kidney cultures. The virus was filterable, sensitive to heat and organic solvents, and immunologically distinct from a number of other chloroform sensitive agents. Neutralizing antibody often was present in acute phase sera of patients with rubella and regularly increased significantly during convalescence in patients yielding the virus.

Ultracentrifugations were made by Dr. Richard Hartman, Dept. of Molecular Biology, of this Institute, and cultures for PPLO, by Dr. Ruth Whittler, Dept. of Bacteriology.

1. Buescher, E. L., Parkman, P. D., Artenstein, M. S., Halstead, S. B., *Fed. Proc.,* 1962, v21, 466c.

2. Parkman, P. D., Artenstein, M. S., McCown, J., Buescher, E. L., *ibid.,* 1962, v21, 466d.

3. Hunter, D. H., Blair, E. B., Rust, J. H., *Bact. Proc.,* 1962, 81.

4. Vogel, J., Shelokov, A., *Science,* 1957, v126, 358.

5. Koher, J., *Nature,* 1959, v183, 1055.

6. Feldman, H. A., Wang, S. S., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 241.

7. Andrewes, C. H., Horstman, D., *J. Gen. Microbiol.,* 1949, v3, 290.

8. Hitchcock, G., Tyrrell, D. A. J., *Lancet,* 1960, v1, 237.

9. Hamparian, V. V., Ketler, A., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 444.

10. Ho, M., *New England J. Med.,* 1962, v266, 1313.

11. Weller, T. H., Neva, F., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 215.

Received July 20, 1962. P.S.E.B.M., 1962, v111.

Coenzyme Q and Succinate-Tetrazolium Reductase Activity of Neonatal Rat Kidney.* (27751)

PETER J. STRAND AND LEE W. WATTENBERG
Department of Pathology, University of Minnesota School of Medicine, Minneapolis

Fisher and Gruhn reported a very low succinate-tetrazolium reductase activity in the outer cortex of the neonatal rat kidney(1). The succinate-tetrazolium reductase system contains multiple components including a specific dehydrogenase, an electron transport mechanism and a tetrazolium salt which is reduced to a highly colored, water insoluble formazan. Initial evidence for this complexity was derived from studies with blue tetrazolium and neotetrazolium which could be shown to require a component which is removed or destroyed by incubating sections with lipase(2). More recently coenzyme Q has been demonstrated to be an intermediate in the succinate-tetrazolium reductase reaction(3). It is evident that a low succinate-tetrazolium reductase activity could result from a low activity of primary dehydrogenase, a deficiency of available coenzyme Q or a combination of these factors. That different factors actually do cause a low succinate-tetrazolium reductase activity has been shown for various types of proliferative lesions of the liver(4,5). The present work is designed to find the cause of the low succinate-tetrazolium reductase activity in the neonatal rat kidney. Evidence will be presented to show both primary dehydrogenase and coenzyme Q are involved in this low activity.

Methods and materials. The animal tissues were obtained from male and female Sprague-Dawley rats. The rats were sacrificed at age intervals of one day beginning with 2 days of age and continuing for 25 days. Animals of 60 days of age were also used. Both kidneys were removed, one was fixed in Zenkers-formalin solution and embedded in paraffin. The other kidney was mounted on a block holder and quick frozen in dry ice. The frozen tissue was then sectioned in a cryostat at 16 μ, mounted on a coverslip and dried. The sections were then stained for succinate-tetrazolium reductase activity with 2,2′-di-p-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (Nitro BT) being employed as the tetrazolium salt. Alternate sec-

* This investigation was supported by grant from Nat. Cancer Inst., USPHS, Bethesda, Md.

tions were run with and without addition of coenzyme Q(3). The paraffin blocks were sectioned at 4 μ and stained with hematoxylin and eosin for general morphology or with phosphotungstic acid hematoxylin for mitochondria.

Results. Effects of coenzyme Q_{10} on succinate-tetrazolium reductase activity of the neonatal rat. In sections run without addition of coenzyme Q_{10}, the kidneys of rats of 2 to 5 days of age showed a complete absence of succinate-tetrazolium reductase activity in the outer band of cortical tissue, Fig. 1 (A). In the inner cortical area some nephrons showed no reductase activity. In other nephrons activity was present, but of a variable intensity, *i.e.*, elements of some nephrons stained very darkly whereas in others the staining was light. Various portions of the same nephron stained with different intensities(6). The distal portion of the proximal convoluted tubules, the thin limb of the loop of Henle and the collecting tubules showed little or no staining. The remainder of the nephron stained relatively intensely and about equally. At about the age of 5 days a noticeable change in the staining pattern occurred in that reductase activity became apparent in the outer cortical zone. The reductase activity of this region was at first very low but as the animals became older the staining became more intense. Also with aging more of the tubular elements of the inner cortex demonstrated activity. At about 14 days of age all of the convoluted tubules showed a pronounced activity. This is the staining pattern which persists throughout adult life.

