not appear to predispose the animals to spontaneous infection in the abnormal kidney.

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Hepatic Glucose Production in Developing Chicken Embryo. (25894)

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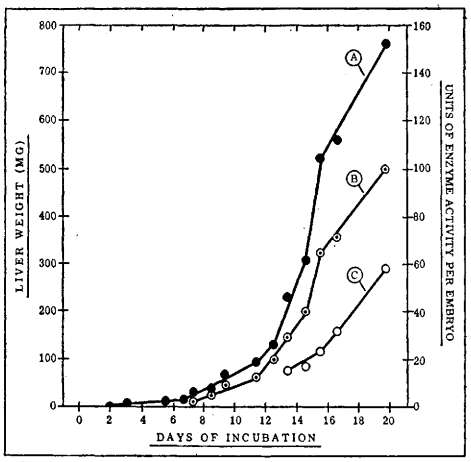
Previous studies by Nemeth(1) and Weber and Cantero(2) have demonstrated an absence of glucose-6-phosphatase activity in fetal guinea pig and rat liver. This enzyme is also absent or of low activity in Von Gierke's disease and is believed to be the primary enzymatic defect which leads to glycogen storage and hypoglycemia characteristic of this disease(3). Since glucose-6-phosphatase activity is not found in fetal liver of 2 species examined the suggestion has been made that Von Gierke's disease may represent a prolongation of the fetal state(1). On 3 occasions we were able to examine specimens of human fetal liver obtained by therapeutic abortion during the fourth to fifth months of gestation. In all cases appreciable glucose-6-phosphatase activity was found. These studies have prompted an investigation of embryonic development of hepatic gluconeogenesis and hepatic glucose formation. Since the avian embryo develops as an isolated system without a constant supply of glucose from the maternal circulation, it seemed possible that glucose-6-phosphatase activity would appear early in embryonic development. In the present study the onset and extent of glucose-6-phosphatase activity during development of the chicken embryo has been investigated. The activity of β-glucuronidase was also determined since both glucose-6-phosphatase and β-glucuronidase are localized in the microsomal subcellular fraction of liver. The overall metabolic potential of embryonic liver for gluconeogenesis and glucose formation was investigated. Liver slices were incubated with pyruvate-2-C^{14} and incorporation of labeled carbon into glucose, glycogen, fatty acids and CO_2 was determined.

Materials and methods. Chicken embryos of one to 7 days of development were rapidly separated from the yolk sac and chorioallantoic membrane and homogenized in water. After the seventh day of incubation the liver was large enough to be quantitatively removed from the embryo. Livers were frozen in a dry ice bath, weighed and homogenized with water, 1 ml/100 mg of tissue. Microsomes were prepared from pooled samples of livers from several dozen 13- to 15-day chicken embryos, homogenized in cold 0.25 *M* sucrose, and the microsomal fraction collected in the Spinco Ultracentrifuge (Model L) according to the method of Hogeboom *et al.*(4). The microsomal fraction was resuspended in isotonic KCl to make a final volume equivalent to 1.0 to 1.5 ml/g of liver. *Enzyme assays.* Glucose-6-phosphatase activity was assayed by release of inorganic phosphate from glucose-6-phosphate incubated in citrate buffer, pH 6.5 as previously described(5). Glucuronidase activity was determined using phenolphthalein glucuronide as a substrate and acetate buffer, pH 5.0 according to the method of Fishman and Bernfeld(6). *Liver incubations.* Approximately 500 mg (wet wt.) of tissue slices were incubated with 5 ml of Hastings medium(7) containing pyruvate-2-C^{14} (40 mM) as added substrate. All flasks were gassed for 5 minutes with 95% oxygen: 5% carbon dioxide and incubated for 90 minutes at 38°. The incubation medium was analyzed initially and finally for glucose(8) and pyruvate(9) and the tissue slices analyzed for glycogen(10) and fatty acids(11). For radioassay pyruvate was plated and counted as the dinitrophenylhydrazone, glucose as phenylglucosazone, CO_2 as barium



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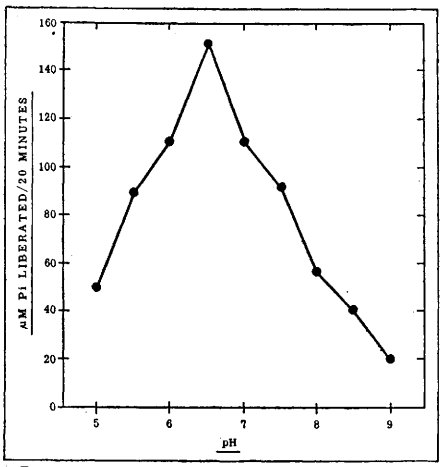

