is of the same order of magnitude as the one found by Benacerraf and Kabat(2) for the reverse Arthus reaction with anti-Ea in guinea pigs of 250 ± 50 g.

By intravenous sensitization with anti-SIII followed by the intradermal injection of 0.20 mg SIII, direct Arthus reactions of medium severity (++ to +++) were obtained with 800 μg, + reactions with 400 μg and ± reactions with 200 μg AbN. For the minimal observable reaction (±) the amount of antibody N required per gram of rat corresponded therefore to approximately 2 μg, while for the guinea pig(2) only 0.4 μg are required per unit weight.

In the passive cutaneous anaphylaxis experiments a value of 0.5-1.0 μg AbN was found for the threshold of the reaction with both the SIII-anti SIII and Ea-anti Ea systems, in agreement with earlier findings(6). The ratios between the amounts of antibody required for minimal reverse Arthus reactions and for passive cutaneous anaphylaxis in the rat and in the guinea pig were respectively 10 and 1000-2000. Since the skin-sensitivity to histamine is about the same in the two species, it is suggested that the higher amounts of antibody required for passive cutaneous anaphylaxis in the rat be ascribed to a lesser ability of the rabbit antibody to become fixed to the dermal tissue.

The enormous difference in the minimal amounts of antibody required for passive cutaneous anaphylactic reactions, as contrasted with the essentially identical values found for the passive reverse Arthus reaction in the rat and in the guinea pig indicates once again that the two reactions are basically different in their mechanisms.

Summary. 1. The severity of the Arthus reaction in the rat was studied using known amounts of antigen and antibody. 2. Reverse Arthus reactions of minimal severity were obtained by intradermal sensitization with 5-10 μg anti-Ea or anti-SIII. 3. By intravenous sensitization direct Arthus reactions of medium severity were obtained with 800 μg and minimal reactions with 200 μg anti-SIII. 4. The threshold of the reaction in passive cutaneous anaphylactic experiments corresponded to 0.5-1.0 μg AbN for both the Ea-anti-Ea and SIII-anti-SIII systems. 5. The meaning of these findings is briefly discussed.

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Nephrotoxic Globulin Nephritis. III. Prompt Death after Administration of Nephrotoxic Globulin to the Rat.* (19950)

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Some recent studies from this laboratory have concerned the pathogenesis of nephritis produced in the rat by administration of rabbit anti-rat-kidney gamma globulin (nephrotoxic globulin, NTG)(1,2). It was noted from the start of these studies that NTG administration produced extrarenal effects, such as intravascular hemolysis. The latter could not be eliminated completely by absorption of the NTG with homologous or heterologous erythrocytes. A variable proportion of the animals in each experiment died within the first 12 to 24 hours after NTG administration and, at autopsy, their kidneys

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TABLE I. Schedule of Groups and Procedures.

Group	Title	No. animals	Sex	Material inj.	Additional procedures
1	GC-nephrectomy	10	♂	GC	Nephrectomy
2	NTG	10	♂	NTG	
3	NTG-nephrectomy	10	♂	NTG	Nephrectomy
4	NTG-cortisone	10	♂	NTG	Cortisone admin.
5	NTG-unabsorbed	10	♂	NTG-unabsorbed	
6	NTG-PBZ	4	♂	NTG	Tripelennamine admin.
7	NTG-unabsorbed-PBZ	6	♂	NTG-unabsorbed	" "
8	NTG-female	10	♀	NTG	
9	NTG-female-PBZ	9	♀	NTG	" "

appeared to be normal on gross examination. It had been known from previous experiments that rats of the same colony will live approximately 48 hours after bilateral nephrectomy if maintained on stock diet. In view of the complex of antigens used for the production of NTG (whole rat kidney), it seemed most likely that the prompt deaths after NTG administration were the result of extrarenal factors, although other less probable explanations could also be advanced. The present experiments were designed to separate the renal and extrarenal effects of NTG administration so that their separate relation to prompt death could be evaluated.