When coenzyme Q_{10} was added to the reaction varying degrees of enhancement of reductase activity were seen. This enhancement was most prominent in the very young animal. In rats 2 to 5 days of age, the outer cortex which showed no reductase activity without quinone addition now showed a weak but clearly demonstrable activity when this quinone was added. Those cortical elements which had a very low but demonstrable activity showed an intense activity in the presence of added quinone, Fig. 1 (B). In cortical elements which showed high activity without quinone addition, little or no increase in activity was seen when coenzyme Q_{10} was added. In older animals in which intense staining of the convoluted tubules occurs, coenzyme Q_{10} has no demonstrable effect on the reaction. Short incubation of kidney sections from these animals was run to eliminate the possibility that the deep staining intensity was preventing the demonstration of a real difference in staining between the sections with and without coenzyme Q_{10} addition. The results with short incubation time were the same as those with longer incubation with regard to lack of coenzyme Q_{10} enhancement. Although a marked increase in activity is brought about in young animals by coenzyme Q_{10} the staining intensity remains significantly less than that of the adult. This would suggest that a lack of primary dehydrogenase as well as coenzyme Q is involved in the low reductase activity in the neonatal rat kidney.

Paraffin sections stained with hematoxylin and eosin were studied to determine what correlation, if any, existed between the maturity of the kidney tissues and the reductase activity. In the neonatal kidney the outer band of cortical tissue which showed weak or absent succinate-tetrazolium reductase activity was immature as indicated by the presence of intensely basophilic cells arranged in clusters frequently without a lumen being present. Sections stained with phosphotungstic acid hematoxylin for the demonstration of mitochondria did not reveal stainable mitochondria in these elements, Fig. 1 (C) and (D). In the neonatal kidney there was a well defined correlation between a lack of mitochondrial staining and the absence of reductase activity without quinone addition. In rats 14 days and older mitochondrial staining and intense reductase activity existed throughout the cortical tubules.

Discussion. The present study has been concerned primarily with the factors involved in the low succinate-tetrazolium reductase activity observed in the cortex of neonatal rat kidney. The results obtained indicate that a lack of optimum concentration of coenzyme Q and a low activity of primary dehydrogenase both participate in the low over-all reductase activity. The marked enhancement which is obtained by addition of coenzyme

