To what degree does the test permit us to distinguish between the 2 groups, one schizophrenic and the other non-schizophrenic? Chi-square analysis yields a value well in excess of that corresponding to P value of .001 defining the great unlikelihood that this distribution could have occurred by chance. The distinction between schizophrenics and either non-schizophrenic patients or normals is equally non-fortuitous. All of these differences are more striking than can be accounted for by age and sex differences alone though these factors have not been completely controlled in our study.

As a diagnostic test the reaction has obvious limitations; it does not occur in all schizophrenics, its specificity is open to question, and its reproducibility has not been studied.

Conclusions. A positive result with glyoxylic acid test was obtained in 62.5% of schizophrenic patients, while only 8% of non-schizophrenics gave a positive test. Though non-specific the test affords a statistically significant distinction which cannot be explained by the non-experimental variables. Because of the chance that the test has a significant association with the disease, schizophrenia, further investigation seems warranted.

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Maturation of Succinic Dehydrogenase and Cytochrome Oxidase in Neonatal Rat Kidney. (25093)

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It is well recognized that functional differences exist between the kidneys of immature and adult animals(1). Although differences are apparent in organization and structure of such kidneys(2) integration of this data has not been accomplished. A depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase and depressed oxygen consumption have been recently demonstrated in the kidneys of nephrotic rats by Fisher and Gruhn(3,4). These changes could be correlated with the degree of proteinuria exhibited by these animals although it was not evident whether this enzymatic alteration was a causative factor or a sequel of proteinuria. Since several references(5,6,7) to occurrence of proteinuria in newborns have been made it appeared worthwhile to explore the relationship of proteinuria and these respiratory enzymes in kidneys of maturing rats. Information derived from such a study also appeared pertinent in providing cytochemical evidence concerning differences in renal function of neonates and adults since oxidative enzymes are considered essential for proper function of the tubular portion of the nephron. Developmental changes in renal alkaline phosphatase have been noted previously(8).

Material and methods. Four to six littermates of the Wistar strain were sacrificed at 1, 7, 12, 15, 18, 24, 27, 30, 39, 63 and 72 days after birth. One kidney was immediately frozen on dry ice and the other placed in Zenker's acetic fluid. Blocks of fresh frozen material were stored at –20°C until the last animal was sacrificed and all fresh frozen material was sectioned at 6 μ in a cryostat and simultaneously stained for demonstration of succinic dehydrogenase by the method of Rosa and Velardo(9) and cytochrome oxidase (G-nadi oxidase)(10). Previous experience indicated that storage of fresh frozen tissue for similar periods did not alter the appearance of these enzymes. Sections prepared from tissue fixed in Zenker's acetic fluid were cut at 5 μ and stained with hematoxylin and eosin, periodic acid-Schiff and phosphotungstic acid hematoxylin technics. Each group contained

