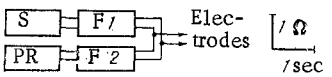

Fig. 1

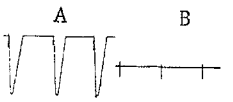

Fig. 2

Fig. 1. Block-diagram of apparatus. S — Stimulator, R) high-frequency ohmmeter, F_1, F_2) high and low frequency filters.

Fig. 2. Trace of electrical response. A) Responses of intact rabbit muscle to threshold pulse (15 V); B) absence of responses to above-threshold stimuli (3 V) in area of necrosis. Vertical lines — markers of stimulating pulses.

tively, in response to electrical stimulation. In necrotic zones of skeletal muscles, despite doubling the voltage of the stimulating pulse, no characteristic change in interelectrode impedance could be observed.

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MASUGI'S NEPHRITIS: PREPARATION OF AN ACTIVE NEPHROTOXIC SERUM

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Production of experimental glomerulonephritis (GN) is necessary in order to study its pathogenesis and the metabolic disturbances which arise in this disease, as well as to evaluate new methods of treatment. In nephrology a model of GN, induced with the aid of antikidney antibodies, suggested in 1900-1901 by the Russian scientist V. K. Lindeman [1], and elaborated in more detail by the Japanese scientist Masugi [8] in 1933-1934, is widely used in nephrology. The development of Masugi's nephritis is induced by injecting an experimental animal with a specific nephrotoxic serum (NTS), obtained from another animal after its immunization with the kidney of the first animal. After intravenous injection of NTS into a rat, antikidney antibodies are quickly fixed on the basement membrane of the glomerulus, and within 1 h after the injection, marked proteinuria develops [5]. This is the first, heterologous phase of the disease. Later, starting with the 10th-14th day, the kidney lesion is maintained as a result of fixation of the animal's own antibodies, produced to rabbit antikidney γ-globulin, bound with the basement membrane, in the glomeruli (the second, autologous phase). A definite role in the pathogenesis of Masugi's nephritis may also be played by autoantibodies formed to antigens of the damaged basement membrane in the second phase [2].

If the serum has high nephrotoxicity, the GN which develops closely resembles, in its clinical picture (edema, hypertension) and its laboratory features (massive proteinuria, hypoproteinemia, hyperlipidemia, azotemia), severe human GN of nephrotic or mixed type. However, preparation of the active NTS is associated with certain difficulties. According to our own observations, when rabbits are immunized with a suspension of rat renal cortex, an NTS capable of inducing a nephrotic syndrome was produced in only one-third of rabbits. This

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TABLE 1. Proteinuria and Some Serum Biochemical Parameters of Rats with Masugi's Nephritis (M ± m)

Experimental conditions	Proteinuria, mg/18 h	Blood serum			
		total protein, g/liter	albumin, g/liter	cholesterol, millimoles/liter	triglycerides, millimoles/liter
Control (n = 7)	1,62±0,34	64,1±2,0	27,7±0,5	1,7±0,21	0,77±0,06
Masugi's nephritis (*n*=9)	119±0,26	36,4±1,4	10,9±0,6	6,26±0,75	3,5±1,02
P	<0,001	<0,001	<0,001	<0,001	<0,05

