sults from experiments with physiological saline, now under way, substantiate this contention.

Summary. A simple method is presented which permits accurate comparative studies on the therapy of experimental renal obstruction from sulfadiazine. Under the conditions of this method, the results of experiments completed up to now, suggest that in fully developed renal obstruction from sulfadiazine, the generally recommended therapeutic measures—"forcing of fluids" with or without massive alkalinization—can be useless or even harmful, whereas, under identical conditions, solutions of neutral salts or of moderately alkalinizing salt mixtures are able to reopen the urinary pathways quickly and thus to save life.

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Effect of Epinephrine on the Synthesis of Acetylcholine.*

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Stimulation of the sympathetic nervous system or administration of epinephrine is often followed by manifestations similar to those that follow administration of acetylcholine. Epinephrine was found to inhibit choline esterase *in vitro*.[1,2] In the following it was ascertained whether the presence of epinephrine modifies the synthesis of acetylcholine *in vitro*.

Method. The synthesis of acetylcholine was studied following the method of Quastel, Tennenbaum, and Wheatley[3] with minor modifications.[4] Hundred mg samples of homogenized fresh frog brains were used as a source of the enzyme, while human serum and human spinal fluid (1 cc) and the frog brain itself supplied the substrate. Three mg physostigmine salicylate, to prevent the enzymatic hydrolysis of the acetylcholine, 4.8 mg glucose, to supply an excess of the precursor of the acetyl-radical and 2 cc Ringer's solution were added to each mixture. Varying amounts of epinephrine (0.03 μg to 30 μg) were added to the mixtures, and the pH of the mixtures was adjusted to 7.4. Identical mixtures without epinephrine served as controls. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After shaking the mixtures were diluted to 10 cc with Ringer's solution and centrifuged. The acetylcholine content was assayed biologically using the sensitized rectus abdominis muscle of frog; the amount of acetylcholine synthesized was computed by subtracting the acetylcholine content of identical, non-incubated mixtures from the acetylcholine content of incubated mixtures. The free acetylcholine synthesized was determined from the acetylcholine content of the supernatant fluid; the total acetylcholine† synthesized, from a solution obtained by resuspending the precipitate in the supernatant fluid, boiling for 2 minutes at pH 6.8, centrifuging and cooling. Since epinephrine in the concentrations used did not induce muscle contraction by itself, and since it did not modify the effect of acetylcholine in inducing muscle contraction, the use of the rectus abdominis muscle was a valid tool for determin-

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1 Ammon, R., *Pflüger's Arch.*, 1933-34, **233**, 486.

2 Waelsch, H., and Rackow, H., *Science*, 1942, **96**, 386.

3 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.*, 1936, **30**, 1668.

4 Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944 in press.

† Total acetylcholine is the sum of free and bound intracellular and extracellular acetylcholine.

TABLE I.
Amount of Acetylcholine Synthesized in the Control Mixtures.

Mixtures containing frog brain (100 mg), Ringer's sol. (2 cc), physostigmine salicylate (3 mg), glucose (4.8 mg) and 1 cc of	Acetylcholine synthesized in μg/100 mg of frog brain	
	Free acetylcholine	Total acetylcholine
Spinal fluid	2.11 ± 0.053	3.16 ± 0.049
Serum	1.45 ± 0.011	2.08 ± 0.029
Ringer's solution	0.41 ± 0.013	1.02 ± 0.033

TABLE II.
Effect of Epinephrine on the Synthesis of Acetylcholine *in vitro*.

Final conc. of epinephrine	Amount of acetylcholine synthesized in % of control*					
	Free acetylcholine			Total acetylcholine		
	Spinal fluid	Serum	Ringer's sol.	Spinal fluid	Serum	Ringer's sol.
0	100	100	100	100	100	100
1.10^{-8}	145	139	151	139	138	147
1.10^{-7}	149	153	160	152	169	172
1.10^{-6}	163	168	178	180	190	104
1.10^{-5}	206	230	115	210	225	250

*Each value represents the average of 8 separate experiments. The S.E. of the mean for each value was less than ±10%.

ing the acetylcholine content of the mixtures.

Results. The amounts of acetylcholine synthesized in the controls are summarized in Table I, those synthesized in the presence of epinephrine in Table II. Epinephrine in concentrations from 1.10^{-8} to 1.10^{-5} increased the synthesis of acetylcholine by 40 to 150% respectively above the control. The increase of synthesis of acetylcholine was observed in mixtures containing frog brain alone as substrate and also in those containing an additional amount of serum or spinal fluid.

Discussion. Epinephrine increases the synthesis of acetylcholine *in vitro*. This increase is significant and manifests itself even with low concentrations of epinephrine. An adequate explanation of the mechanism of this potentiation by epinephrine cannot be offered yet. The lowest concentrations used in the above experiments are within the concentrations of epinephrine in the blood.[5]

Assuming that similar increase of the synthesis of acetylcholine may occur in the body, it is possible that the succession of manifestations of stimulation of the sympathetic nervous system by manifestations similar to those that follow administration of acetylcholine is due to both an increase in synthesis of acetylcholine and an inhibition of choline esterase.

Summary. 1. The synthesis of acetylcholine *in vitro* in the presence of epinephrine was investigated. 2. Epinephrine in concentrations used (1.10^{-8} to 1.10^{-5}) increased the synthesis of acetylcholine by 40 to 150% respectively above the control.

[5] Stewart, G. N., and Rogoff, J. M., *Endocrinology*, 1922, **2**, 127.