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ATTEMPTS TO EXERT AN INFLUENCE ON EXPERIMENTAL GLOMERULONEPHRITIS*

EDIT BEREGI

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Most research workers agree in regarding glomerulonephritis as a form of allergic disease. SCHICK who was the first to recognize this allergic character, asserted that glomerulonephritis subsequent to scarlet fever manifests itself after a certain period of latency. Whereas the evidence both from humans and from test animals seems to support his statement, opinions about the course of development from a specific infection to glomerulonephritis are at variance. STREHLER, for instance, suggests that in response to a streptococcal infection the organism produces antibodies belonging to the globulin group. In the antigen-antibody reaction these globulins suffer considerable changes as a result of which they will be no more accepted as "self". Henceforth this antibody acts as antigen, to release a secondary course of defensive reaction, followed by a secondary process of antibody formation and a secondary antigen-antibody reaction, until glomerulonephritis develops. According to KURTZKE in streptococcal infection the streptococci enter into a reaction with the kidney proteins to produce an antigen which assumes foreign albumin character and provokes antibody formation. In one or two weeks the antibody titre attains its peak, the antibody reacts with the kidney proteins and gives rise to acute glomerulonephritis. MELLORS *et al.* discovered gamma globulins in the glomeruli and thus provided immuno-morphological proof of the antigen-antibody reaction occurring within the kidneys.

The significant part played by the reticuloendothelial system in inducing allergic reactions and in producing immune substances has long been established. In the study to be reported our aim has been to establish whether and by what means the development of experimental glomerulonephritis could be controlled by influences exerted on the reticuloendothelial system.

In a previous series of experiments we have sensitized rabbits with horse serum, thereafter treated them with pilocarpine and immersion into cold water, with the result that some of the animals died exhibiting anaphylactoid symptoms, the rest showed the clinical and anatomical changes characteristic of

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acute or subacute glomerulonephritis. The aim of the present studies was to discover whether the method described gives rise to an immunological reaction. The crucial point therefore was to establish the effect of the reticuloendothelial system on the entire process. The experiments were carried out in several groups as follows.

(i) In the first group, 10 rabbits, weighing 2500 to 3500 g each, were sensitized on two consecutive days with 2 ml of horse serum and one week later their spleens were removed. They were subjected to pilocarpine administration and cold water bath two weeks after the operation. None of the animals succumbed to anaphylactoid shock. One rabbit became somnolent after the sixth treatment and showed the histological features of acute glomerulonephritis with albuminuria, haematuria and a NPN of 90 mg per cent. The remaining 9 animals were killed by air embolism after the 18th treatment (see Table I).

Table I

Rabbit No.	Experimental period, days	Urinanalysis		NPN mg per 100 ml	Histology
		Alb.	Sedim.		
1.	6	++	RBC, casts	90	Proliferative gln.
2.	18	+++	RBC, casts	62	Chronic gln.
3.	18	++	RBC, casts	58	Exudative gln.
4.	18	+++	∅	34	Nephrosis
5.	18	+++	∅	38	Nephrosis
6.	18	++	∅	34	Nephrosis
7.	18	+++	∅	36	Nephrosis
8.	18	∅	∅	36	Normal
9.	18	∅	∅	38	Normal
10.	18	∅	∅	36	Normal

Two out of the nine showed albuminuria, haematuria and cylindruria with a NPN of 58 mg and 62 mg per cent, respectively. In one animal histology revealed glomerulonephritis with marked exudative changes. Another animal displayed hypertrophic glomeruli, many of them with hyaline degeneration (Fig. 1), with signs of incipient chronic glomerulonephritis, the like of which had not occurred in previous experiments. In four of the 9 rabbits there was mere albuminuria, normal NPN level and thickening of the basal membrane. Three rabbits showed nothing abnormal.

Summing up the results in this group, anaphylactoid shock never followed splenectomy, but glomerulonephritis and nephrosis sometimes presented themselves. The question suggests itself what difference would it make if the animals were sensitized not before but after the spleen had been removed.