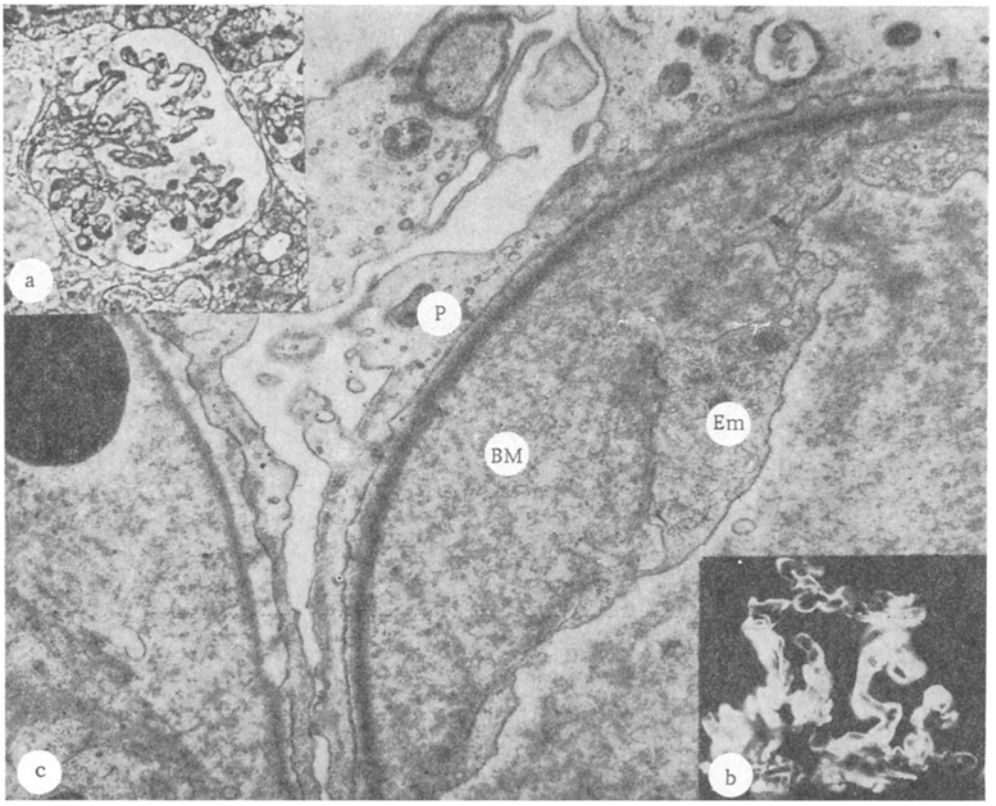

Fig. 1. Masugi's nephritis, 5th day after injection of NTS into rats: a) moderate congestion of glomerulus, focal thickening of capillary basement membranes. Methylene blue — azure II — fuchsin. 200 ×; b) fixation of IgG on basement membranes of glomerular capillaries is linear in character. Direct Coomb' method. 200 ×; c) accumulation of finely granular material in lamina rara interna of basement membrane (BM). Abundance of pinocytotic vesicles in cytoplasm of endothelial cells (En). Fusion of pedicles of podocytes (P). 22,000 ×.

required seven intravenous injections and five intraperitoneal injections of the antigen. The serum obtained in a similar way by other workers [3] had relatively low activity: after injection into rats, the proteinuria amounted to only 10-15 mg/day. More recently, to obtain an active NTS, the basement membrane of the glomerular capillaries has been used as the antigen [4]. However, the isolation of this membrane is very laborious, requires an expensive technique, and is therefore not a practical propositon for every laboratory.

In the present investigation, to obtain the NTS, whole glomeruli isolated from the rat renal cortex were used. To stimulate immune processes, Freund's complete adjuvant was used. Immunization of rabbits with rat glomerular tissue by the scheme described below [7] yielded an active NTS in the majority of animals.

EXPERIMENTAL METHOD

To obtain kidney antigen, male albino rats weighing 400-500 g were used. The kidneys were perfused *in situ* with sterile physiological saline, buffered with phosphate buffer (0.1 M,

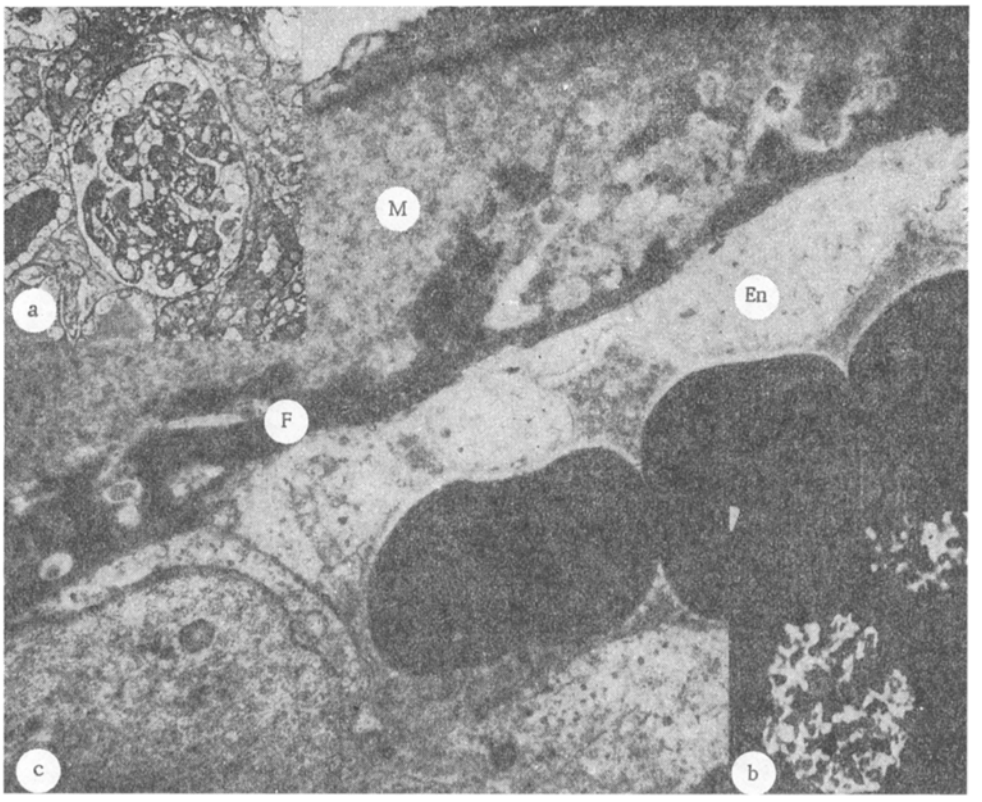

Fig. 2. Masugi's nephritis, 15th day after injection of NTS: *a*) picture of extracapillary proliferative glomerulonephritis. Methylene blue — azure II — fuchsin. 200 ×; b) fixation of IgG on basement membranes of glomerular capillaries is in the form of large granules. Direct Coombs' method. 200 ×; c) floccular material (M) and fibrin (F) in lamina rara interna, swelling of endotheliocytes (En), sludging of erythrocytes (E) in lumen of glomerular capillaries. 20,000 ×.

