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Effect of Temperature (and Substrate Concentration) on Chick Lactate Dehydrogenase Activity.* (31621)

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It has been reported that total lactate dehydrogenase (LDH) activity of several human tissues reacts more similarly toward pyruvate and lactate at 25°C than expected from marked differences in substrate inhibition at this temperature between isolated and purified LDH 1 and 5(1). Furthermore, at 37°C LDH 5 closely resembled LDH 1 in extent of substrate inhibition. It was concluded that these results are incompatible with the theory that differences in the degree of isozyme inhibition have resulted in the predominance of LDH 5 in anaerobic tissues and LDH 1 in aerobic tissues.

Previously we have reported that at 30°C it was possible to distinguish between the activities of chick lens LDH isozymes at high and low pyruvate concentrations, and that the results were in agreement with the starch gel data(2). The purpose of the present experiment was to test the effect of temperature on LDH activities of chicken heart, breast muscle, and LDH 1 and LDH 5 isolated from these tissues respectively.

Heart and breast muscle of 1 year old chickens was obtained immediately after death, and homogenized in a Waring blendor at 4°C using one gram of tissue to 3 ml of 0.9% saline. The homogenates were centrifuged at 20,000 *g* for 30 minutes. Enzyme activity in the supernatant was assayed on a Beckman DB spectrophotometer at 340 mμ at 25°C, 30°C and 40°C. The temperature of the reaction chamber was stabilized by the use of a temperature controlled water bath. The final dilution of heart muscle supernatant was 1:600 and 1:1200 for breast muscle.

The assay mixtures, as described previously, were at pyruvate concentrations of 3.3×10^{-4} M and 6.6×10^{-3} M respectively (2). Sufficient activity was obtained by using .05 ml of the diluted supernatant, and the assay volume was kept constant at 3 ml. To isolate LDH 1 and LDH 5, 8 samples of heart muscle supernatant and breast muscle supernatant (1:4 dilution) were subjected to electrophoresis on starch gel for 16 hours. The section of the gel containing LDH 1 and that containing LDH 5 was cut out and the enzyme eluted.

The effect of temperature on the activity of heart and breast muscle supernatant measured at high and low pyruvate concentrations is shown in Fig. 1. At all temperatures breast muscle showed greater activity at high pyruvate concentration than at low. With increasing temperatures there was greater activity at both pyruvate concentrations, but this increase was especially marked at high pyruvate concentration measured at 40°C over that at 25°C, while at low pyruvate concentration the increase was small. Heart muscle was also more active at higher temperatures, but the increase was slight compared to that of the breast muscle. Furthermore, the difference between activity at high and low pyruvate concentrations was more marked at 25°C than at 40°C for heart muscle. The data obtained for isolated LDH 1 and LDH 5 (Fig. 2) corresponded to that obtained for the heart and breast muscle respectively.

The results of this experiment show that the LDH in the supernatant of chicken breast

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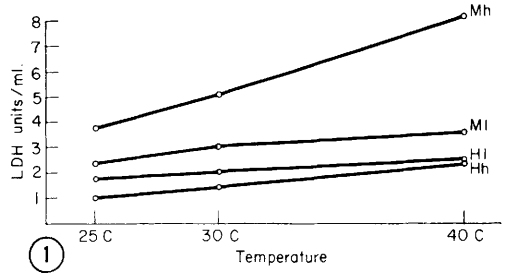

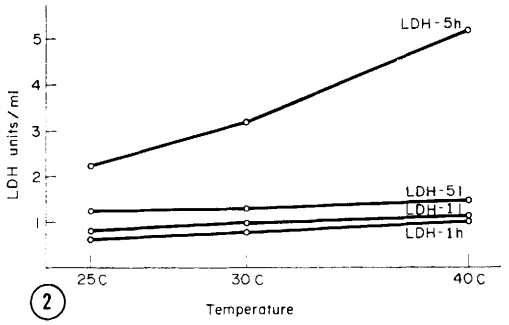

FIG. 1. Effect of temperature on LDH activity. M = breast muscle, H = heart muscle. l = 3.3×10^{-4} M pyruvate, h = 6.6×10^{-3} M pyruvate. One unit of LDH equals the amount of enzyme utilizing 1 μmole of NADH per minute.

FIG. 2. Effect of temperature on LDH 1 and LDH 5 at high and low pyruvate concentration. The individual isozymes were obtained by elution from starch gel after electrophoresis of heart and muscle supernatants for 16 hr at 4°C. h = 6.6×10^{-3} M pyruvate, l = 3.3×10^{-4} M pyruvate.

muscle homogenate, and isolated breast muscle LDH 5 both have greater activity at high pyruvate concentration than at low, especially at the physiological body temperature of the chicken (40°C). The LDH in the supernatant of a chicken heart muscle homogenate, and isolated LDH 1 react best at low pyruvate concentration, the difference, however, is less marked at 40°C. These data are in accord with the observation of Cahn *et al* (3) on the differential sensitivity of LDH 1 and LDH 5 to pyruvate concentration.

Summary. Spectrophotometric analysis of chick lactate dehydrogenase activity showed that the LDH in breast muscle supernatant and isolated LDH 5 had greater activity at high pyruvate concentration than at low. The difference was more marked at 40°C than at lower temperatures. Heart muscle LDH, and isolated LDH 1 reacted best at low pyruvate concentration. The difference was less marked at 40°C than at lower temperatures.

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Production of Gonadotrophin Antibodies in Mouse Peritoneal Fluid.* (31622)

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The production of antibodies against protein hormones has provided the basis for sensitive and specific assays for these hormones. A variety of animal species has been used to produce antibody, ranging from the common rabbit to guinea pigs, sheep, goats and horses. Use of the mouse as a source of antibody has been limited primarily because of the difficulty in obtaining large amounts of serum. Munoz(1), however, reported that antigens (egg albumin or beef serum albumin) mixed with Freund's adjuvant and injected intraperitoneally into mice caused the development of large amounts of peritoneal fluid (ascites fluid) containing specific antibody in high concentration. Subsequent in-

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