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1. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., *Circ.*, 1952, v6, 276.

2. Moskowitz, M. S., Moskowitz, A. A., Bradford, W. L., Jr., Wissler, R. W., *A.M.A. Arch. Path.*, 1956, v61, 245.

3. Malinow, M. R., Pellegrino, A. A., Ramos, E. H., *ibid.*, 1958, in press.

4. Malinow, M. R., *Ciencia e Invest.*, 1956, v9, 395.

5. Ludden, J. B., Bruger, M., Wright, I. S., *Arch. Path.*, 1942, v33, 58.

6. Mininni, G., Contro, S., Chechia, C., *Circ. Research*, 1955, v3, 191.

7. Fisher, R. A., Yates, F., *Statistical Tables for Agricultural, Biological and Medical Research*, Oliver and Boyd, ed., Edinburgh, 1953.

8. Duff, G. L., McMillan, G. C., Lautch, E. V., *Am. J. Path.*, 1954, v30, 941.

9. Swedin, B., Aberg, B., *Scand. J. Lab. Clin. Invest.*, 1952, v4, 68.

10. Malinow, M. R., Bur, G. E., *Ciencia e Invest.*, 1958, in press.

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Effect of Nephrosis on Renal Q_{O_2} and Histochemical Appearance of Cytochrome Oxidase and Succinic Dehydrogenase. (23771)

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Investigation of the appearance and distribution of certain histochemically demonstrable renal tubular enzymes in rats with nephrosis induced by injection of anti-rat kidney-rabbit serum revealed an appreciable depletion of cytochrome oxidase (G-nadi oxidase) and succinic dehydrogenase(1). Certain physiologic and morphologic considerations suggested that these alterations were related to degree of proteinuria rather than to a nephrotoxic effect of the serum employed. It therefore appeared worthwhile to evaluate such enzymatic activity in nephrosis produced by another etiologic agent and to attempt to quantitate any changes if present, manometrically. In addition, this information would also provide an opportunity for correlation of results obtained by the *in vitro* and histochemical staining technics. In the present study nephrosis was induced by appropriate doses of aminonucleoside, an agent which has been demonstrated to produce such a state with relative ease and a high degree of consistency(2,3).

Methods. Forty-two adult female Wistar rats weighing 150-200 g were utilized. Group A consisted of 21 animals that received 0.003 ml of a 0.5% aqueous solution of aminonucleoside* per g of body weight subcutaneously for 9 consecutive days and were sacrificed 5 days after the last injection. There were 8 animals in Group B that received a similar dose of aminonucleoside but were sacrificed after only 6 injections. Group C was comprised of 13 untreated rats of similar sex, strain and weight. Protein content of the urine was determined daily and the serum cholesterol, total lipid and total protein at the time of sacrifice by methods described elsewhere(1). All animals were sacrificed by decapitation. Their kidneys were quickly removed. One was placed immediately in ice after removing its capsule and ureter. Thin slices consisting of the cortex and medulla were prepared according to the method of Deutsch(4). These were incubated in Krebs-Ringer-phosphate-glucose buffer, pH 7.2 at 37 C in a total volume of 3.0 ml. The gas phase consisted of 5% CO_2 and 95% O_2. Liberated CO_2 was absorbed by 20% KOH placed in the center well. The oxygen consumption was followed from 30 to 60 minutes, after which time the tissues were removed and dried at 108°C for 2 hours. Their dry weights were obtained after desiccation for 18 hours. 0.6 ml of a 0.5% solution of aminonucleoside, previously neutralized and

* Generously furnished by Dr. Stanton Hardy, Lederle Laboratories.

TABLE I. Renal Qo_2 of Nephrotic and Normal Rats.

Group	No.	Qo_2 (ml/mg dry wt)
A. (Treated, nephrotic)	21	14.5 ± 3.0
B. (" , not nephrotic)	8	17.4 ± 2.4
C. (Untreated, " ")	13	18.7 ± 1.9

Difference between groups	P value
A & B, A & C	$<.01$
B & C	$>.1$

dissolved in the buffer, was added from a side arm to the kidney slices from 6 additional untreated controls twenty minutes after normal respiration was observed. Qo_2 was determined by appropriate calculations and the statistical significance between groups was obtained by utilization of Fisher's "t". The remaining kidney was longitudinally sliced and one-half immediately frozen on dry ice, sectioned at $-20^{\circ}C$ in a cryostat and stained for the demonstration of cytochrome oxidase utilizing a-naphthol and dimethyl-p-phenylenediamine hydrochloride(5) and succinic dehydrogenase with a substrate of neotetrazolium chloride and sodium succinate(6). The remainder of the kidney was fixed in Zenker's acetic fluid and processed for histologic study in the usual manner.

Results. All of the animals of Group A developed the nephrotic syndrome. Proteinuria, exceeding 100 mg/18 hours first appeared on the 8th day following the initial injection and was followed generally in 2 days by the presence of ascites. Hyperlipemia, hypercholesterolemia and hypoproteinuria were also present at the time of the sacrifice. The microscopic appearance of the renal lesions produced by aminonucleoside will be described in detail elsewhere. On the other hand, those rats which were sacrificed after only 6 injections (Group B) failed to exhibit any chemical, clinical or morphologic manifestations of the nephrotic syndrome.

