phosphorylate creatine is probably a secondary phenomenon related to the marked pathological condition of that tissue in advanced dystrophy. However, the possibility cannot be excluded that vit. E plays some role in these enzymatic processes *in vivo*, which were not detected by our analytical procedures.

It is of interest that with test system used the rate of pyruvate oxidation was increased 60 to 80% by addition of either glucose or hexokinase to the otherwise complete system. This demonstrates that in such preparations substrate oxidation may be controlled by the presence of phosphate acceptors.

Summary. 1. An assay system is described for the direct measurement of the ability of heart preparations to couple esterification of phosphate with oxygen uptake. With this system a high efficiency of phosphorylation is obtained with normal rabbit heart tissue. 2. Heart tissue preparations from rabbits made dystrophic by lack of vit. E showed no decrease in the ability to carry out oxidative phosphorylation.

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Effect of Nitrogen Mustard on Nephrotoxic Nephritis in Rats. (18692)

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The hypothesis that glomerulonephritis is a manifestation of an abnormal immune response(1,2) has led to attempts to alter its characteristics by pharmacological means (3,4). Nitrogen mustard protects rabbits against bovine gamma globulin nephritis and has been reported of value in treatment of the nephrotic stage of human glomerulonephritis (5-7). Since nephrotoxic nephritis in rats is believed to be the consequence of an antigen-antibody reaction(8-10), the effect of nitrogen mustard, which inhibits antibody production(4,11), in preventing the development of this type of nephritis has been studied.

Methods. Male Wistar rats, weighing 60-80 g were used. They were fed dog food cubes and given tap water *ad lib*. Three lots of nephrotoxic anti-rat kidney rabbit serum were prepared by Smadel's method(12), which produced "mild," "moderate," and "severe" nephritis as judged by proteinuria, edema, and survival when 0.1-0.2 ml per 100 g of rat was given intravenously on 3 successive days. Methyl-bis (beta-chloroethyl) amine hydrochloride (HN_2) 0.3-0.4 mg/kg was given intravenously for 4 or 5 successive days beginning 2 or 3 days before the first injection of nephrotoxic serum. The minimal leucocyte depression averaged 67% and usually coincided with the time of administration of the nephrotoxic serum. Urines were collected for 16-hour periods for quantitative protein determinations by the Shevky and Stafford method and for microscopic examination of the sediment. The animals were weighed and examined for evidence of edema

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on alternate days for 2 weeks and then weekly for periods up to 2 months.

Results. Fifty-six rats were divided into 3 groups of 14-22 and given one of the 3 nephrotoxic sera. Approximately half of each group served as controls, the others being treated with HN_2. Survival was influenced by the toxicity of HN_2 as well as by the severity of the induced nephritis. There was no mortality in the control groups given "mild" and "moderate" nephritis, while 6 of 9 rats with "severe" nephritis died in renal failure from 6-14 days after the first injection of nephrotoxic serum. Of 8 rats given HN_2 and "mild" nephrotoxic serum, 5 died apparently from HN_2 intoxication. All 10 rats receiving a slightly smaller dose of HN_2 and "moderate" nephrotoxic serum survived. Four of 11 treated rats with "severe" nephritis died apparently from HN_2 intoxication and 3 from nephritis, leaving only 4 survivors. Edema was noted only in rats with "severe" nephritis. Subcutaneous edema and ascites were manifested within the first week and persisted 3 to 18 days. In 6 of 11 rats pretreated with HN_2 edema was definitely less than in the control group.