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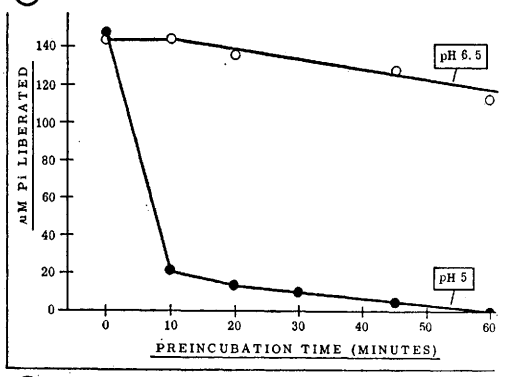

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FIG. 1. Glucose-6-phosphatase and β-glucuronidase activity in developing chicken embryos. Curve A, glucose-6-phosphatase; 1 unit equals amount of enzyme which will split 1 μM of substrate in 20 min. Curve B, liver wt. Curve C, glucuronidase; 1 unit equals amount of enzyme that splits 0.1 μM of substrate in 60 min.

carbonate and fatty acids plated directly on lens paper(11).

Results. Glucose-6-phosphatase activity is present in chicken embryo of 60 or more hours of age (Fig. 1). Since development of the liver begins around the 48th hour it is presumed that the appearance of glucose-6-phosphatase activity is more or less coincident with development of liver cells. That the activity observed in the early embryos was localized in the liver is suggested by the observation that no appreciable glucose-6-phosphatase activity was found in 7-day embryos from which the liver was removed.

Liver glycogen and fat content as well as β-glucuronidase and glucose-6-phosphatase activities of embryonic and adult livers are compared in Table I. Glycogen content of the liver increases during 6th to 21st days of incubation, but markedly decreases during hatching. Fat content of the liver appears to remain relatively constant during the process of embryonic development. Glucose-6-phosphatase and β-glucuronidase activities per g wet liver remain relatively constant, although total activity of both enzymes is increased in proportion to the increase in liver weight. That 2 microsomal enzymes of presumably quite different function increase in direct proportion to liver mass suggests that the hepatic cells formed may be fully functional.

Properties of microsomal glucose-6-phosphatase. To determine whether the microsomal glucose-6-phosphatase activity from embryonic chicken liver was similar in properties to enzyme found in human and rat liver, pH optimum, substrate specificity and inactivity at pH 5 were studied. The pH activity curve (Fig. 2) and substrate specificity (Table II) of chick embryo glucose-6-phosphatase was found to be the same as that observed in human and rat(12) liver.

FIG. 2. pH activity curve—Glucose-6-phosphatase activity of embryonic liver of microsomal fraction expressed as μM of inorganic phosphate (P_i) liberated during a 20 min. assay is plotted as a function of pH.

FIG. 3. pH inactivation of glucose-6-phosphatase from chicken embryo liver microsomes. Activity, expressed as μM of inorganic phosphate (P_i) liberated in a standard assay (pH 6.5) has been plotted as a function of time after preincubation of homogenates at pH 6.5 and 5.0.

TABLE I. Glycogen, Fatty Acid, Glucose-6-Phosphatase and β-Glucuronidase Content of Embryo and Adult Chicken Liver.

No. obs.	Age, days	Embryo wt, g	Liver wt, mg	L/E × 100	Liver: Glycogen, μmoles/g	Liver: Fatty acids, mg/g	Liver: Glucuronidase, mg/g	Liver: G-6-Pase, μmoles P_i/g
4	6- 7	.8 ±0	8 ± 1	1.09 ±.15	5.2 ± 2.7	61.2 ±11.4		
4	8- 9	2.1 ± .1	31 ± 3	1.49 ±.12	18.7 ± 5.6	27.7 ± 5.8		
4	9-10	2.9 ± .1	35 ± 2	1.19 ±.06	51.3 ± 4.0	31.4 ± 7.0		
3	12-13	6.8 ± .1	125 ± 9	1.84 ±.10	22.0 ± 6.3	47.0 ± 4.7	3.5 ± .3	365 ± 2
5	13-14	9.5 ± .5	170 ± 9	1.80 ±.07	73.0 ±10.3	39.4 ± 1.5	4.3 ± .7	355 ± 15
4	14-15	12.6 ± .9	238 ±28	1.88 ±.11	81.2 ±13.3	36.6 ± 2.1	4.4 ± .1	323 ± 16
4	15-16	14.8 ±1.1	293 ±23	1.98 ±.07	160.0 ± 7	41.2 ± 2.9	4.7 ± .8	286 ± 10
5	16-17	20.3 ±1.4	402 ±27	1.98 ±.04	158.0 ±20	35.7 ± 1.5	3.4 ± .7	300 ± 12
2	21-22	43.7 ±2.9*	635 ± 7*	1.46 ±.11*	46.5 ± 5.5*	32.2 ± 1.6*	4.5 ± .2*	307 ± 51*
4	Adult	808 ± 39	16800 ± 500	2.08 ±.07	141.0 ± 16	33.0 ± 1.8		251 ± 15

* Spread instead of stand. error.

