vanced to telophase (Table II). This possibility, however, can not be supported without additional data obtained at earlier times after injection.

The main points of this report are the following: First, the administration of cortisol in 1000 γ doses, as well as that of 9α-fluorocortisol in 10 γ doses, results in a significant reduction in number of mitoses in pinna epidermis. Second, doses of the compounds which exhibit significant antimitotic effects do not change significantly the proportions of mitotic stages. Accordingly, these compounds may not interfere with the mitotic process *per se*, but rather, they may act upon some aspect of interphase. Third, a comparison of those doses of cortisol and of 9α-fluorocortisol which exhibit antimitotic activity supports the suggestion that the substitution of a fluorine atom in the 9α position results in a marked enhancement of the antimitotic activity of cortisol. The fluorine-substituted compound has more than 10 times the activity of cortisol, under the conditions of this study. This suggestion is in keeping with the enhancement of other biologic activities of cortisol by fluorine-substitution in the 9α position(7-13).

Summary. Mitotic activity in pinna epidermis of male ZBC mice was studied under environmental conditions standardized for evaluation of 24-hour periodicity, during the period of day of relatively high mitotic activity. Under these circumstances, antimitotic effects, exerted apparently during interphase, were noted for hydrocortisone and for 9α-fluorohydrocortisone. The latter compound had more than 10 times the antimitotic potency of the former.

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Plasma Enzyme Actvity in Myocardial Infarction in Dog and Man.* (22344)

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Increases of plasma transaminase(1) and lactic acid dehydrogenase in liver disease(2) and myocardial infarction(3) suggested the probability of the release of other enzymes from the myocardium or the liver as a result of hypoxia or tissue necrosis. This laboratory has been interested in the immediate changes following experimental myocardial infarction in dogs. For example, a higher concentration of pyruvate in coronary sinus than in arterial blood (negative myocardial balance of pyruvate) was observed within the first thirty minutes following coronary embolization(4). This phenomenon has also been observed in hemorrhagic shock(5) and in ventricular fibrillation(4). It is likely that the reduction in coronary flow which immediately follows

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embolization of the coronary arteries is responsible for the metabolic changes. Furthermore, myocardial ischemia leads to necrosis, with concomitant changes in cell permeability and loss of enzymes into the blood. The present report is concerned with variations in the plasma levels of transaminase, isomerase, aldolase, and malic acid dehydrogenase in dogs with experimental coronary occlusion and in man suffering from myocardial infarction.

Methods. Pyruvate was determined by the method of Friedemann and Haugen(6). Experimental coronary occlusion was produced by following essentially the method of Agress (7). However, human albumin was substituted for gum acacia as a vehicle for suspension of the plastic spheres (size of spheres: 325 mu; amount injected: 2 mg/kg weight). This modification was necessary since it was found that gum acacia alone can produce infarction with typical pathologic and biochemical alterations. Plasma transaminase activity was determined by the method of Karmen *et al.*(1) and plasma aldolase by that of Bruns (8); the latter is expressed as micrograms of triose phosphate formed per ml of plasma per hour at 38°C. Plasma isomerase activity was determined by the method of Bodansky(9) expressed in activity units as therein described. The determination of malic acid dehydrogenase in the presence of other unidentified enzymes and metabolites presents considerable difficulties. The reaction of malate, in the presence of DPN and malic acid dehydrogenase comes to a rapid standstill presumably due to accumulation of oxalacetic acid. The addition of glutamate, in the presence of excess transaminase removes this difficulty(10). Since, however, the reaction is greatly in favor of oxalacetate $\longrightarrow$ malate(9) and involves only a few components, it was tested as follows: To 0.1 - 0.05 ml of plasma was added 2 ml of 0.1 M phosphate buffer pH 7.55 and 0.1 ml $DPNH_2$ (2.5 mg/ml); the volume was made up to 3.0 ml with distilled water. After 10-15 minutes 0.1 ml of 0.5 M oxalacetic acid (Sigma) at pH 7.0 in 0.1 M phosphate buffer was added. The change in optical density at $\lambda 340$ of $DPNH_2$ was observed for 3 to 4 30-second periods to assay the reaction rate. The oxalacetate solution was prepared just before use and was kept in a glass stoppered test tube in ice water. In addition to decarboxylation reactions(11) which would result in some lactic acid dehydrogenase activity being measured simultaneously, oxalacetate can enter many