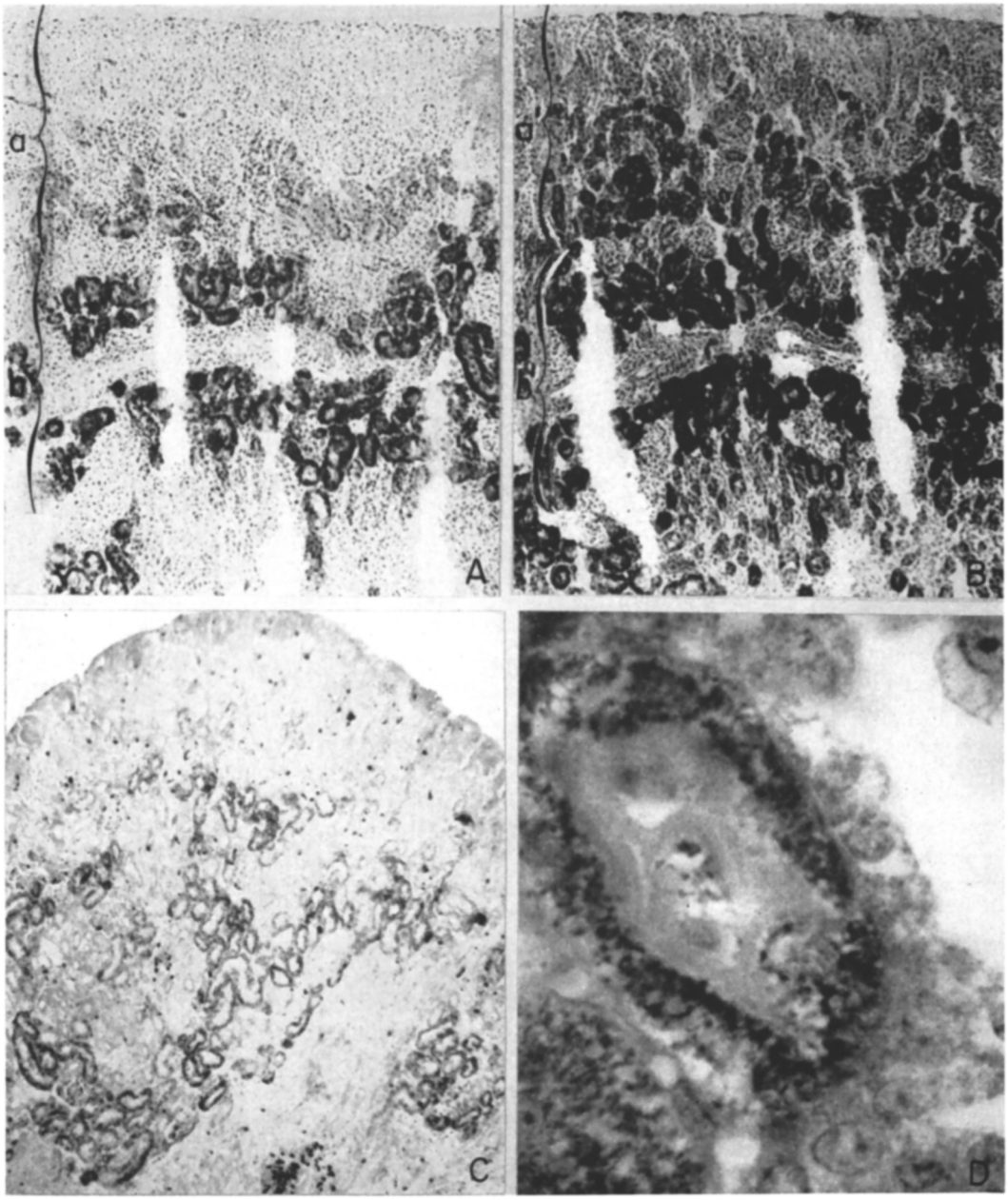

FIG. 1. A. Section of cortex of neonatal rat kidney stained by the succinate-tetrazolium reductase method with no coenzyme Q_{10} added to reaction mixture. (a) Outer cortical zone containing tubules which have little or no staining; (b) inner cortical zone containing a group of mature tubules which stain intensely and immature lighter staining elements. B. Adjacent section stained by succinate-tetrazolium reductase method with coenzyme Q_{10} added to reaction (a') note the increase in staining over the comparable area (a), (b') note immature elements showing an increase in staining whereas mature tubules are minimally affected as compared to (b), ×100. C. Section of neonatal rat kidney stained for mitochondria by the phosphotungstic acid hematoxylin method. Note absence of stained mitochondria in the immature outer cortical zone and the immature elements of the inner zone, ×50. D. High power photomicrograph of neonatal rat kidney cortex stained for mitochondria by the phosphotungstic acid hematoxylin method. Note mature tubules with stained mitochondria in left portion of the field and adjacent immature tubular elements without staining mitochondria in the right portion, ×1000.

Q_{10} to sections of neonatal rat kidney as contrasted to the negligible effect in the mature kidney indicates that lack of available quinone is a rate limiting factor for the reductase activity. However, the fact that even with addition of coenzyme Q_{10} the activity in immature renal elements does not reach that of mature ones indicates that the activity of primary dehydrogenase is likewise low. In this study there has been found a close relationship between the stainability of mitochondria by phosphotungstic acid hematoxylin and the properties of the reductase system. Immature elements with mitochondria which do not stain have a low succinate-tetrazolium reductase and show pronounced enhancement of this reductase activity by additional coenzyme Q_{10}.

The nature of the factors required for mitochondrial maturation are not known. The neonatal rat kidney cortex appears to be a convenient object for study of these factors and the observations reported here have the potential of serving as parameters for further investigation.

Summary. Histochemical studies of the low succinate-tetrazolium reductase activity found in tubular elements of the neonatal rat kidney cortex have shown that the low reductase activity is due to a low activity of the primary dehydrogenase and a lack of availability of optimum amounts of coenzyme Q. In addition, a correlation has been found between the low reductase activity, morphologic immaturity of renal tubular elements, and a lack of mitochondrial staining of these elements.

The technical assistance of J. Lionel Leong and Mrs. Mary Erickson is gratefully acknowledged.

1. Fisher, Edwin R., Gruhn, John, PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 781.

2. Farber, E., Bueding, E., *J. Histochem. Cytochem.*, 1956, v4, 357.

3. Wattenberg, Lee W., Leong, J. Lionel, *ibid.*, 1961, v8, 4.

4. Wattenberg, L. W., Gronvall, J. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 394.

5. Osterberg, K. A., Wattenberg, L. W., *ibid.*, 1961, v108, 300.

6. Wachstein, M., Meisel, E., *Am. J. Path.*, 1954, v1, 1947.

Received May 28, 1962. P.S.E.B.M., 1962, v111.