Differentiation of alkaline phosphatase and glucose-6-phosphate dehydrogenase in rat yolk-sac

By E. MARSHALL JOHNSON & RALPH SPINUZZI

From the Department of Anatomical Sciences, University of Florida College of Medicine

INTRODUCTION

During early gestation in the rat, at the time of rapid embryonic differentiation and prior to the full formation of the chorioallantoic placenta, the function of 'placental' nutrition was attributed to the yolk-sac by Brunschwig (1927). Such a paraplacental function of the yolk-sac would assume that nutrients pass through the parietal wall into the yolk-sac cavity and thence into the embryo via the visceral yolk-sac epithelium and its underlying vitelline vessels. Supporting this concept were the findings of Everett (1935) who demonstrated in 13-day embryos that toluidine blue was able to pass into the omphalomesenteric vessels more rapidly than it could reach the umbilical veins via the chorioallantoic placenta. Furthermore, the visceral entodermal cells appeared to exert some selectivity in that trypan blue did not pass into the embryo but was localized in the apical cytoplasm. More recently, Padykula, Deren & Wilson (1966) demonstrated that the rat yolk-sac concentrated both vitamin B12 and vitamin B12 plus intrinsic factor throughout most of gestation. Also, the rabbit yolk-sac has been shown by Deren, Padykula & Wilson (1966) to take up several amino acids. During late gestation, from day 19 to term, Brambell & Halliday (1956) demonstrated that maternal antibodies pass into the rat fetus via the yolk-sac entoderm. The relative importance of the yolk-sac as a 'placental' organ late in gestation, however, is questioned by the facts that ligation of the omphalomesenteric vessels at this period produces no detrimental effect to the embryo, and ligation of the umbilical vessels results in embryonic death (Noer & Mossman, 1947).

Both at the light microscopic and the ultrastructural levels Dempsey (1953) and Wislocki & Dempsey (1955) observed that the yolk-sac has the characteristics of an absorptive membrane which undergoes continual morphological and biochemical changes during gestation; these data were confirmed by

1 Authors' address: Department of Anatomical Sciences, University of Florida College of Medicine, Gainesville, Florida 32601, U.S.A.
Padykula (1958) and Padykula & Richardson (1963). Consistent with these observations were the findings of alteration in the electrophoretically mobile enzyme forms of lactate and malate dehydrogenase, acid phosphatase, and non-specific esterase throughout gestation (Johnson & Spinuzzi, 1966). Furthermore, we demonstrated that when the pregnant rat was fed a synthetic diet deficient in folic acid but containing the potent teratogen 9-methyl pteroylglutamic acid the enzymic differentiation of the yolk-sac was selectively altered.

The purpose of the present study was to determine the enzymic differentiation of the visceral yolk-sac with respect to alkaline phosphatase and glucose-6-phosphate dehydrogenase, both of which are enzymes implicated by Moog & Wenger (1952) and Karnofsky (1962) as being important in transport phenomena. The study included visceral yolk-sacs from normal control pregnancies and from pregnant rats treated with the folic acid antagonist 9-methyl pteroylglutamic acid in order to determine the extent to which this teratogenic procedure was able to alter the normal differentiation of these enzymes.

METHODS

To initiate the period of folic acid deficiency, pregnant black-hooded rats were intubated with 1 mg of 9-methyl pteroylglutamic acid (9-mePGA) on day 10 of gestation. The vitamin deficiency (Johnson, Nelson & Monie, 1963) was maintained from day 10 to 13 by a diet of purified foodstuffs which contained 10 mg/100 g of 9-mePGA. The deficiency was terminated by feeding a diet lacking the antagonist but containing a high level of the crystalline vitamin.

Experimental and normal control rats were killed on days 10, 11, 12, 13, 14, 16, 18 and 20 of gestation. The visceral yolk-sac was dissected free from the embryos and all remnants of the parietal yolk-sac and chorioallantoic placenta were removed. The visceral yolk-sacs were pooled, homogenized in an equal volume of triple-distilled water and subjected to zone electrophoresis in either starch or polyacrylamide gels.