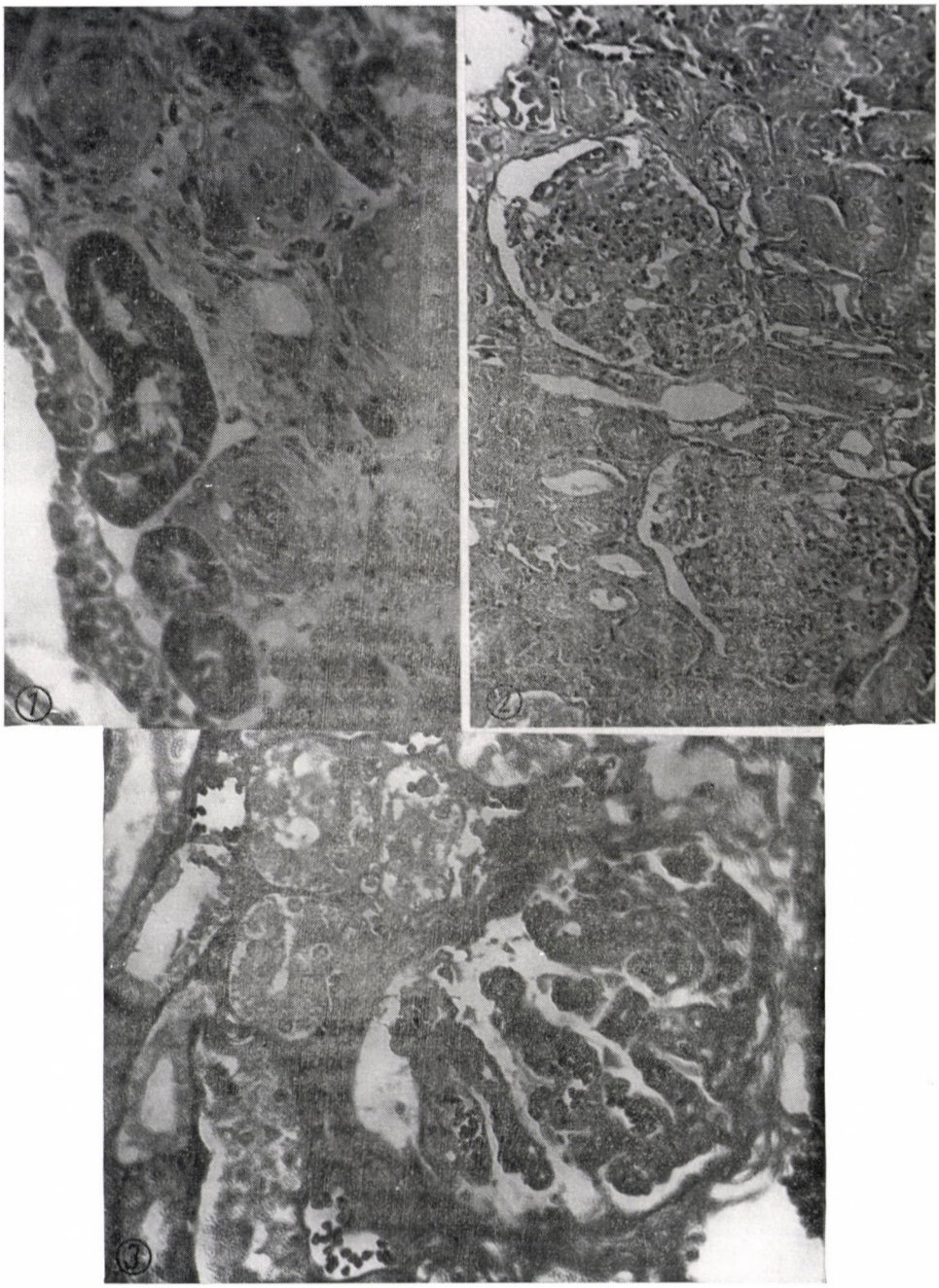

Fig. 1. Numerous contracted glomeruli, some of them with hyaline changes (Haematoxylin-eosin)

Fig. 2. Swollen and cellabundant glomeruli, the loops adhering in some places to the wall of Bowman's capsule (Haematoxylin-eosin)

Fig. 3. The glomerular basal membrane is thick with widened basal membrane of Bowman's capsule (Heidenhein's azan stain)

(ii) To study the effect under such conditions, 10 rabbits were sensitized two weeks after splenectomy and subjected three weeks later to pilocarpine treatment and cold water bath. Neither anaphylactoid shock nor glomerulonephritis was observed in any of them.