Since glucose-6-phosphatase activity of rat liver microsomes is rapidly destroyed below pH 5.5(13), this process was used to separate activity of glucose-6-phosphatase from that of acid phosphatase. When embryonic chicken liver microsomes are preincubated at pH 5 there is a rapid loss of phosphatase activity (Fig. 3), thus eliminating the possible contribution of acid phosphatase to activity measured at pH 6.5.

TABLE II. Relative Rates of Hydrolysis of Various Substrates by Liver Microsomes. Conditions used for hydrolysis of all substrates were the same as those described for glucose-6-phosphate in text. Rates of hydrolysis of various substrates are expressed in relation to glucose-6-phosphate hydrolysis, arbitrarily expressed as 100.

Substrate	Rate
Glucose-6-phosphate	100
Galactose-6- "	28
Glucose-1- "	5
β-Glycerol "	4

Utilization of C^{14} pyruvate. Pyruvate is readily utilized by embryo liver (Table III) and labeled carbon from this substrate is incorporated into glycogen, glucose, fatty acids and CO_2. These data demonstrate the ability of embryo liver to form glucose both by glycogenolysis and gluconeogenesis. The appearance of radioactivity in CO_2 suggests the presence of an active citric acid cycle and respiratory enzymes. The slight incorporation of C^{14} into fatty acids is not too surprising when one considers that the yolk which serves as the energy store for the embryo is primarily lipid.

These studies suggest that early in development the chicken embryo liver is functionally complete with respect to the various parameters measured and probably is capable of releasing glucose with the appearance of the first hepatic cells.

Summary. Hepatic glucose-6-phosphatase activity appears in developing chicken embryo almost simultaneously with appearance of first hepatic cells. Glucose-6-phosphatase and β-glucuronidase activity of embryo increase in direct proportion to liver weight. Properties of embryonic liver glucose-6-phosphatase are similar to those found in adult liver of other species. Studies with liver slices indicate that the embryo liver is capable of glycogenolysis and glucose formation.

TABLE III. Incorporation of Carbon from Pyruvate into Glycogen, Glucose, Fatty Acids and CO_2 by Liver Slices from Embryonic Chickens.

Age of embryo, days	No. obs.	Pyruvate utilized	Glycogen: Net change	Glycogen: Pyruvate to	Glucose: Net change	Glucose: Pyruvate to	Pyruvate to: F.A.	Pyruvate to: CO_2
13	4	111 ± 7	−14.1 ± .5	.9 ± .1	26 ± 2	18 ± 1	.2	18.3 ± .6
15	8	96 ± 9	−55 ±4	.4	54 ± 6	34 ± 3	.2	14 ± .2*

All values as μmoles/g wet liver/90 min. Values with ± sign are mean and S.E.

* Avg of 4 determinations.

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Teratogenic Action of a Hypocaloric Diet and Small Doses of Cortisone.* (25895)

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Cortisone acetate produced cleft palate in offspring of treated pregnant mice(1), in various frequencies depending on genetic and certain non-genetic variables involved(2,3,4). Supplemental treatment with riboflavin, folic acid, protein, or carbohydrate did not influence the incidence of the defect(5). The following study was undertaken to investigate the effect on fetal development of restricted daily food intake and smaller doses of cortisone than have usually been used in the past.

Materials and methods. Mice were young adult B6AF1 females, from the cross C57BL/6J ♀ x A/J ♂. They were bred to A/J males and put into individual cages upon observation of a vaginal plug, a sign to indicate that ⅓ day had passed since conception. Females were weighed at this time and divided into 5 groups of approximately similar weight composition. The groups were treated as follows: Group 1, 0.5 mg, Group 2, 1 mg, cortisone acetate/day for 4 consecutive days, beginning 11⅓ days after conception (called 0.5 and 1 mg cortisone, respectively). Group 3, given a pellet of 2.5 g food/day for 5 consecutive days, beginning 10⅓ days after conception (called restricted diet). Group 4, 0.5 mg cortisone + restricted diet. Group 5, 1 mg cortisone + restricted diet. Cortisone (Merck) was administered intramuscularly in the thigh. The food, Purina Lab Chow, was fed *ad lib.* at all times, except to females when on restricted diet. Fresh tap water was available always. Females were killed at 17⅓ days after conception, *i.e.*, about 2 days prepartum, resorption sites counted, and young removed, dried, weighed to nearest hundredth of g, and examined.

Results. Fig. 1 shows mean weights of 5 groups of females during pregnancy. Females treated only with cortisone continued gaining weight throughout treatment period, but mice fed the restricted diet lost weight early in treatment period, then became stabilized in weight, until *ad lib.* feeding was resumed, and animals given both treatments concurrently lost more weight and for a longer period than those of Group 3. The Fig. also shows that females given the restricted diet attained at time of sacrifice about the same weight as those given cortisone alone, but that animals given combined treatments did not catch up (mean weight in g, at 17⅓ days after concep-

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