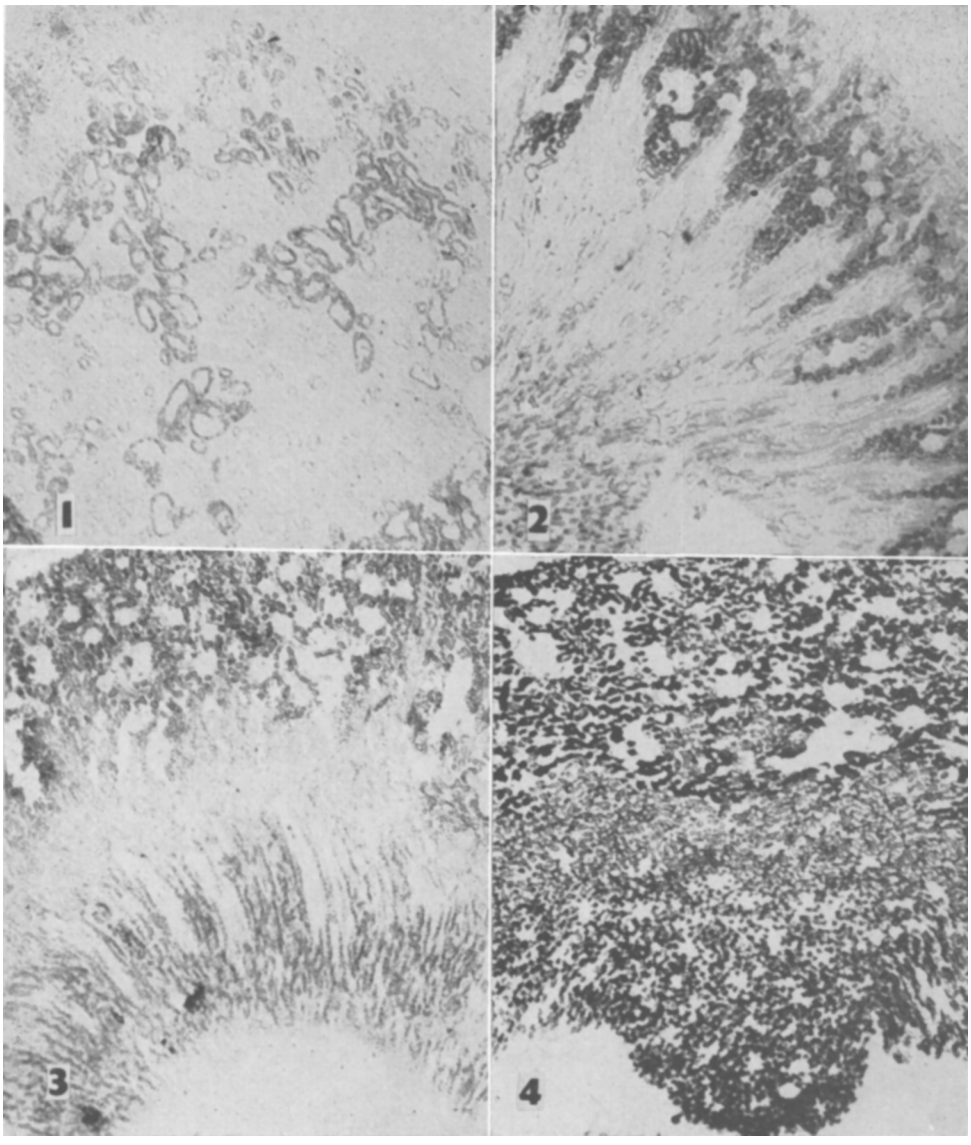

FIG. 1. Succinic dehydrogenase (appearing black) in isolated tubules of cortex of kidney of rat 1 day old. (× 65)

FIG. 2. Succinic dehydrogenase (appearing black) in outer cortical zone with slight reaction in neocortical tubules of neonatal rat 15 days old. (× 30)

FIG. 3. Cytochrome oxidase (appearing black) in cortical zones and moderate activity in outer medullary area corresponding to ascending loops of Henle in neonatal rat kidney 22 days old. (× 30)

FIG. 4. Succinic dehydrogenase (appearing black) with adult distribution and staining intensity in kidney of neonatal rat 39 days old. (× 30)

at least one female. Amount of protein excreted by newborn rats was determined by placing a suture ligature about the external genitalia of groups of 4 rats at 2, 4 and 16 days after birth. Animals were subjected to laparotomy 24 hours later and urine aspirated from their urinary bladders into a clean syringe through a #23 needle. Total protein was estimated according to method of Looney and Walsh(11). Estimation of protein reabsorption within the kidney was performed on 4 newborns 4 days after birth. Each re-

TABLE I. Distribution and Staining Intensity of Succinic Dehydrogenase and Cytochrome Oxidase in Neonatal Rat Kidney.

Age (days)	OCZ	ICZ	OMZ
1	1+ *		
7	2–3+*	±	±
12	2–3+*	"	"
15	2–3+	"	"
18	4+	1+	"
24	"	"	1+
27	"	2+	1–2+
30	"	"	3+
39	"	"	4+
63	"	"	"
72	"	"	"
Adult	"	"	"

* Neocortical tubules negative.

OCZ, outer cortical zone; ICZ, inner cortical zone; OMZ, outer medullary zone.

ceived an intraperitoneal injection of 2.5 mg of Evans blue dye and sacrificed 24 hours later.

Results. Distribution and staining intensity of both succinic dehydrogenase and cytochrome oxidase were similar. A few tubules in the outer cortical zone which includes convoluted segments of proximal convoluted and distal convoluted tubules exhibited faint enzymatic activity 24 hours after birth (Table I and Fig. 1). Tubules in the neogenic area, however, were devoid of such activity until 15 days after birth (Fig. 2). At this time and shortly thereafter tubular components of the outer cortical zone assumed an adult appearance (Fig. 3). Complete maturation of tubules in inner cortical zone corresponding to straight segments of proximal convoluted tubules and portions of the ascending limbs of Henle was not apparent until 27 days of post natal life. The last zone to acquire mature characteristics corresponded to that topographical area comprised of ascending limbs of Henle located in the outer medullary zone at 39 days (Fig. 4). The variable lack of enzymes prior to 39 days was accompanied by absence of mitochondria. Brush borders, as evidenced by periodic acid-Schiff stain, were absent in the proximal convoluted tubules of the neogenic zone but became evident with its maturation. There was no apparent difference in male or female newborns.