The mean protein excretion per 16 hours in 80 control urinalyses on 65 rats was 0.91 $\pm$ 0.57 mg. Maximal increase in proteinuria occurred within 4 days of the first injection of nephrotoxic serum in the 3 control groups, and averaged 3.9, 35.8, and 52.5 mg per 16 hours, respectively. In rats with "mild" nephritis the initial 4-fold increase in proteinuria was maintained during the 2-month period of observation. After the initial peak in protein excretion, rats with "moderate" nephritis exhibited a decrease in proteinuria during the first month followed by stabilization at levels 4 to 10 times those of the control period. Proteinuria in the 3 rats surviving the early phase of "severe" nephrotoxic nephritis was not only maintained but increased during the second month. The early increase in proteinuria manifested by the control rats was not observed in the rats pretreated with HN_2. The 8 animals injected with "mild" nephrotoxic serum failed to develop a significant increase in proteinuria until the fourteenth day. After this time, the 3 surviving rats exhibited increases in protein excretion which were slight but did not differ from those of the control animals with nephritis of comparable severity. In the animals pretreated with HN_2 injection of "moderately" potent nephrotoxic serum failed, likewise, to elicit a sudden, maximal increase in urinary protein excretion; rather, there occurred in the 10 rats a gradually increasing proteinuria, which became maximal and equal to that of the untreated animals on the fourteenth day. The urinary protein excretion of the pretreated rats subsequent to that time did not differ from that of the control animals. Similarly, pretreatment with HN_2 altered the degree of proteinuria during the acute phase of "severe" nephritis. Although a marked increase in urinary protein excretion occurred shortly after the injection of serum, it was but 30-50% of that of the control group. Comparison of the treated and untreated groups during the established phase of the "severe" nephritis is difficult because of the smaller number of surviving animals, but the proteinuria of the pretreated animals remained consistently about 50% of their controls. Urinary sediments contained increased numbers of formed elements in all three groups, both treated and controls. Histologic sections of the kidneys of animals dying during the experiments resembled those described by Smadel(13). No differences were apparent in the control and treated groups.

Effect of HN_2 on Chronic Nephrotoxic Nephritis in Rats. In order to study the influence of HN_2 on chronic nephrotoxic nephritis in rats, nitrogen mustards were given intravenously, 0.3 mg per kg per day for 5 days to 8 animals showing a stabilized increase of urinary protein excretion, ranging from 5.4 to 60.6 mg per 16 hours, 3 or 4 months after injection of nephrotoxic serum. A reduction in average proteinuria per 16 hrs occurred in 7 of the 8 rats, varying from +2.0 to -31.9%, with an average depression of -16.0%. There was no apparent relationship between the decrease in average urinary protein per 16 hrs and the intensity of the HN_2 effect in terms

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of maximal leucocyte depression. Changes of the magnitude noted cannot be considered significant in view of the observation that day to day protein excretion in individual rats varied widely and not necessarily in the same direction as the general trend.

Discussion. It is apparent from our experiments that pretreatment of young Wistar rats with nitrogen mustards failed to protect against the development of nephrotoxic nephritis produced by intravenous injection of rabbit antirat kidney serum. In the 2 groups of animals injected with nephrotoxic serum of either "mild" or "moderate" potency both the pretreated animals and those receiving serum alone exhibited approximately the same degree of proteinuria after the first 14 days of the disease. Although the rats pretreated with HN_2 and injected with a nephrotoxic serum capable of producing a "severe" nephritis excreted less protein during the 8 weeks of observation than the untreated rats injected with the same serum, all the surviving animals manifested evidence of chronic nephritis. The small number of animals permits no statistical evaluation of the apparent difference within this group.

Although HN_2 failed to prevent the occurrence of nephritis, some modification of the early phase of the disease was apparent. The delay of approximately 14 days in the onset of marked proteinuria in the pretreated animals suggests that the HN_2 may have interfered with the initial antigen antibody reaction in the kidney in such a fashion as to delay the manifestations of this combination. Nitrogen mustards are reported to have no effect on antigen-antibody reactions *in vitro* (11), but this does not preclude such interference *in vivo*.

Nitrogen mustards are known to exert profound inhibitory effects on the metabolism of various tissues, including renal tissue, of experimental animals(14). It is possible that this property of HN_2 might effect a reduction in proteinuria during the period of its maximal action. This would account also for the delay in onset of increased proteinuria, but it is not consistent with the failure of nitrogen mustard to reduce regularly the abnormal proteinuria of rats with chronic nephritis.

Conclusions. 1. Pretreatment with nitrogen mustards failed to protect rats injected with nephrotoxic serum against the development of acute or chronic nephritis but did appear to modify certain early manifestations of the disease. 2. No consistent effect on the proteinuria of rats with chronic nephritis was produced by the administration of nitrogen mustards.

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Antibiotic Effects of Circulin Against Certain Gram-negative Plant Pathogens. (18693)

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Circulin, a mixture of polypeptide antibiotics produced by *Bacillus circulans* Q-19, (like the polymyxins to which it is closely related) is very active against gram-negative bacteria, both *in vivo* and *in vitro*(1-4).

This note calls attention to the fact that among the organisms inhibited *in vitro* are

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