taken together, that the follicle ovulating about the time of lay exhibits some of the properties of the ruptured follicle.

It has been shown that the terminal egg of a clutch remains in the oviduct for an appreciably longer time than do mid-clutch eggs.[6] The 2 birds of Group II and the 4 of Group IV, in which lay was delayed only a few hours, were entirely dependent upon the most recently ruptured follicle for lay, next-due ovulation having been precluded by operation or regression. The postponement in time of lay by only a few hours is thus approximately comparable with the behavior of the terminal egg of a clutch, which egg also is laid in the absence of closely ensuing ovulation.

It would be premature to speculate at this time upon the mechanism underlying the action of the ruptured follicle in determining the hour of lay of its ovulated yolk. We believe, however, that the results presented here provide adequate proof that the avian ruptured follicle is not an organ without function, but that this structure, like its mammalian homologue, exerts an influence on the oviducal history of the ovum it once contained. Since the oviducal histories of ova of the two classes of vertebrates are so dissimilar, it will be interesting to observe what parallels and divergences exist in physiological behavior of the two organs.

Summary. Removal of the ruptured ovarian follicle or of this follicle together with the oldest maturing follicle of the hen at the time when the egg taking its origin from the ruptured follicle was in the oviduct almost invariably resulted in the holding of the egg beyond the expected time of lay. Most eggs were held from 1 to 7 days. Removal of other parts of the ovary at comparable times, without simultaneously removing the most recently ruptured follicle, practically never resulted in comparable holding of the oviducal egg. The ruptured follicle of the hen is believed therefore to be an important factor in determining the time of lay of the egg formed from its previously contained ovum.

[6] Heywang, B. W., *Poultry Sci.*, 1938, **17**, 240.

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Treatment of Experimental Renal Obstruction from Sulfadiazine. I. "Forcing of Fluids" and Alkalinization.*

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The value of massive alkalinization in the prevention of renal complications from intratubular precipitation of sulfadiazine and its acetylated product has been established clinically[1,2,3,4] as well as in the animal experiment.[5,6] The application of this procedure in combination with the "forcing of fluids" has also been suggested in fully developed renal obstruction and anuria occurring during the course of sulfadiazine therapy,[7] but its usefulness and advisability lacks experimental foundation.

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Gilligan, D. R., Garb, S., and Plummer, N., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 248; Gilligan, D. R., Garb, S., Wheeler, C., and Plummer, N., *J. A. M. A.*, 1943, **122**, 1160.

[2] Barnes, R. W., and Kawaichi, G. K., *J. Urol.*, 1943, **49**, 324.

[3] Jensen, O. J., Jr., and Fox, C. L., Jr., *J. Urol.*, 1943, **49**, 334.

[4] Fox, C. L., Jr., Jensen, O. J., Jr., and Mudge, G. H., *J. A. M. A.*, 1943, **121**, 1147.

[5] Lehr, D., *Bull. New York M. College, Flower and Fifth Ave. Hosps.*, 1943, **6**, 70.

[6] Jensen, O. J., Jr., *Am. J. Med. Sci.*, 1943, **206**, 746.

[7] Lowell, F. C., *M. Clin. North America*, Sept., 1943, p. 1247.

It was the purpose of this investigation to evaluate the merits of alkalinization in conjunction with the "forcing" of water, after the production of a renal block by intratubular precipitation of sulfadiazine under the standard conditions of the animal experiment. Included in this study for the purpose of comparison was an evaluation of the effects of "forcing" either water alone, or solutions containing an acidifying salt or a salt mixture.

Procedure. Young albino rats from our own standard colony, weighing 150 to 200 g, were used in all experiments. The animals were kept on a standard diet (Rat Food, Rockland Farms) and had free access to drinking water.

Drug precipitation in the kidney tubules—in accordance with a finding reported originally for sulfathiazole[8]—was produced by intraperitoneal injection of a single fatal dose of sodium sulfadiazine† (1.5 g per kg body weight). If left untreated, the animals without exception develop, within a few hours, pronounced and long lasting renal obstruction from intratubular precipitate of sulfadiazine, and 70 to 80% succumb to this complication after 2 to 3 days. (This observation is based on chemical findings in blood and urine, and post mortem examination of 50 animals.) A detailed analysis of the mechanism causing death has been presented elsewhere.[9]

Treatment consisted in stomach tube feedings of fixed amounts of fluid (water or salt solutions), twice daily, starting shortly before the sulfadiazine injection and continuing for at least one or several more days. All salt solutions were tolerated by control animals for several days with little ill effect and with no significant change of the blood pH.

Throughout the experimental period, volume, specific gravity, pH (measured with a Cambridge Electron-Ray pH meter), and sulfadiazine concentration of the urine were recorded daily, and determinations of nonprotein nitrogen, drug level and pH were carried out in the blood at various intervals. All surviving rats were killed by exsanguination in ether narcosis, usually 8 to 10 days after completion of each particular experiment. Thorough post mortem examinations were performed on these animals and also on those succumbing during the experiment. Special attention was given to the appearance of the urinary tract. The most important organs of representative animals were fixed in formaldehyde for histological study.

Results. The results of different experiments although similar in their main features, varied considerably in accordance with the duration of the treatment. Undue prolongation of therapy usually increased the mortality. For the purpose of this report it seemed sufficient to present some significant findings of one typical experiment, performed with 60 closely bred, 3 months old, female rats. The data are summarized in Table I.