Protein excretion/24 hours, as estimated from bladder aspirates following ligation of external genitalia, was 7-15 mg. Our repeated control determinations revealed a normal adult value of 7-29 mg/24 hours.

Kidneys of newborns receiving Evans blue were only faintly stained macroscopically. Their urines also were only mildly colored by the dye. Microscopic examination of fresh frozen sections failed to disclose intracellular droplets. These findings are similar to those of normal adult rats receiving intravenous injections of Evans blue, but are unlike those of nephrotic animals in which renal cortex and urine are intensely stained and blue droplets may be readily discerned within epithelium of portions of proximal convoluted tubules(3).

Discussion. Failure to observe proteinuria or alteration in renal tubular reabsorption of protein in the neonatal rat precludes comment concerning relationship of this phenomenon and depletion of respiratory enzymes noted. However, it does indicate the necessity for reevaluation of references to proteinuria in newborn. It also appears significant in the light of recent electron microscopy relating the ultrastructure of neogenic glomeruli to that observed in glomeruli considered characteristic of the nephrotic syndrome(12).

Absence of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase in various tubular segments of the immature kidney of the rat complements the findings of Cutting and McCance(13) who noted decreased oxygen consumption by such kidneys. Recognition of the segmental development of these respiratory enzymes provides further cytochemical evidence for differences encountered in neonatal and adult renal function and offers an opportunity for more precise physiologic and structural correlation.

Summary. Succinic dehydrogenase and cytochrome oxidase undergo maturation in respect to distribution and staining intensity in the neonatal kidney of the rat. Recognition of this event provides cytochemical evidence of functional immaturity in the neonatal kidney. Proteinuria or altered tubular reabsorption of protein was not observed in newborns of this species.

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Effect of Lipoprotein Lability on Immunological Properties of Human Low Density Lipoproteins.* (25094)

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The effects of aging, freezing and thawing, and heating on immunological properties of human low density lipoproteins were studied to estimate the extent of changes produced by these procedures. Various reports(1-6) have indicated that lipoproteins are labile molecules readily denatured by auto-oxidation and aggregation reactions which may also alter the serological properties of the lipoproteins.

Methods. S_f 3-9 and S_f 10-400 lipoprotein fractions were prepared from human serum by ultracentrifugal flotation procedures(7), characterized by chemical composition(3), and injected into rabbits to produce antisera (7). The lipoprotein antisera did not react with human albumin, γ-globulin, high density lipoproteins, or lipoprotein free serum(7). Agar diffusion and precipitin ring tests were used in the immunological studies. The agar diffusion experiments were performed by plate method of Rheins *et al.*(8). In precipitin ring tests, doubling dilutions of an S_f 3-9 lipoprotein fraction in 0.2 ml volumes were layered over 0.2 ml of anti S_f 3-9 serum in precipitin tubes. These tubes were incubated at room temperature 30 minutes, then observed for appearance of a precipitin ring. Electrophoretic patterns were obtained with Spinco Model R paper electrophoresis system. Duplicate strips were stained with bromphenol blue for protein and sudan black B for lipid. The absorbance of lipoprotein solutions was measured with a Coleman Junior spectrophotometer at 430, 460, and 490 mμ against a distilled water blank. A standard S_f 3-9 lipoprotein fraction containing 1 mg of protein/ml was used in aging, freezing and thawing, and heating experiments. This lipoprotein solution was dialyzed against 15 volumes of 0.15 *M* NaCl containing no ethylenediaminetetraacetic acid (EDTA). In aging studies, S_f 3-9 lipoprotein fractions were stored at 4°C, then titrated at stated intervals. Lipoprotein aliquots were stored with and without aqueous merthiolate, added to a final dilution of 1:10,000, as a preservative. Two procedures were used in freezing and thawing experiments. Procedure I: Five ml of the S_f 3-9 lipoprotein fraction was frozen at –20°C in the deepfreeze and then thawed immediately at 37°C in a water bath. Aliquots were removed for analysis before the first and after first, fifth, and tenth freeze-thaw cycles. Procedure II: Aliquots of an S_f 3-9 lipoprotein fraction, 2.5 ml, were frozen at –40°C in a dry ice-acetone bath, then thawed immediately at 37°C in water bath. Samples were removed for analysis after first, fifth, and tenth freeze-thaw cycles. An S_f 3-9 lipoprotein fraction was warmed at 56°C in water

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