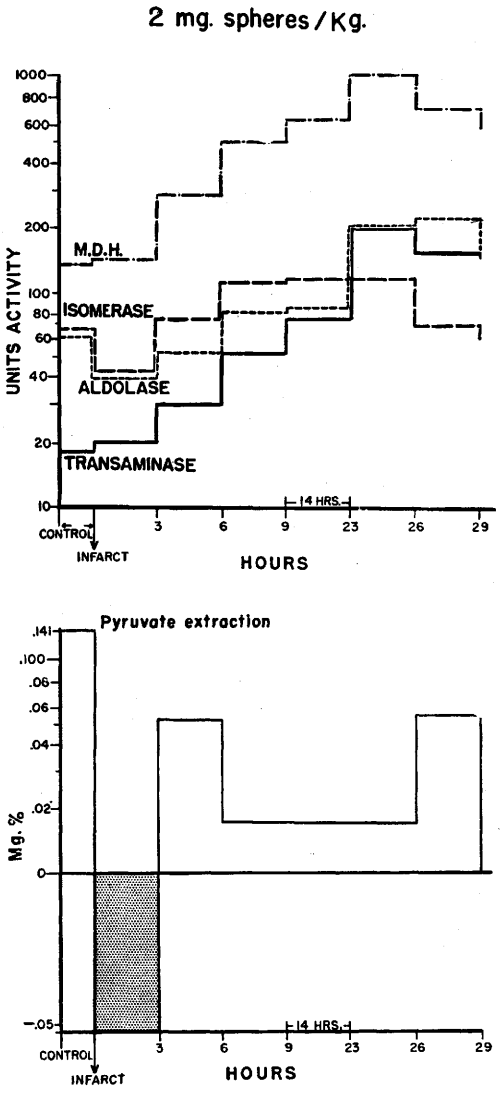

FIG. 1. Variations in plasma activity of malic acid dehydrogenase, isomerase, aldolase, and transaminase. Changes in plasma enzyme activity are correlated with myocardial pyruvate extraction. It may be seen that there is an immediate drop in myocardial pyruvate extraction; however, the negative myocardial balance is of short duration. Enzyme activity in plasma commences to rise 3 hr after coronary embolization. Peaks in plasma enzyme activity occur 24 hr following embolization.

other pathways(12). While a sharply rising activity with pH has been reported for lactic acid dehydrogenase(2) with a maximum at pH 9.0, the reaction described has an almost constant activity between pH 7.5 and 8.1. The range of normal activity units and the standard deviation are also less than that reported for lactic acid dehydrogenase(3). Since the reaction may not be entirely specific for malic acid dehydrogenase, it will be referred to in quotation marks.

Results. The average, range and standard deviation for the plasma concentrations of these enzymes in normal dogs are as follows: transaminase, 23, (10-37), 9; "malic acid dehydrogenase", 108, (42-195), 47; isomerase, 33, (16-68), 17; aldolase, 42, (24-68), 18.

Fig. 1 is representative of the results obtained after coronary embolization. In a series of 9 experimental animals, 7 were examined and showed postmortem evidence of myocardial infarction. Four animals of the experimental group were subjected to coronary sinus catheterization and showed negative myocardial pyruvate balances. In 3 of these animals myocardial pyruvate balances were negative within 15 minutes after the infarction and then became positive (Fig. 1). One showed a negative balance at 3 hours. It is seen from Fig. 1 that the significant release of enzymes began by the third hour. At 24 hours the increase in enzyme activity was maximal for all animals. At that time, the coronary flow and the coronary vascular resistance which had been diminished immediately following embolization of the coronary arteries had returned to their control levels. At 48 hours a rapid fall in plasma enzyme activity ensued. After 24 and 48 hours both transaminase and "malic acid dehydrogenase" showed increases of comparable degree, with aldolase and isomerase exhibiting increases of a lesser order.

