stroma serum. Granules of platelets fail to take up the dye at low temperatures and at an acid pH, both of these conditions being reversible. Platelets stored for 10 days at 5°C had normal staining of their granules.

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Developmental Changes in Dehydrogenase Activities in Rabbit Eggs.* (32586)

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Cytochemical techniques have been employed to study developmental changes occurring during maturation, fertilization and cleavage of several vertebrate eggs. The cytochemical characteristics of many of mammalian egg components, DNA, RNA, lipids and polysaccharide staining-positive materials, depend on the species and stage of development(2,10,11,12,14).

The metabolic pathways are markedly unique at different developmental stages (8,15). Studies in mammalian eggs have demonstrated the existence of multiple enzyme systems responsible for the respiratory activity in the rat(1,18,20) and in the rabbit (5,6,7,17). Some enzymes (phosphates and dehydrogenases) have been histochemically identified(3,4,13,21) and their activities appear to vary with the stage of cleavage; moreover their intracellular localization is different in the inner cell mass and trophoblast cells, perhaps reflecting the specific function of the cells.

To date, little is known about the dehydrogenase systems in the egg. Thus, this investigation presents a histochemical analysis of various dehydrogenase activities or their role in energy production during very early developmental stages of the rabbit embryo. From the data the possible role(s) of these enzymes in metabolism is discussed.

Material and methods. Adult New Zealand White rabbits weighing 7 to 10 lb were bred to fertile bucks, injected intravenously with 15 i.u. of HCG (Hafez, 1961) and sacrificed at specified times.

The different dehydrogenase activities were determined by a modification of Nachala's method (1957); the concentration of substrate was 0.1 M, the concentration of the electron acceptor was 0.2%, and the incubation period was 30 to 90 minutes. 5 to 7 eggs were used per culture vessel, which contained 0.3 to 0.5 ml of the incubation medium (1 ml of 0.1 M phosphate buffer at pH 7.4 and 1 ml of 0.2% nitroblue tetrazolium per

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TABLE I. Intensity of Various Dehydrogenase Activities in the 2-Cell Stage Rabbit Embryo.

Substrate	No. of embryos	Number of embryos staining			
		Zero	Weak	Moderate	Strong
None	10	10	0	0	0
Glucose 1-phosphate	10	0	8	1	1
Glucose 6-phosphate	11	0	6	5	0
Glutamate	10	0	2	7	1
Glycerophosphate	10	0	7	3	0
Lactate	10	0	7	3	0
Malate	11	0	8	3	0
Isocitrate	10	0	8	2	0
Succinate	10	0	0	2	8

Each sample was incubated for 90 min. The ova were examined microscopically at 400×.

0.3 ml of the desired substrate solution). The samples were incubated at 37°C in a moisture chamber. In controls, eggs were incubated in a similar medium, but without the substrates. Also, formazan formation from the chytochrome system was inhibited by addition of cyanide.

After incubation, the eggs were fixed with 10% buffered formalin for 10 minutes and then transferred with a capillary pipette (inner diameter of 200 to 300 μ) to an orange peel immersed in 70% alcohol. The orange peel-eggs were embedded in paraffin and sectioned at 7 or 10 μ. The sections were then examined microscopically to localize formazan formation.

Results. Dehydrogenase activity in the two-cell egg. The dehydrogenase activities in the eggs were classified into 1 of 4 arbitrary categories (zero, weak, moderate, and strong) according to the intensity of the reduced dye's blue color. These classifications were all relative to eggs (control) incubated under identical conditions, except in the absence of any exogenous substrate. The various activities found in 2-cell eggs incubated in specific substrates are summarized in Table I. Succinic dehydrogenase gave the strongest color development, glucose 6-phosphate dehydrogenase and glutamic dehydrogenase were intermediate and the remaining enzymes gave a relatively weak reaction.

Examination of the formazan that occurred in the cytoplasma of the egg (50×) showed that the blue pigment was evenly distributed throughout the vitellus. When examined under a higher magnification (400×), it was clear that the formazan formation took place primarily in the vitelline granules. Furthermore, the difference in color intensity was apparent in the different, as well as the same substrates.

