problems raised by the coordination model for aggregation which has been proposed. Coordination requires that the apposing surfaces are within 15A—and probably closer to 5A—at the coordination sites. It is difficult, with evidence now available, to evaluate distances between cells in cell-cell aggregates, but it seems unlikely that large areas of the surfaces are juxtaposed this closely. However, in cell-matrix-cell aggregates, the restriction on spacing of cells is eliminated. The hypothesis also requires that water of hydration on monovalent cations can be displaced by other ligands, both 'fixed' on the cell surface and 'mobile.' A variety of studies(6,7), have now indicated that the structure of water is ordered in the vicinity of these macromolecules and this ordering may be such as to restrict hydration of surface-bound cations. The coordination hypothesis has explicitly stated that there is a coupling between the state of aggregation of cells and electrolyte exchange. In this process, highly conformable macromolecules situated at the cell surface which orient water and other electrolytes (8,9) may be expected to play a significant role.

Summary. 1. Partial and slow dissociation of mouse liver *in vitro* that occurs on treatment with phosphate indicates a K^+ independent factor involved in aggregation of cells. 2. The presence of low concentrations of hydrocortisone during dissociation in phosphate causes the aggregation into long fibers of an intercellular material. 3. Dissociation effected with phosphate has been attributed to a 'destabilization' of the intercellular matrix.

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Effects of Hyperbaric Oxygenation on Lactic Dehydrogenase Isoenzymes in Rats in Noble-Collip Drum Shock.* (30955)

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Inasmuch as dehydrogenases are affected in the brains of shocked rats(1) and hyperbaric oxygenation (OHP) increases survival after challenge in the Noble-Collip drum(2), it appeared of interest to study the behavior of dehydrogenases in different organs of the rat in shock and in OHP.

This report compares electropherograms of isoenzymes of lactic dehydrogenase (LDH) from brain, heart, kidney, liver, mucle (quadriceps) and plasma of the rat under the conditions mentioned.

Materials and methods. Young adult male Wistar rats weighing 190 to 250 g were used. Shock was produced by 640 turns in the Noble-Collip drum(3), with the fore and hind limbs of the rats fettered with adhesive tape. They were unfettered immediately after drumming and while one group was left in room air, another group was placed in a hyperbaric tank, a horizontal cylinder, 65 cm long, inner diameter 20 cm.‡

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‡ Built by Dixie Manufacturing Co., Baltimore, Md.

Pure oxygen was supplied from a commercial source and the pressure was quickly and evenly increased and maintained at 3 atmospheres for 3 hours with oxygen constantly flowing through the tank. The pressure was measured with standardized gauges the first of which was attached to the cylinder and the second one to the outflow from the pressure chamber. All animals were decapitated 3 hours after drumming. The controls consisted of animals which had not been drummed.

Tissue preparations. Blood was collected in heparinized tubes and plasma was separated by centrifugation of heparinized blood in a clinical centrifuge. Brain, heart, liver, and quadriceps muscle were quickly dissected, washed with a small amount of cold 0.9% saline to free them from adhering blood, chopped under ice, and homogenized in Potter-Elvehjem homogenizers of 10 ml capacity fitted with teflon pestles using Tris-barbiturate buffer pH 8.9, ionic strength 0.05 M. Two ml of the buffer were used to homogenize 1.5 g of brain, 0.7 g of heart, and 1.0 g each of kidney, liver and muscle. The homogenates were spun in a Spinco preparative ultracentrifuge for 40 minutes at 127,000 *g*. The supernates were diluted 1:25 with Tris-barbiturate buffer.

Electrophoresis and identification of LDH isoenzymes: Samples of the diluted tissue extracts and undiluted plasma were electrophoresed with marker dye on strips of cellulose polyacetate, 2.54 × 17.15 mm§ after these had been soaked in the buffer. The electrophoresis was accomplished within 40 minutes at 315 V, 15 mA‖ in a chamber supplied by Gelman Instrument Co. After electrophoresis each strip was covered with a strip of cellulose polyacetate which had been soaked in a freshly prepared solution containing 12 mg Tetranitro Blue Tetrazolium, 280 mg sodium-L-lactate, 25 mg nicotinamide adenine dinucleotide (Sigma) and 600 μg of phenazine methosulfate (Sigma) in 20 ml of water. The double strips were sandwiched between glass plates and incubated for 30 minutes at room temperature in the dark. The zones of LDH isoenzymes thus became visible as purple bands.

The developed strips were washed 2 or 3 times in 5% acetic acid to remove background color and were dried between layers of filter paper. They were then made transparent by soaking in heavy mineral oil and mounted between microscope slides. The zones of enzyme activity were numbered beginning with that zone which had migrated farthest from the origin. The optical densities of the zones were determined with an integrated reflectance densitometer.¶ These values were analyzed for statistical significance.

Results. Table I gives the results with the P values for the differences between controls and shock, and between controls and shock followed by treatment with hyperbaric oxygen.