It may be observed in passing that there has been a tendency in the current literature for nephritis produced by nephrotoxic serum administration to be considered as the equivalent of human glomerular nephritis or lipoid nephrosis(3,4). This tendency is not adequately justified. The pathogenesis and etiology of human nephritis and nephrosis are unknown, although it is generally believed that the diseases involve mechanisms of hypersensitivity. There are readily observed differences between human nephritis, nephrosis, and nephrotoxic globulin nephritis in the rat. Among these differences are the paucity of hematuria, lipoid deposition in the kidney, and periodic acid-Schiff reagent stainable material in the rat. For the sake of clarity, it would seem desirable to consider nephrotoxic globulin nephritis and the human diseases mentioned as independent entities which are not necessarily related to one another.

Methods and materials. In this experiment 60 male and 19 female rats of the Slonaker-Addis strain were used. The animals were selected to weigh 150 g within a small range of variation. The stock diet for this colony, which contains 17% protein, was used before and during the experiment, with water *ad libitum*. The preparation of nephrotoxic globulin (NTG)† by immunization of rabbits against a suspension of whole rat kidney, and the preparation of control rabbit globulin (GC) previously has been described in complete detail(2). In order to eliminate the influence of variable potency, observed in NTG preparations, all these experiments were performed with a single lot (8-9-51). Some experiments were performed with NTG which had not been subjected to absorption with erythrocytes to remove anti-rat-erythrocyte antibody (NTG-unabsorbed). NTG, NTG-unabsorbed, and GC (Lot 3-3-52) were administered by injection in a foot vein, in equivalent amounts: 23.0 mg protein in 1.0 ml 0.85% sodium chloride solution. Bilateral nephrectomy was performed under light ether anesthesia, before injection of the globulin. The entire procedure, which utilized the transperitoneal approach through a flank incision on each side, required about 7 minutes for each animal. Muscle and peritoneum were closed in one layer with silk, while the skin was closed with Michel clips. After the experimental manipulations, the animals were returned to the cages and were inspected at intervals to ascertain the number of survivors. After 50 hours from the beginning of the experiment the remaining animals were discarded and the experiment was concluded.

The various groups and experimental con-

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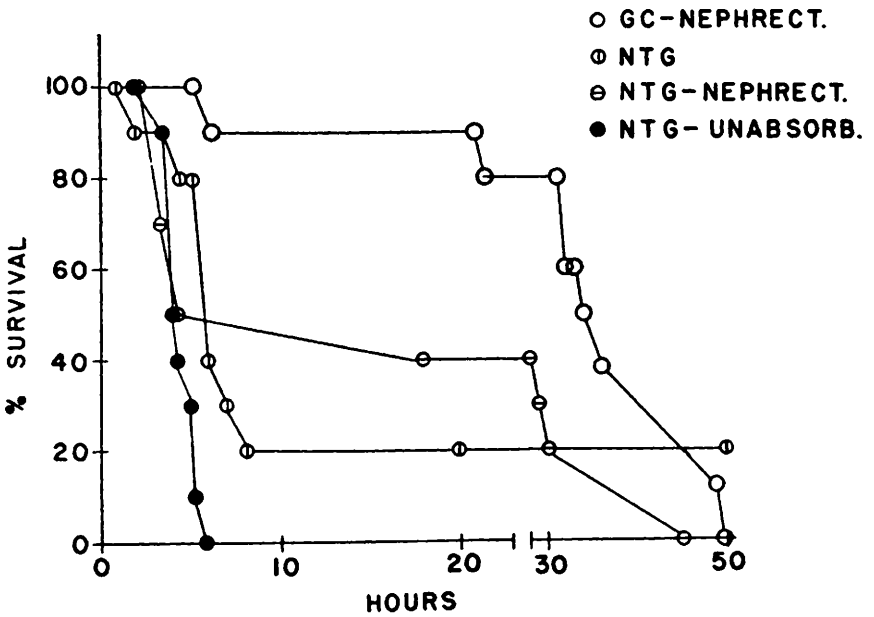

FIG. 1. Survival of rats after nephrectomy and after injections of NTG and GC.

ditions for each are noted in Table I. In group 4 cortisone acetate‡ (2.5 mg in 0.1 ml) was administered by intraperitoneal injection 30 minutes before NTG administration. In groups 6 and 7 tripelennamine hydrochloride§ (1.0 mg in 0.2 ml) was administered by intraperitoneal injection 30 minutes before NTG administration. In group 9 the same dose of tripelennamine was repeated for a total of 3 doses at intervals of 2 hours.

