activity but did not alter plasma MDH activity.

Discussion. From the results reported here, it is apparent that acute exercise on a treadmill caused a significant increase of plasma MDH activity as did swimming exercise(3). Indeed, whereas swimming exercise caused an increased plasma MDH activity of approximately 80%(3), treadmill exercise caused an increase of about 300%. Similarly, the increase of basal plasma MDH activity consequent upon treadmill training (107%) was greater than the insignificant increase which we observed with swimming training (20%). As with swimming exercise(3), acute treadmill running did not cause an elevation of plasma MDH activity in trained rats. It would seem from these observations that treadmill exercise under our conditions, represents a more severe exercise than does swimming for a 2-hour period. Unfortunately, the latter exercise cannot be quantitated with any accuracy.

The degree of cold exposure encountered in the present experiment would be at least as great as that encountered in the swimming exercise(3). The observation that cold exposure (1 hour at 6°C) caused a small but significant increase of liver, but not plasma, MDH activity suggests that, in swimming exercise, effects of cold probably are not important in determining the enzyme response to the exercise.

Possible factors in the increased activity of serum of plasma enzymes induced by exercise include: increased permeability of the cell membrane, hypoxia, and increased en-

TABLE II. Effect of Exposure to Cold (6°C for 1 Hour) on Liver and Plasma Malic Dehydrogenase Activity (MDH) in the Rat. Results expressed as mean ± S.E.M. for 6 rats.

Group	Liver MDH, units/mg wet tissue	Plasma MDH, units/ml plasma
A Control	217 ± 8.30	92 ± 10.6
B Cold-exposed	267 ± 20.1	96 ± 10.1
Probability, P	<.02.	

zyme synthesis. The present experiment does not provide information from which the mechanism of increase can be determined. Increased enzyme synthesis might account for the increased MDH activity observed with training but would not seem to be a likely mechanism in the increased activity associated with acute exercise. Whatever the mechanism, it is noteworthy that, in the rat, an increased activity of plasma MDH is elicited by two quite different forms of exercise, namely swimming and treadmill running and as stated previously(3), the failure of acute exercise to increase plasma MDH activity in the trained animal offers possibility of using plasma MDH activity as a criterion of training.

Any form of exercise, particularly if unusual to the rat, could constitute a form of stress and this, rather than the physical activity *per se*, might conceivably cause the observed alterations of enzyme activities. It would seem worthwhile to investigate the possible role of stress by carrying out experiments inducing stress in the absence of physical activity, using adrenergic blocking agents, and using various hormones. Such experiments are currently planned.

The author is indebted to Miss R. Childs and Miss M. J. Gooley for their able technical assistance.

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Received July 22, 1966. P.S.E.B.M., 1966, v123.

Sodium and Potassium Excretion in Rats Treated Chronically with Morphine.* (31553)

CLAUDE R. MARCHAND† AND JAMES M. FUJIMOTO
Department of Pharmacology, Tulane University School of Medicine, New Orleans

Several investigators have shown that rats developed tolerance to the antidiuretic action of narcotic analgetics with ensuing polyuria as treatment was continued (1,2,3,4). Since the acute antidiuretic action of morphine in the rat is mediated in part by release of antidiuretic hormone(5), one suggestion is that this tolerance may arise by virtue of the rats becoming less responsive to endogenous antidiuretic hormone(1,2,3). Chronically morphine treated rats were less sensitive to the antidiuretic action of vasopressin(1,2). Also Shimai *et al*(1) found decreased concentrations of ADH in the blood and decreased Gomori staining granules in the hypothalamo-hypophyseal system. Newsome *et al*(3) working with levorphanol found that the blood from tolerant rats which were polydipsic and polyuric bioassayed for large concentration of ADH-like material. If indeed this material were ADH, the hypothesis of tolerance through a change in ADH mediated mechanism would be further supported.

Since sodium and potassium ion depletion is known to diminish responsiveness to vasopressin(6), the present study investigated the excretion of these ions in rats developing tolerance to the antidiuretic effect of morphine.

Material and methods. Sprague-Dawley male rats weighing between 250 and 280 g were treated subcutaneously at 8 a.m., 4 p.m. and 12 p.m. with morphine sulfate, 8 mg/kg. This t.i.d. dose was increased by 8 mg/kg every day, except on the fifth day when the increase was 16 mg/kg. By the 15th day, the

* This investigation was supported by grant AM 6515-04 from Nat. Inst. of Arthritis and Metab. Dis., USPHS.

† This investigation was in partial fulfillment of the Ph.D. degree and was performed while this investigator was on USPHS Training Grant 2T1 GM363. His present address is Dept. of Pharmacology, Faculty of Medicine, Univ. of Montreal, Canada.