The equipment and procedure for electrophoretic separation in starch (Solomon, Johnson & Gregg, 1964) and polyacrylamide gels (Johnson & Spinuzzi, 1966) have been described previously. Alkaline phosphatase was visualized by standard biochemical means (Johnson, 1965) with sodium α-naphthyl acid phosphate. Isozymes of glucose-6-phosphate dehydrogenase were visualised with 0.1 M glucose-6-phosphate as substrate employed in conjunction with 0.2 mg/ml. phenazine methosulfate, 10 mg/ml. nicotinamide adenine dinucleotide phosphate (NADP), and 1 mg/ml. nitro-blue tetrazolium in 0.2 M phosphate buffer at pH 7-4. Since photographs of similarly stained gels have been published previously (Johnson & Spinuzzi, 1966), semidiagrammatic representations of the zymograms were prepared for Figs. 1 and 2.
RESULTS

In yolk-sacs from both normal control and PGA-deficient pregnancies, a maximum of three mobilities of alkaline phosphatase were resolved by electrophoretic separation in starch gel (Fig. 1). In the controls band number 1 was uniform in zone width and staining intensity throughout gestation, but band number 2 appeared on day 13, remained a uniform density to day 16, and then increased in staining activity on days 18 and 20. Zone number 3 was the slowest moving of the electrophoretic mobilities. It was uniformly present from days 10 to 13, only weakly reactive on days 14 and 16 and undetected thereafter. Anodal migration (+0) from the origin was detected throughout gestation.

The effects of the folic acid deficiency on this group of enzymes were observed first on day 11. This was only 24 h after commencement of the PGA-deficient regimen, but the anodal migration at the origin was absent and band number 1 was undetected in over half of the homogenates. There also occurred a precocious disappearance of band 3 on days 13 and 14 but staining returned to this area by day 16 and attained a greater intensity on day 18 than was encountered in any other stage. It should also be noted that by day 18 normal material no longer showed any staining in this area. Origin staining was absent on day 11 and was above the control levels on both days 16 and 18.

A maximum of four molecular species of different mobilities of glucose-6-phosphate dehydrogenase in normal control yolk-sacs was detected by electrophoretic separation in polyacrylamide gel. Band 4 appeared to be the major isozyme throughout gestation (Fig. 2). It reached its maximum staining reaction during days 12-14; although this zone persisted through term, it was a minor
band on day 16 and day 20. Isozymes numbers 1, 2 and 3 all attained their greatest staining intensity during the third week of gestation. A positive origin staining (+0) was seen throughout gestation at a relatively constant level but because of the nature of the homogenate employed in these polyacrylamide gels it was not considered a definite molecular species.

The effects of folic acid deficiency on the glucose-6-phosphate dehydrogenases were extensive. This teratogenic treatment from days 10 to 12 produced a 24 h delay in the appearance of isozyme number 1 and an acceleration of its dis-

![Diagram](image)

Fig. 2. Diagrammatic representation of glucose-6-phosphate dehydrogenases from visceral yolk-sac as seen in acrylamide gels.

appearance by day 20. In addition, the staining intensity by this molecule was reduced from the control levels during the third week. A similar reduction was caused in zones 2 and 3 in the third week. A more consistent reduction in the PGA-deficient yolk-sac was detected in isozyme number 4 which equalled or exceeded the intensity of the controls only on days 16 and 20.

**DISCUSSION**

Previous histochemical observations by Padykula (1958) have demonstrated a rise in alkaline phosphatase activity between days 12 and 14 of gestation. (The day of finding sperm in a smear of vaginal contents is considered as day 0 of gestation. Literature cited has been altered to standardize comparison of gestational age.) A second increase in activity was detected on day 16 but was followed by a precipitous decline by day 19. These findings were confirmed by the present study to the extent that the greatest number of electrophoretic bands were present on days 13 to 16. The precipitous decline in activity observed by Padykula, however, would appear to result from the decline of anodal (+0) staining and the absence of band 3 toward term. Alkaline phosphatase activity was localized in the brush border and apical cytoplasm of the columnar visceral...
entoderm, and in agreement with the observations of Moog & Wenger (1952), one may conclude that the visceral yolk-sac epithelium has at least part of the enzyme repertory anatomically associated with transport.

The teratogenic insult used in the present experiments appears to affect the yolk-sac during the critical period of paraplacental function. The delay in appearance of band 1 may constitute a major deficit in this enzyme repertory at the period in which the yolk-sac epithelium appears to be the only available site for transport. The asynchronous appearance and disappearance of molecular forms of alkaline phosphatase in response to the folic acid deficiency is in accord with previously reported observations for a number of other enzymes (Johnson, 1965; Johnson & Spinuzzi, 1966).