Results and discussion. The curve for group 1 (Fig. 1) indicates the mortality from bilateral nephrectomy in the rat, under the conditions used for these experiments. Most of the animals died between 22 and 36 hours after operation and the remaining ones succumbed before the 50th hour.

For the purposes of this discussion we can consider the NTG preparation to contain 3 classes of antibodies: anti-rat-kidney, anti-rat-erythrocyte, and anti-rat-tissues. Anti-rat-kidney is intended to include only antibodies formed to specific kidney proteins from renal parenchymal cells. Anti-rat-tissues is intended to include all other antibodies (excluding anti-rat-kidney and anti-rat-erythrocyte) formed in response to serum protein, connective tissue, nervous tissue, fat, and other elements which are contained in a whole kidney suspension but are not specific to it. In Fig. 1 it can be seen that the injection of NTG-unabsorbed, which contains all the 3 classses of antibodies defined above, lowers the survival curve in a striking manner, so that all of the animals died within 6 hours of the injection. From this it is clear that the prompt death after injection of NTG-unabsorbed cannot be explained even by the assumption that there has been total cessation of renal function.

Not all the animals died when NTG (which was absorbed with rat erythrocytes) was administered. The survival time was somewhat extended, although still very significantly lower than the survival time after bilateral nephrectomy. This led to the conclusion that, while a definite fraction of the prompt lethal effect resides in the anti-rat-erythrocyte antibody, the principal effect is associated with the other 2 antibody classes. However, administration of NTG after nephrectomy yields a survival curve nearly identical with that obtained after NTG administration in the

‡ Cortone acetate, kindly provided by Dr. Leo V. Curtin, Merck and Co., Rahway, N. J.

§ Pyribenzamine hydrochloride, kindly provided by Dr. Ernst Oppenheimer, Ciba Pharmaceutical Products, Summit, N. J.

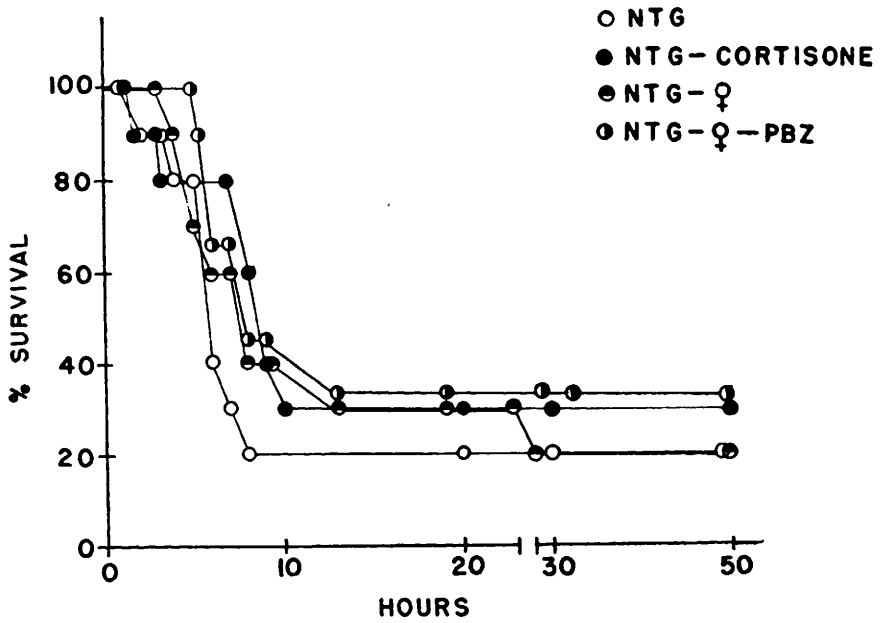

FIG. 2. Survival of rats after injection of NTG; influence of cortisone and tripelennamine on survival.

intact rat. The latter observation eliminates the possibility that the prompt lethal effect is the result of interaction between anti-rat-kidney antibody and kidney tissue. Therefore, the final conclusion is inescapable that the principal prompt lethal effect of NTG must reside in the anti-rat-tissue antibodies, with an additional effect contributed by the anti-rat-erythrocyte antibody.