pH 7.4) until a uniform grayish-brown color was obtained. They were then detached from the renal pedicle and the capsule removed. The cortical layer of the kidneys was removed and transferred into a sterile metal sieve, with pore diameter of 105 μ. The kidney tissue was pressed through the sieve, mounted on a beaker, using the bottom of a small sterile flask. Glomeruli which clogged the pores of the filter were washed off into the beaker with a jet of cold physiological saline. Merthiolate was added at the rate of 1 mg to 10 ml as antiseptic to the saline solution mentioned at this stage and later. The suspension was transferred to centrifuge tubes with a capacity of 30-40 ml and centrifuged at 4°C and 500g (2000 rpm, radius of rotor 11 cm) for 3 min. The supernatant with tissue fragments was discarded and the residue resuspended in 15-20 ml of physiological saline and centrifuged under the same conditions. The procedure of washing the residue to remove nonglomerular impurities was repeated 3 times. The last centrifugation was carried out at 4000 rpm for 10 min. The residue consisted mainly of glomeruli, as could be clearly seen during microscopic examination of unstained preparations. The residue was transferred into a graduated test tube by means of physiological saline. The total volume of the suspension in the tube was made up to 10 or 15 ml. The weight of antigen was determined after drying of 1ml of the suspension, by weighing the dry residue.

Six male rabbits weighing 2.5-2.7 kg were immunized by the following scheme. In the first stage of immunization 20-25 mg of antigen in 1 ml of suspension was mixed with 1 ml of Freund's complete adjuvant and injected intramuscularly into a rabbit at several points of the animal's body. After 4 weeks the antigen was injected intraperitoneally in a dose of 100 mg, in a volume of 5 ml, during three consecutive days. To avoid anaphylactic reactions, the intraperitoneal injection was given fractionally.

EXPERIMENTAL RESULTS

On the 7th-8th day after the last injection of antigen, 10-12ml of blood was withdrawn from the auricular vein of the rabbits to determine activity of the serum. Incidentally, as-

sessment of activity of the NTS on the basis of the titer of antikidney antibodies, determined in the ring-precipitation test or the complement fixation test, is insufficiently objective, for the nephrotoxicity of the serum does not correlate with the titer of antikidney antibodies [6]. Activity of the serum was therefore determined in a biological test, by injecting the serum into the caudal vein of rats weighing 180-200 g, in a dose of 0.8 ml/100 g body weight on two consecutive days. A highly active NTS was obtained in four of the six rabbits: on the 3rd day after injection the proteinuria in the rats reached 20-30‰ (normally 0.17-0.66‰). The serum of the other two rabbits possessed weaker nephrotoxicity: the proteinuria in the rats amounted to 0.99-1.5‰. Blood was collected from the auricular vein of rabbits with active NTS on the 9th-10th, 14th-15th, and 23rd-24th days after final immunization, in a volume of 30-40 ml. Altogether 60-75 ml of NTS was obtained from each of the four rabbits. The serum was inactivated by heating to 56°C for 30 min, then poured 2ml at a time into ampuls, and freeze-dried. After 3 months, the three rabbits from which active NTS was obtained were reimmunized by intraperitoneal injection of kidney antigen in a dose of 20-40mg in 5ml of physiological saline on three consecutive days. Of the three rabbits only one responded to this immunization by the production of active NTS.

GN was produced in 17 male rats weighing 170-200 g. The freeze-dried NTS was dissolved in distilled water (2 ml per ampul) and injected into the caudal vein of the rats as stated above. Marked proteinuria developed as early as on the 3rd day after the first injection of NTS. The dynamics of the proteinuria was studied in eight rats in the course of 40-50 days. The protein concentration in the urine was determined every 5-7 days by the sulfosalicylic acid method. Excretion of protein with the urine during 18 h amounted to 60-320 mg (normally 0.7-3.0 mg), and considerable variations were observed in individual animals. By the 5th-7th day of the disease, all the rats had marked edema.

Several serum biochemical parameters were determined in nine rats on the 10th-15th days after the first injection of NTS on a "Technicon" automatic analyzer. Blood was obtained from the animals by decapitation. The results, given in Table 1 show that besides high proteinuria, the rats also had marked hypoproteinemia and hyperlipidemia, i.e., they indicate the development of a nephrotic syndrome. Histological and electron-microscopic study of the kidney tissue of rats killed on the 5th and 15th days of the disease showed changes characteristic of acute GN (Figs. 1 and 2).

The use of rat renal glomeruli and Freund's adjuvant for immunization of rabbits thus enabled an active NTS, capable of inducing GN of nephrotic type in rats, to be obtained from the majority of animals.

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