(iii) The purpose of the third series of experiments was to inhibit the reticuloendothelial function not of the spleen alone but possibly of the entire organism. Blocking of the reticuloendothelial system was attempted on the basis of JANCSO's observation that a 0.06 per cent electrocolloidal copper solution injected intravenously in rats and mice paralyzed the reticuloendothelial function for 24 to 48 hours. It turned out, however, that colloidal copper at such a concentration killed the rabbits within a few hours, so a weaker concentration had to be applied. Ten rabbits, weighing 2500 to 3500 g each, were therefore sensitized with horse serum and after three weeks each animal was given 10 ml of a 0.02 per cent colloidal copper solution intravenously. Two hours later pilocarpine and a cold bath were administered. These treatments were repeated on every other day several times. The animals suffered them badly, though symptoms of shock did not appear. Three animals died after the second treatment, 3 after the third, 2 after the fourth and 2 after the fifth treatment. In 6 rabbits albuminuria was observed, combined in 4 with haematuria and cylindruria. Four rabbits showed no changes in the urine. In 3 the NPN level was elevated (87, 95 and 76 mg per cent). The findings are given in Table II. Necropsy of the clinically affected rabbits revealed swollen kidneys, greyish yellow in colour, with superficial pin-head haemorrhages; the cut surface showed a confluent margin between cortex and medulla. In the renal glomeruli of those animals which survived only two treatments there was intercapillary oedema and protein in Bowman's capsule. In the animals which had died on the fourth and fifth days proliferative glomerulonephritis was

Table II

Rabbit No.	Experimental period, days	Urine		NPN mg per 100 ml	Histology
		Alb.	Sedim.		
1.	2	∅	∅	34	∅
2.	2	∅	∅	38	∅
3.	2	∅	∅	36	∅
4.	3	∅	∅	38	∅
5.	3	+++	∅	38	Nephrosis
6.	3	++++	∅	36	Nephrosis
7.	4	++	RBC, casts	36	Proliferative gln.
8.	4	+++	RBC, casts	87	Proliferative gln.
9.	5	+++	RBC, casts	95	Proliferative gln.
10.	5	++	RBC, casts	76	Proliferative gln.

observed (Fig. 2), with grave regressive changes in the tubuli and sporadic necrosis of the epithelium. In two rabbits this was associated with calcification in the necrotic cells. Two rabbits with nephrotic changes were conspicuous for thickening of the glomerular basement membrane (Fig. 3).

The results seemed to indicate that in response to the usual treatment combined with the administration of the specified concentration of colloidal copper the rabbits developed severe acute glomerulonephritis. It remained to discover whether or not the dose of copper used sufficed to paralize reticuloendothelial function, furthermore what renal changes the administration of colloidal copper alone is able to induce.

(iv) To test reticuloendothelial activity according to Jancsó's method, 5 rabbits were given a 0.02 per cent solution of colloidal copper and 2 hours later a solution of colloidal gold. After a second period of 2 hours the animals were killed. The presence of gold in the Kupffer cells indicated that the applied amount of copper was unable to inhibit reticuloendothelial function. With the use of 0.06 per cent colloidal copper there was no storage of gold in the Kupffer cells but this concentration was not long tolerated by the rabbits.

(v) As a control, 10 rabbits were treated with 0.02 per cent colloidal copper every other day for 18 days. No sign of glomerulonephritis appeared; regressive changes with necrosis and calcification were only noticeable in the tubules.

(vi) In an earlier series of experiments a difference has been observed in the pathological course of pilocarpine nephritis between old rabbits and middle-aged ones weighing 3000 to 3500 g. The difference has been attributed to the senile modification of immunological behaviour.