The effect of NTG administration was studied after administration of cortisone and tripelennamine. In Fig. 2 it can be seen that neither cortisone nor tripelennamine significantly affected the survival curve. Experiments with NTG-unabsorbed and tripelennamine gave a similar result. It can also be seen that there is not a significant sex difference in the survival curves (Fig. 2, NTG and NTG-female), contrary to the sex difference observed in manifestations of NTG nephritis after 4 weeks have elapsed(5).

It has been shown by Pressman and his associates(6,7) that anti-rat-kidney serum prepared by the same method as NTG but unfractionated and unabsorbed with erythrocytes, will localize in a variety of organs and tissues. These include kidney, liver, lung, spleen, blood and, to a lesser degree, brain and heart. Previous work has also shown a toxic effect of NTG on tissue culture explants of kidney, heart, and brain(8). Such results may reasonably be explained by the complex antigen composition of ground, whole kidney suspension, previously mentioned. The minor constituents of kidney tissue may be expected to become more significant antigens during the prolonged series of injections required to produce sera of high titer, and this circumstance will increase the occurrence of significant antibody titers to extrarenal tissues. In addition, several investigations have shown that multiple injections of a single purified antigen may also result in diminished specificity of the high titer antisera(9). Even those antisera produced with antigens obtained from partial tissue fractionation(10,11) must have a complex composition. The glomerular antigen of Greenspon and Krakower(11) contained "minimal nonglomerular contaminants" as well as soluble material liberated during the fractionation process from crushed cells. Glomeruli themselves contain endothelium, epithelium, basement membrane, and other tissue constituents, and consequently may contain multiple antigens, not necessarily peculiar to the kidney.

These considerations support the proposition that nephritis produced by NTG administration cannot be compared directly with human glomerular nephritis and lipoid nephrosis; the possibility that a study of NTG nephritis may lead to generalizations which are relevant to the study of human nephropathies cannot be denied. Further, the prompt deaths that follow NTG administration in the rat cannot be considered to result from the nephrotoxic action of NTG, but are the result of extrarenal effects.

Summary. Administration of nephrotoxic globulin (NTG) to the rat produces death in less than 12 hours if the amount given is sufficient. Bilateral nephrectomy usually does not lead to death in less than 24 hours, when the animals are maintained on a stock diet containing 17% protein. The prompt death is related principally to anti-rat-tissue antibodies other than anti-rat-kidney. Anti-rat-erythrocyte is of definite but minor significance in the production of prompt death. Cortisone and tripelennamine exert no protection against the prompt death described. It is evident that nephrotoxic globulin (NTG) has important extrarenal effects which must be considered in comparing NTG nephritis with other similar diseases of the kidney, such as those of natural occurrence in man.

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The Chemical Nature of Encephalitogenic Proteolipide A and B. (19951)

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The brain proteolipide A and B (PL, AB), a new type of tissue lipoprotein recently discovered by Folch and Lees(1), deriving from the mouse, was found to be capable of inducing acute disseminated encephalomyelitis in mice indistinguishable from that brought about by the injection of whole brain(2,3). It was also shown that PL. AB possessed a degree of encephalitogenic power similar to that of whole brain and that the latter, after this constituent had been extracted, was inactive. It was therefore assumed that all of the encephalitogenic agent was present in the PL, AB fraction. An attempt was then made to test for activity the remaining fractions of the extract; to break up the PL into smaller units and to determine whether the active agent could be identified with any one of these components. The present paper describes these investigations.

Materials and methods. The PL, AB was prepared from mouse brain and the H-line W-Swiss mice(4) were employed as experimental animals. All fractions were mixed with the Freund-type adjuvant and inoculated into mice subcutaneously, 6 times or less, at weekly intervals. The methods of preparation, inoculation and observation of reactions in animals have already been described(2-5). Proteolipide C (PL, C) was obtained following closely the technic of Folch and Lees(1):

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