Comparison. Significant increases occur in shock in isoenzyme No. 1 for heart, kidney and plasma; the increase in brain is statistically not significant. This isoenzyme seems to disappear from muscle; however, muscle tissue of the control animals contains little of it. Isoenzyme No. 2 shows, in the shock situation, a statistically significant decrease in kidney and muscle. The slight decrease in brain is statistically not significant. Plasma shows a significant increase of this isoenzyme in shock. Isoenzyme No. 3 is significantly decreased in brain, heart, kidney and muscle as a result of shock. It is also increased in plasma. Isoenzymes 1, 2 and 3 are not observed in the liver extract. Isoenzyme No. 4 decreases significantly in the kidney only due to shock. It is significantly increased in plasma only. Isoenzyme No. 5 is significantly increased in muscle of shocked animals only; other organs, namely, brain, heart, and kidney also show an increase which is however, statistically not significant. There is no change in this isoenzyme in the liver. This fraction is significantly decreased in plasma after shock.

Treatment of shocked rats with OHP produces some significant changes from the isoenzyme electropherograms obtained in shock;

§ Sepraphore III, Gelman Instrument Co., Ann Arbor, Mich.

‖ Heathkit Power Supply, Model PS-3.

¶ Chromoscan made by Joyce Ltd.

TABLE I. Distribution of Lactic Dehydrogenase-Isoenzyme as Percent of Total.

	Tissue	N	Peak value 1	P	Peak value 2	P	Peak value 3	P	Peak value 4	P	Peak value 5	P
Control	Brain	16	25.9		20.1		16.5		22.3		15.1	
	Heart	21	29.8		34.0		23.1		10.3		2.9	
	Kidney	20	33.4		23.1		7.9		12.4		23.3	
	S. muscle	16	3.4		7.2		8.5		20.8		60.2	
	Liver	6	0		0		0		9.2		90.8	
	Plasma	5	1.8		3.6		3.6		14.4		76.5	
Shock	Brain	16	28.4	*<.2*	18.2	*<.05*	13.6	*<.05*	23.2	*<.4*	16.6	*<.4*
	Heart	21	36.4	*<.01*	33.6	*<.8*	17.8	*<.001*	7.7	*<.2*	4.6	*<.2*
	Kidney	20	42.3	*<.01*	18.7	*<.02*	2.2	*<.001*	9.4	*<.05*	27.1	*<.1*
	S. muscle	16	0	*<.001*	2.4	*<.02*	4.0	*<.02*	18.9	*<.3*	74.8	*<.01*
	Liver	6	0		0		0		13.3	*<.5*	86.7	*<.5*
	Plasma	5	6.4	*<.05*	9.2	*<.05*	12.9	*<.01*	22.5	*<.01*	48.2	*<.01*
Shock and OHP	Brain	16	21.1	*<.001*	19.8	*<.4*	20.5	*<.01*	20.9	*<.2*	17.7	*<.1*
	Heart	21	29.8	NSD	33.3	*<.7*	21.3	*<.3*	10.5	*<.9*	5.1	*<.05*
	Kidney	20	30.7	*<.2*	24.1	*<.3*	8.4	*<.7*	12.8	*<.7*	23.8	*<.8*
	S. muscle	16	.3	*<.01*	.9	*<.001*	2.4	*<.001*	17.7	*<.2*	78.7	*<.001*
	Liver	6	0		0		0		0		100	*<.01*
	Plasma	5	5.6	*<.2*	7.7	*<.2*	8.1	*<.1*	18.5	*<.02*	60.1	*<.05*

Legend: N = No. of animals in group.
Italicized "P" values indicate significant differences in comparison to control values, i.e., P <.05 as campared with controls.

the following fractions are significantly changed back toward normal levels: 1 and 3 in the heart, and 1, 2, 3, and 4 in the kidney. Isoenzymes of liver and of muscle are not affected by OHP treatment after shock. The response of brain isoenzymes to OHP is not consistent.

Discussion and conclusions. Significant changes in lactic dehydrogenase isoenzyme patterns of plasma and of extracts of several organs were seen in rats which had been shocked in the Noble-Collip drum. The changes were observed 3 hours after drumming. Increase of lactic dehydrogenase in the plasma of dogs and of rabbits in hemorrhagic shock has been described by Vessell, Feldman and Frank(4), who have also carried out electrophoresis of lactic dehydrogenase in dog plasma and have observed 4 fractions. No difference in the relative activity of each fraction was seen in shock: all fractions were elevated to approximately the same degree.

We find that after administering tumbling shock to rats the isoenzymes of LDH measured in extracts from different organs are differently affected. In agreement with numerous other observations the plasma enzyme is elevated throughout suggesting damage to several organs and leakage of enzymes into the blood stream. Individual organs, however, do not show uniform responses. In the kidney the response to both challenge and recovery are most outspoken: 3 of 5 isoenzymes show significant increases and one significant decrease, in shock. In the OHP treated shocked rat all differences of kidney isoenzymes from the normal animal disappear. Two of the isoenzymes from shock heart preparations show return to the normal range after OHP treatment. No consistent effects were noted by us in the brain in shock, with and without subsequent OHP treatment.

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