Dehydrogenase activity in the morula and blastocyst. The various dehydrogenase activities in the 8 to 16-cell eggs were not detectably different from those in the 2-cell stage. However, at both the morula and blastocyst stages, the activities were marked, as measured by the intensity of the color and the time required to stain the samples (Table II). All of the activities examined at this time gave either moderate or strong color development, except malic dehydrogenase which gave a weak to mederate reaction.

Each of the dehydrogenase activities was stronger in the inner cells of the blastocyst than in the trophoblastic cells. However, it was frequently observed that the amount of formazan was different in blastomeres and in some cases, the color development took place on only one side of the blastomere.

Dehydrogenase activity in the unfertilized ova. The various dehydrogenase activities were also present in unfertilized ova recovered from oviducts at 8 to 10 hours after ovulation (Table III). Lactic dehydrogenase and glucose 6-phosphate dehydrogenase gave strong reactions, succinic and glutamic dehydrogenase gave moderate intensity and the remaining enzymes gave either weak or no activity. Comparable unfertilized ova 30 hours after ovulation had less glutamate and succinate dehydrogenase activity than those 8 to 10 hours post ovulation; all dehydrogenase activity disappeared 48 hours after ovulation. (Table III).

Discussion. The formation of formazan in the presence of substrates such as glucose 1-

TABLE II. Intensity of Various Dehydrogenase Activities in the Morula and Blastocyst Stage.*

Substrate	Stage of embryo†	No. of embryo	Number of embryo staining			
			Zero	Weak	Moderate	Strong
Glucose 1-phosphate	M	11		0	1	10
	B	12		0	0	12
Glucose 6-phosphate	M	10		0	3	7
	B	10		0	1	9
Glutamate	M	10		0	2	8
	B	10		0	1	9
Glycerophosphate	M	—	None observed	—	—	—
	B	10		0	2	8
Lactate	M	—		—	—	—
	B	12		0	2	10
Malate	M	11		8	3	0
	B	12		0	8	4
Isocitrate	M	10		0	1	9
	B	11		0	3	8
Succinate	M	10		0	1	9
	B	11		0	2	9

* The morula stage was 4 days old; the blastocyst stage was 5 days old.
† The morula stage embryos were stained 15-30 min.

phosphate, glucose 6-phosphate, glycerophosphate, glutamate, isocitrate, malate, lactate and succinate, suggest that these substrates are oxidized by enzymes that can be classified as dehydrogenases. It is difficult to establish that formazan is formed by specific enzymes concerned with each of the substrates. Another assumption (perhaps more critical) that is made for the following discussion is that the rate of transfer of both the dye and exogenous substrates from the incubation solution into the cell is constant and, moreover, is independent of the stage of development.

With the present histochemical technique, the various dehydrogenase enzymes investigated were detectable in unfertilized eggs, and in 2-cell, 8 to 16-cell, morula and blastocyst stages. However, the amount of the various enzymes present or the amount of substrate (whichever is rate limiting), as judged by the intensity of the stain and length of time required to stain the eggs, was independent of each other and furthermore, appeared to be related to the stage of development. The lowest activity for any of the enzymes was in unfertilized eggs, (Table IV). It should also be pointed out that the amount of activity in eggs incubated in the absence of any exogenous substrate also increased during development, indicating the enhancement of endogenous metabolism.

TABLE III. Intensity of Various Dehydrogenase Activities* in the Unfertilized Ova.

Substrate	Hours after ovulation		
	8–10	30	48
Glucose 1-phosphate	W	W	Z
Glucose 6-phosphate	S	M	Z
Glutamate	M	M	Z
Glycerophosphate	Z	Z	Z
Lactate	S	W	Z
Malate	W	Z	Z
Isocitrate	W	W	Z
Succinate	M	M	Z

* S, strong; M, moderate; W, weak; Z, zero.

Oxygen consumption of rabbit and rat embryos gradually increases between the 1-cell and morula stages, whereafter, it increases tremendously(1,5,6,18,19,20). It has been suggested that rabbit oviducal eggs utilize glucose mainly *via* the hexose monophosphate shunt prior to the blastocyst stage and after this stage, the Embden-Meyerhof-Parnas (EMP) and tricarboxylic acid (TCA) cycles are the predominant carbohydrate catabolic pathways (8,15). The absence of malic and isocitrate dehydrogenase activity and the presence of a considerable amount of glucose 6-phosphate dehydrogenase activity in the pronuclear stage contrasted to a high activity of all 3 enzymes in the blastocyst support this proposal of differentially active pathways. The presence of

TABLE IV. Intensity of Various Dehydrogenase Activities* in Rabbit Embryo at Different Pre-Implantation Stages.