As indicated in Table I there was a statistically significant decrease of oxygen consumption by the kidney slices from rats with nephrosis (Group A) as compared to those of the untreated controls or those animals sacrificed prior to the onset of the nephrotic state. The addition of aminonucleoside to the kidney slices from normal animals was without statistically significant effect on Qo_2 when compared with the internal control values.

Similarly, a depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase (G-nadi oxidase) was apparent only in those animals with the nephrotic state (Fig. 1 and 2). Histochemically, the loss of these respiratory enzymes appeared in those topographic areas corresponding to the greatest concentration of proximal convoluted tubules and ascending limbs of Henle, sites which also exhibited a depletion of mitochrondrial elements (unpublished). The degree of depletion noted histochemically usually appeared greater than might be expected from the manometric findings although in many instances changes observed were of the same order of magnitude.

Discussion. Reduction of renal tubular succinic dehydrogenase has been noted following a variety of experimental procedures (7). However, it appears unlikely that the reduction in renal Qo_2 and histochemically demonstrable succinic dehydrogenase and cytochrome oxidase (G-nadi oxidase) activity observed in nephrosis induced by aminonucleoside is the result of a direct toxic effect of this agent. The addition of an excessive quantity of this drug *in vitro* failed to significantly affect the Qo_2 of fresh kidney slices from normal rats. In addition, no effect on enzymatic activity was apparent in rats receiving aminonucleoside which were sacrificed prior to the onset of nephrotic manifestations. On the other hand, the histochemical findings are identical to those previously noted in animals with nephrotoxic serum nephrosis with massive proteinuria and ascites. Such information suggests that the mechanism responsible for suppression of cellular respiration in the nephrotic state produced by these diverse modalities may be similar and related to the massive proteinuria. Increased tubular reabsorption of protein which also is apparent in both nephrotic states(1), further suggests that such enzymatic depletion may result during an accentuated phase of this tubular activity. Kretchmer and Dickerman(8) have noted a decrease in succinic oxidase and cytochrome oxidase activity in the droplet and

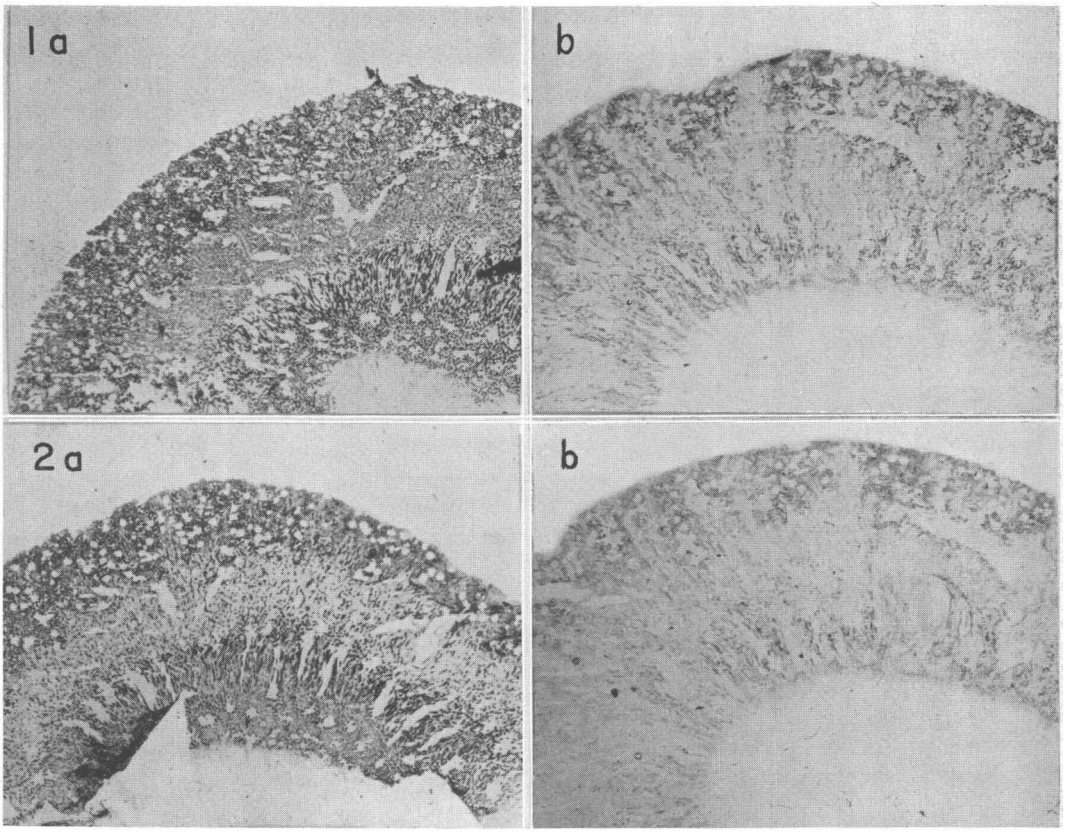

FIG. 1. a. Appearance of renal succinic dehydrogenase in untreated control. b. Nephrotic. (×10).