The next group of 10 young rabbits, 1200 to 1500 g in weight, was subjected three weeks after sensitization to the usual treatment with pilocarpine combined with cold bath. None succumbed to anaphylactoid shock. From the fourth day onwards protein appeared in the urine of 4 animals. In 2 of these erythrocytes and casts were also present. One of the latter animals died on the 10th day, showing an NPN level of 98 mg per cent; the remaining 9 rabbits were killed by air embolism after the 18th treatment. These had a normal NPN level, only one showed a slightly elevated value (58 mg per cent). The 2 rabbits with elevated NPN displayed acute glomerulonephritis but the glomerular changes, similarly to those in the aged animals, were predominantly of exudative character, with little proliferation. The 4 rabbits with albuminuria exhibited a thickening of the basal membrane, with protein in Bowman's capsule and the tubules. Four rabbits were free from pathological changes.

The final conclusion seems to be that it is possible to control the renal process through the reticuloendothelial system. Development of the nephritis was inhibited if the rabbits were sensitized after their spleen had been removed. Colloidal copper at a concentration sufficient to paralize reticuloendothelial

function killed the animals. Lower concentrations were tolerated but failed to inhibit reticuloendothelial activity and so to prevent development of the acute glomerulonephritis. In young rabbits no anaphylactoid symptoms appearad and the renal changes were much slighter than in adults. This seems to confirm ERDMANN's statement that to develop glomerulonephritis, young rabbits need a much higher dosage of nephrotoxic serum than adult rabbits and that the histology of juvenile glomerulonephritis is different from the mainly proliferative glomerulonephritis of adult animals.

Summary

An attempt has been made to determine the effect of the reticuloendothelial system on the course of experimental glomerulonephritis. Pilocarpine nephritis has been prevented if the animal was sensitized after splenectomy. If the spleen had been removed before sensitization, glomerulonephritis developed. Colloidal copper at a concentration high enough to paralyze reticuloendothelial functions was found to kill the animals. Lower concentrations were tolerated but failed to inhibit reticuloendothelial activity and thus the development of acute glomerulonephritis.

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UNTERSUCHUNGEN ÜBER DIE BEEINFLUSSBARKEIT DER EXPERIMENTELLEN GLOMERULONEPHRITIS

EDIT BEREGI

Es wurde versucht, den Einfluß des RES auf den Verlauf der — nach einer früher beschriebenen Methode herbeigeführten — experimentellen Glomerulonephritis klarzustellen. Wie die Untersuchungen ergaben, kommt die Hemmung der Nephritis mit Pilocarpin in dem Falle zustande, wenn vor Sensibilisierung der Kaninchen die Splenektomie ausgeführt wird. Bei Anwendung kolloidaler Kupferlösung war festzustellen, daß die Kaninchen von der die RES-Funktion lähmenden Dosis rasch getötet wurden. Die von den Tieren besser tolerierte niedrigere Dosis brachte keine Aktivitätshemmung in den RES-Zellen zuwege, so daß akute Glomerulonephritis auftrat. Bei jungen Kaninchen entwickelten sich erheblich leichtere Nierenveränderungen als bei ausgewachsenen Tieren.

ИССЛЕДОВАНИЯ ПО ВОЗДЕЙСТВИЮ НА ЭКСПЕРИМЕНТАЛЬНЫЙ ГЛОМЕРУЛОНЕФРИТ

Е. БЕРЕГИ

Автор старается выяснить роль РЭС в протекании экспериментального гломерулонефрита, вызванного описанным им раньше методом. В ходе своих исследований он выявил, что торможение нефрита пилокарпином имеет место в том случае, если до сенсибилизации кроликов проводится спленэктомия. При применении коллоидного раствора меди было установлено, что парализующая функцию РЭС доза — ввиду большой токсичности раствора — вызывает быструю гибель животных. Меньшая доза, которую животные лучше переносят, не вызывает торможения активности в клетках РЭС, и в таких случаях развивается острый гломерулонефрит. У молодых кроликов изменения развивающиеся в почке были гораздо меньшей степени чем у взрослых кроликов.

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