Substrates	Developmental stage					
	1-cell	2-cell	8-16 cell	Morula	4-day blastocyst	5-day blastocyst
Glucose 1-phosphate	M	M	M	S	S	S
Glucose 6-phosphate	M	M	M	M	S	S
Glycerophosphate	Z	W	S	M	S	S
Glutamate	S	S	S	S	S	S
Lactate	M	M	M	S	S	S
Malate	W	W	M	M	S	S
Isocitrate	W	W	S	S	S	S
Succinate	S	S	S	S	S	S

* S, strong; M, moderate; W, weak; Z, zero.

high amounts of succinic dehydrogenase at all stages of development, however, appears to be in conflict with these ideas and is presently not understood. The appearance of glycerolphosphate dehydrogenase, which catalyzes the formation of an EMP intermediate from glycerol phosphate, is also temporally correlated with the appearance of the EMP pathway.

Even though the O_2 consumption increases between the 1-cell and morula stage, several studies directed toward enhancing the respiratory rate with TCA and EMP intermediates have shown no detectable effect(7, 19). The present findings that many of the intermediates of the 2 pathways do get oxidized, presumably by the dehydrogenases mediating O_2 consumption, suggest that another enzyme or enzymes related to O_2 consumption is rate-limiting.

The present results suggest that the appearance of enzyme activities in rabbit embryos is correlated with their development and carbohydrate metabolism. Even though it is tempting to assume that the increases in enzyme activity reflect *de novo* synthesis, the possibility of enzyme activation cannot be ruled out. Further experimentation may clarify this point.

Summary. Rabbit embryo (pronuclear to blastocyst stage) dehydrogenase activities (as measured by the reduction of nitroblue tetrazolium) were measured histochemically in the presence of the following substrates: glucose 1-phosphate, glucose 6-phosphate, glycerolphosphate, lactate, glutamate, succinate, isocitrate and malate. The various dehydrogenases were found at all stages of development, not, however, in equivalent amounts. The succinic and glutamic dehydrogenase activities in the one-cell and 8 to 16-cell stage eggs were qualitatively higher than the other enzymes at these stages, and furthermore quantitively constant. At the morula stage, all of the enzymes analyzed had increased slightly, and after blastocyst formation, they had increased dramatically. In unfertilized ova, the activity of the dehydrogenases decreased with the progression of time. After 48 hours, all activity had disappeared completely.

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Inhibition of a Hepatic Microsomal Enzyme System After Head X-Irradiation of Rats.* (32587)

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Studies in the recent past from various laboratories have shown that the activity of hepatic drug metabolising enzymes can be influenced by a variety of factors: for example, species, sex, age, nutritional status, drugs, chemicals and alterations in hormonal level (1,2). Exposure to ionising radiation may also be added to this long list of modifiers of enzyme activity. Hietbrink and DuBois(3) have demonstrated the inhibitory effect of x-irradiation on the development of hepatic microsomal enzymes that metabolise organophosphates. Using weanling rats irradiated to the head alone they also found evidence for an abscopal inhibitory effect. Recent investigations in our laboratory have revealed that *in utero* exposure of rats to low doses of x-irradiation (25 or 50 R) produced in the male offspring a significant suppression of the development of the hepatic microsomal enzyme system that metabolises hexobarbital (4). Irradiation at 21 days of age (total body or head alone) also suppressed the developmental increase of enzyme activity normally seen in the males, but to a lesser extent than in the prenatal series. Inhibition of the enzyme system, both direct and abscopal, was observed in the adult males also, but only at radiation doses above 500 R (total body or liver) or above 1,000 R (head alone).

Methods. Sprague-Dawley rats were used in these studies. *In the prenatal series,* rats received 25 or 50 R of x-irradiation (at 11-12 R/min) to the pelvic region on the 14th day of gestation. The offspring were examined at 10, 20, 40, and 80 days of age. *In the postnatal series,* 21 day old male rats received a single dose of 200 R (49-50 R/min) to the

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