FIG. 2. a. Appearance of renal cytochrome oxidase in untreated control. b. Nephrotic. (×10).

mitochondrial fractions of renal tubular epithelium of rats subjected to intraperitoneal injections of ovalbumin. Droplet formation, conspicuous in the tubular epithelium of rats with aminonucleoside nephrosis, has been considered as morphologic evidence of increased tubular reabsorption of protein.

Comparison of the results obtained manometrically and histochemically indicates that the latter technic is capable of detecting moderate losses of respiratory activity within the renal tubules and is not dependent upon an all or none phenomenon. In this regard our findings are similar to those of Cascarano and Zweifach(9) who found the oxygen consumption of fresh kidney slices under varying conditions to parallel endogenous values for 2, 3, 5 triphenyl tetrazolium chloride reduction.

Summary. A moderate decrease of Q_{O_2} and a reduction in the intensity of the histochemical stains for succinic dehydrogenase and cytochrome oxidase have been observed in kidneys from rats with nephrosis induced by aminonucleoside. The possible relationship of these alterations to proteinuria is discussed.

1. Fisher, E. R., Gruhn, J., *A.M.A. Arch. Path.*, in press.

2. Frenk, S., Antonowicz, I., Craig, J. M., Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

3. Fiegelson, E. B., Drake, J. W., Recant, L., *J. Lab. and Clin. Med.*, 1957, v50, 437.

4. Deutsch, W., *J. Physiol.*, 1936, v87, 56P.

5. Lillie, R. D., *Histopathologic Technic and Practical Histochemistry*, 1954, Blakiston Co., N. Y.

6. Rosa, C. G., Velardo, J. T., *J. Histochem.*, 1954, v2, 110.

7. Wachstein, M., *J. Histochem.*, 1955, v3, 246.

8. Kretchmer, N., Dickerman, H. W., *J. Exp. Med.*, 1955, v3, 369.
1954, v99, 629.
9. Cascarano, J., Zweifach, B. W., *J. Histochem.*,

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Antibody Production in Mice Exposed Intermittently to Radium Gamma Rays. (23772)

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Recovery processes taking place between successive irradiations are thought to account for the increased LD_{50} and prolonged survival time reported for mice(1,2) and for rats(3) when the radiation was administered in 2 or more exposures made at varying time intervals. Mortality and survival times following radiation exposures in the lethal range and given as divided doses have been considered theoretically to be measurements of the summed injury to several physiological processes(4). Some of these processes, such as hematopoiesis, blood coagulation, defense against infection and antibody production following acute exposures to ionizing radiations have been studied in great detail. Less attention has been given to the study of injury to any of these processes following intermittent irradiation. Observations on the immune response following divided-dose exposures to ionizing radiation are of interest because measurement of injury to this physiological process may be made following sublethal exposures with good accuracy by procedures now available. Furthermore, studies of antibody production following intermittent exposure to radiation provide some information on injury and recovery processes involving the cellular mechanisms of protein synthesis. In the experiments here described antibody production and total circulating leucocyte counts were studied in mice following their intermittent exposure to radium gamma rays.

Materials and methods. Male NIH mice used throughout these experiments were 5 to 8 weeks of age at the beginning of the radiation exposure. Litters were represented in each of the treatment groups and mice exposed to radiation were confined in wood boxes containing 5 to 8 animals each. Control animals were housed in steel cages in the animal rooms and at the end of the exposure both the irradiated and control mice were transferred to individual cages. Mice of parallel groups were bled from the tail for circulating leucocyte counts on the second day after the end of exposure. Immunization was done on the third day and the mice were sacrificed for serums on the 8th day after the end of the irradiation. Mice of another experiment were given acute exposure to X-rays 4 days after the end of the "chronic" accumulation of 450 r from the radium source described below in order to see whether the prior exposures would be additive to the acute irradiation in their effect on antibody production. Immunization was done one week and leucocyte counts 6 days after the acute exposure. Serological procedures followed those previously described(5) except that the serums were frozen and stored at -20°C until titration within 3 to 4 weeks after sacrifice of the mice. Briefly, the serum hemolysin content was estimated after a single intravenous inoculation of a 2.5% suspension of washed sheep erythrocytes. Approximately 4.85 x 10^6 erythrocytes were given per gram of body weight. Serums were titrated by the procedure described by Taliaferro(6) and the hemolysin titers are expressed as the negative log of the serum dilution providing 50% hemolysis in a standard suspension of sheep erythrocytes.

The arrangement for exposing the mice to the radium source was described in detail by Lorenz(7) and provided 8.8 r or 2.2 r per 8 hour day. In the experiments described in this paper the total exposure dose to the mice