Table I gives the normal values on a small series of humans without hepatic or cardiac disease. In man the average value, range and standard deviation for "malic acid dehydrogenase" and isomerase were less than those seen in the dog. Results obtained in patients with myocardial infarction are also illustrated in Table I. As compared to the dog, aldolase activity was least elevated in patients with myocardial infarction. Isomerase activity in plasma increased following in general the trend of transaminase. Similar findings were obtained with "malic acid dehydrogenase."

The high activity of "malic acid dehydrogenase" in normal plasma suggests the possi-

TABLE I. Levels of Plasma Transaminase, Malic Acid Dehydrogenase and Isomerase in Normals and Patients with Myocardial Infarcts.

		Transaminase	Malic acid dehydrogenase	Isomerase* (Bodansky units)	Aldolase as triose-phosphate formed per ml plasma per hr
Normals	Avg	25 (9)	79 (7)	13 (10)	26 (7)
	Range	15–39	50–104	6–19	21–32
	Stand. dev.	8	20	4	5
L.J.	2/ 5	185	333	111	59
	2/ 6	150	200	30	32
	2/ 7	47	163	15	25
	2/ 8	37	120	12	21
V.A.B.	2/ 6	155	300	51	41
	2/ 7	95	208	20	32
P.E.	1/30	123	200	94	65
P.P.	2/11	100	647	374	39
J.P.	2/11	34	240	54	24
	2/13	62	110	7	24
	2/14	43	152	9	24

* Normal values reported on a series of 87 patients by Bodansky: Avg, 20; range, 8–40; stand. dev., 8.

Figure in parenthesis represents No. of animals.

bility that the transaminase reaction could be run adequately without the addition of the expensive and labile malic acid dehydrogenase enzyme preparation. Omitting this enzyme has little effect on the results of the determinations on stored dog plasma, when the transaminase levels were at the limit or beyond the established normal range. However, when transaminase activity is determined in stored human plasma, the omission of malic acid dehydrogenase leads to results which are low and inconsistent.

The system used to determine "malic acid dehydrogenase" activity by means of the rate of oxidation of DPNH in the presence of oxalacetic acid and plasma may not measure malic acid dehydrogenase activity exclusively. Nevertheless, the rate of $DPNH_2$ oxidation appears to be closely related to the increase of activity of other enzymes, which are observed following myocardial infarction. The reaction appears to be particularly useful because of its simplicity and rapidity. Similarly, the isomerase determinations in myocardial infarction may be of clinical importance since they can be carried out without specialized equipment.

Summary. 1. Increased plasma levels of transaminase as well as aldolase, isomerase and "malic acid dehydrogenase" activity occurred in dogs after experimental myocardial infarction and in humans suffering from coronary occlusion. 2. Plasma levels of all enzymes were highest 24 hours after experimental myocardial embolization and had fallen rapidly at 48 hours. 3. Normal human values for plasma isomerase and "malic acid dehydrogenase" were significantly lower than those found in normal dog plasma. 4. In dogs following experimental myocardial embolization plasma transaminase, "malic acid dehydrogenase" and aldolase were increased to a greater extent than isomerase. In humans the plasma aldolase activity was comparatively less elevated than that of transaminase, "malic acid dehydrogenase" and isomerase. In both man and dog, coronary occlusion resulted in comparable increases of plasma transaminase and "malic acid dehydrogenase" activity. In dogs with coronary embolism, there was a reversible decrease in coronary flow myocardial oxygen usage, coronary vascular resistance as well as an early reversible negative pyruvate balance. At 24 hours the coronary flow and myocardial oxygen usage had returned to normal. 5. The negative myocardial pyruvate balance appeared to be an immediate response of an hypoxic myocardium. In contrast, the release of enzymes into the plasma was correlated with muscle necrosis. 6. A rapid and simple method is described for the determination of "malic acid dehydrogenase" activity in plasma.

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