In an extensive study of glycogen storage in the rat placenta (Padykula & Richardson, 1963) the principal period of glycogen accumulation within the yolk-sac epithelium was found to be between days 14 and 17 of gestation. Between 17 and 20 days there was a progressive loss of glycogen within the epithelial cells, although these cells never became devoid of glycogen. The suggestion that the yolk-sac serves the function of a fetal liver is difficult to resolve with the fact that glucose-6-phosphatase could not be demonstrated within these cells. The suggestion of an intrinsic use for this glycogen (Johnson & Spinuzzi, 1966) is supported by the high activity of lactate dehydrogenase within the visceral entoderm. The present findings suggest that the phosphogluconate oxidative pathway (hexose monophosphate shunt) may also be an active pathway within the visceral yolk-sac. The shunting enzyme, glucose-6-phosphate dehydrogenase, reaches its over-all peak activity between days 12 and 18. In addition, staining activity by this enzyme decreases markedly on day 20 which is in agreement with the time of depletion of glycogen from the visceral yolk-sac. Karnovsky (1962) has presented evidence that the principal energy source for phagocytosis in neutrophils is via glycolysis and the phosphogluconate oxidative pathway. Therefore, the presence of active glycolytic and shunt enzymes within the yolk-sac suggests that these enzymes may be responsible for the energy needs of transport across this membrane. The observation that the major band of glucose-6-phosphate dehydrogenase reaches a high level of activity quite early in gestation, which is the period of postulated placental function for the yolk-sac, also supports the latter suggestion. The teratogenic insult during early gestation when rapid organogenesis is occurring produces an enzymic pattern completely foreign to the embryo. A situation other than normal also occurred in late gestation as indicated by the precocious absence of band 1. Such paraplaxia sequence has been reported previously both on a morphological and biochemical level (Johnson, 1965).

The possibility exists that the foreign enzyme repertory found in the experimental animals may result in the interference of operation of the hexose monophosphate shunt as a source of 5-carbon sugars for nucleotide and nucleoside synthesis. The fact that a folic acid-deficient diet causes interference with DNA
synthesis (Nelson & Asling, 1962) as well as an independent interference with cytoplasmic RNA synthesis (Johnson, 1964) lends support to this suggestion.

In view of previous work and the present findings, it appears that the over-all peak activities of alkaline phosphatase and glucose-6-phosphate dehydrogenase do not occur on days 10 and 11 when the yolk-sac is probably the major pathway for embryonic-maternal exchange. This fact, however, does not disprove the concept of paraplacental transport by the yolk-sac, but instead raises questions for future investigation, i.e. which molecular species of an enzyme are required for transport, in what direction is the transport at the different gestational ages, and what is the nature of the substances transported?

SUMMARY

1. Electrophoretic and biochemical techniques were applied to homogenates of the visceral yolk-sac of rat embryos at various gestational ages.
2. Alkaline phosphatase was shown by starch gel electrophoresis of normal yolk-sacs to undergo specific sequential changes from day 10 to day 20 of gestation. The isozyme repertory on any given day of gestation was achieved by deletions from, or additions to, that of the previous day.
3. Glucose-6-phosphate dehydrogenase was shown, by polyacrylamide gel electrophoresis of normal yolk-sacs, to achieve early in gestation a qualitatively constant number of enzyme forms. Quantitative changes among the enzyme forms occurred as gestation proceeded.
4. A teratogenic folic acid deficiency resulted in the delayed appearance of some enzyme forms as well as the premature disappearance of others in the two enzyme systems studied. The effects of the treatment were selective in that not all of the enzyme forms of the treated material were affected.

RÉSUMÉ

Différenciation progressive de la phosphatase alcaline et de la glucose-6-phosphate déhydrogénase dans la vésicule ombilicale du rat

1. Des homogénates de la vésicule ombilicale d'embryons de rats, à des stages variés de la gestation ont été soumis à des techniques électrophorétiques et biochimiques.
2. Il a été ainsi démontré par l'électrophorèse sur gel d'amidon que dans la vésicule ombilicale normale, la phosphatase alcaline subit des changements progressifs du 10e au 20e jour de la gestation. Le répertoire isozymique de chaque jour de la gestation différait de celui du jour précédent soit par des délétions soit par des additions.
3. Par l'électrophorèse sur gel polyacrylamide de vésicules ombrilicales normales, il a été montré que la glucose-6-phosphate déhydrogénase se diversifie tôt dans la gestation, en un nombre qualitativement constant de formes en-
Differentiation in yolk-sac

zymatiques. Des changements quantitatifs de ces formes enzymatiques ont été démontrés au fur et à mesure de la progression du développement.

4. Une déficience tératogénique en acide folique a provoqué un retard dans l'apparition de certaines formes enzymatiques ainsi que la disparition prématurée d'autres de ces formes en ce qui concerne les deux systèmes enzymatiques étudiés. Les effets de ce traitement ont été sélectifs en ce sens que toutes les formes enzymatiques n'ont pas été affectées.

This research was supported by NIH Grants GM 579 and HD 00109.

REFERENCES


(Manuscript received 5 June 1967, revised 18 September 1967)