on the day that the rats were put on the choline-deficient diet. This operation gave protection to the decapsulated kidney in proportion to the completeness of the operation. In 19 out of 34 animals there was complete protection from the renal syndrome of acute choline deficiency on the operated side. This protection seems to be in abeyance if the operation is not performed well in advance of the acute disturbance, since if the decapsulation is not done until the fifth day, no protection is observed.

Wolbach[5] has suggested the possibility that a neurovascular mechanism might be the basis of the renal syndrome observed in acutely choline-deficient rats. There is support for such an assumption in our findings that subcutaneous administration of atropine seems to inhibit the renal syndrome. When rats were treated with 1 mg atropine sulfate daily, from the day they were started on the choline-deficient diet, prevention of the renal syndrome was observed in almost half of the cases. In these preliminary experiments, which lasted 7 days, 10 of 21 choline-deficient young rats failed to show the "hemorrhagic renal syndrome."

Summary and Conclusion. Morphological and experimental studies suggest a vascular disorder to be the primary injury observed in the kidney of the acutely choline-deficient rat.

[5] Wolbach, S. B., and Besser, O. A., *Physiol. Rev.*, 1942, **22**, 233.

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Reticulo-Endothelial Immune Serum (REIS). VI. Production of Potent Serum by Anamnestic Reaction.*

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The development of antiorgan sera has been reported in studies from our laboratories.[1] Whereas in our original work[2] we followed the method of Soviet workers,[3] some modifications in the production of REIS were introduced in the present study. It was thought that an accumulation of antibodies in the recipient host would be enhanced by a slow process of immunization rather than by the rapid method of using massive doses.[4]

In the preparation of antiguinea pig and of antirat sera, constant amounts of tissue, 400 mg of spleen plus 100 mg of bone marrow, suspended in 6 ml of saline were administered to the rabbit. Intravenous antigen injections of 0.25, 0.5, 0.5, and 0.75 ml were given at 4-day intervals, and the same amounts were given by mouth, the total being approximately 133 mg of spleen and 33 mg of bone marrow by each route within 12 days.

Complement-fixation titres illustrate the high values of antirat and antiguinea pig sera following low antigenic dosages.

The achievement and maintenance of adequate potency of the serum was one of the main objectives. Attempts were made, therefore, to provoke an increased antibody production in previously immunized animals by their anamnestic response to a renewed contact with the antigen. A single intravenous injection of the same type of antigen 30-40 days following the last injected dose was administered to rabbits.

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[1] Pomerat, C. M., *Phi Beta Pi Quart.*, 1945, **42**, 203.

[2] Pomerat, C. M., and Anigstein, L., *Tex. Rep. Biol. and Med.*, 1945, **3**, 122.

[3] Marchuk, P. D., *Am. Rev. Sov. Med.*, 1943, **1**, 113.

[4] Straus, R., Runjavac, M., Zaitlin, R., Duboff, G., and Swerdlow, H., *II. J. Immun.*, 1946, **56**, 155.

TABLE I.
Showing Comparative Values of REIS Potency in a Unit Experiment.

Rabbit No.	Antigen source	REIS complement-fixation titers		Spleen explant outgrowth in REIS 1:4	
		Primary	Anamnestic	Primary	Anamnestic
68	Chick	1:60	X	+	X
69	"	1:40	1:640	++	+
70	Guinea pig	1:640	1:1280	+++	+
71	" "	1:1000	1:2000	+	0
72	Rat	1:1000	X	0	X
73	"	1:320	1:160	+	++

X Animal sacrificed.
0 No outgrowth—complete inhibition.
+ Slight outgrowth.

The serum of rabbit 69 (Table I), treated with chick antigen, primarily showed a titre of only 1:40. This rabbit was given a booster dose 30 days later of 1:25 ml suspension of 400 mg of chick spleen and 100 mg of bone marrow in 6 ml saline. The animal was bled 9 days later and at this time the "anamnestic" serum, as compared with the "primary," showed a titre for complement fixing antibodies of 1:640. A 100% rise in the titre was obtained in 2 other rabbits previously treated with guinea pig antigen. On rare occasions there was no response to the booster method (rabbit 73).

The potency of the serum was also evaluated by the tissue culture assay method. It was previously suggested[2,5] that it is possible to measure the action of antiorgan preparations by the degree of inhibition of cellular outgrowth from explants in a medium containing various concentrations of REIS. The outgrowth equivalent to that of untreated explants was designated as ++++. When the serum of rabbit 69 (primary titre 1:40) was tested, the outgrowth of 15- to 17-day chick embryonic spleen fragments was moderately good (++), showing weak inhibitory effect of the serum. However, the serum of the same rabbit, after the anamnestic reaction, reaching the titre of 1:640 almost completely inhibited the outgrowth. Marked clumping of outwandering cells under the influence of the serum of increased potency at dilutions of 1:4, 1:8, and 1:16 was noted. The anamnestic serum totally inhibited the outgrowth in concentrations of 1:4, 1:8, 1:16, 1:32 as compared with a slight inhibition of the primary serum.

Summary. In the preparation of antiorgan sera the administration of small amounts of antigen (spleen and bone marrow) was found more effective than the rapid immunization of rabbits with massive antigen dosage. A single injection of the antigen 30-40 days after the last dose gives rise to an anamnestic response of the host and to a serum of higher potency. This can be evaluated by complement fixation titres and by the outgrowth of tissue explants exposed to the action of the "anamnestic" serum.

5 Pomerat, C. M., and Anigstein, L., *Science*, 1944, **100**, 456.