This particular experiment was run in 2 parallel sets. Subgroups of 5 rats each were placed in separate metabolism cages. Chemical studies were done on the second set (animals No. 6-10) only, with all animals which were still alive 48 hours after the injection of sulfadiazine. Therapy (forcing of fluids) was started 3 hours before the sulfadiazine injection and continued for one more day (4 stomach tube feedings in all).

The most striking result apparent from Table I is the excellent therapeutic success achieved with solutions of sodium chloride and of salt mixture (Groups B and E). They made possible the survival and complete recovery of all rats from an otherwise fatal sulfadiazine intoxication, whereas no benefits were derived from the "forcing" of water alone (Group A). Sodium bicarbonate (Group D), on the other hand, very definitely shortened the time of survival (maximum 48 hr), and ammonium chloride (Group C), in addition to a further reduction of the life span (24 hr), also increased the mortality to 100%.

From the chemical data obtained in this particular test, the consistent impairment of renal function is obvious in groups A, B, E, and F (uniformly pronounced elevation of the nonprotein nitrogen values in the blood); the sulfadiazine blood levels, however, taken at

[8] Lehr, D., Antopol, W., and Churg, J., *Science*, 1940, **92**, 434.

† The sodium sulfadiazine used in this study was supplied by the Schering Corporation, Bloomfield, N.J.

[9] Lehr, D., and Antopol, W., *Urol. and Cutan. Rev.*, 1941, **45**, 545.

TABLE I.
Fatal Intoxication of Albino Rats with Sulfadiazine.
(Single intraperitoneal injection of 1.5 g of sodium sulfadiazine per kg body weight in 5% aqueous solution.)

Forcing of fluids (3 ml per 100 g body wt) by stomach tube twice daily	Rat No.	Died within days			% dead	48 hr after injection of SD			
						Blood		Urine per rat (mean value)	
		1	2	3		N.P.N. mg%	SD mg%	SD mg	Vol. ml
(A) Water	1								
	2		X						
	3		X						
	4		X						
	5		X		80				
	6			X		215	116		
	7					205	124		
	8		X					42	19
	9		X						
	10		X						
(B) Aqueous sol. of NaCl, 3.33%	1								
	2								
	3								
	4								
	5				0				
	6					227	73		
	7					205	71		
	8					199	94	65	38
	9					215	87		
	10					199	91		
(C) Aqueous sol. of NH_4Cl, 1.67%	1	X							
	2	X							
	3	X							
	4	X							
	5		X		100				
	6	X							
	7	X							
	8	X						7	5
	9	X						(24 hr values)	
	10	X							
(D) Aqueous sol. of $NaHCO_3$, 3.33%	1		X						
	2								
	3								
	4		X						
	5		X		80				
	6		X						
	7		X						
	8		X					70	21
	9		X						
	10		X						
(E) Aqueous sol. containing *both* NH_4Cl, 1.67%, $NaHCO_3$, 3.33%	1								
	2								
	3								
	4								
	5				0				
	6					179	54		
	7					199	98		
	8					222	75	107	51
	9					160	51		
	10					222	76		

TABLE I. (Continued)

Forcing of fluids (3 ml per 100 g body wt) by stomach tube twice daily	Rat No.	Died within days			% dead	48 hr after injection of SD			
						Blood		Urine per rat (mean value)	
		1	2	3		N.P.N. mg%	SD mg%	SD mg	Vol. ml
(F)	1								
Control	2								
No treatment of	3		X						
SD intoxication	4		X						
	5			X	80				
	6			X		207	118		
	7		X			216	135		
	8			X		219	131	38	16
	9			X		227	128		
	10		X						

the same time, indicate a much more rapid clearance of the drug in the sodium chloride and salt mixture groups (B and E). The observation is confirmed by the high amounts of sulfadiazine excreted in the urine of these two latter groups, and finds its explanation in the successful abolition of the renal block in the surviving animals. After 4 days most of the survivors showed only traces of sulfadiazine in the blood and the nonprotein nitrogen levels had fallen to almost normal. The disappearance of kidney obstruction can also be inferred from the large volumes of urine. Massive alkalinization, on the other hand, although it increases the solubility of sulfadiazine and thus permits the initial elimination of large amounts of drug (high urinary concentrations), does not succeed in keeping the urinary pathways sufficiently open to prevent the fatal accumulation of alkali in the body.

In experiments, otherwise identical, blood determinations were performed 20 hours after sulfadiazine injection, including estimation of the pH. This shorter interval permitted the inclusion of the sodium bicarbonate and ammonium chloride animals in the blood analysis. The determinations revealed very high and strikingly uniform nonprotein nitrogen and sulfadiazine values (N.P.N., 170-200 mg %; sulfadiazine "total," around 150 mg %) in all groups, indicating that no animal escaped initial renal obstruction. However, there were very significant differences in the serum pH values obtained from heart blood. They indicated the presence of a severe uncompensated acidosis (pH 6.80 to 7.15) in the ammonium chloride group, whereas those from the bicarbonate animals pointed to the development of an uncompensated alkalosis (pH 7.67 to 7.75). The pH values of all other groups remained within the normal range (pH 7.35 to 7.60), with the sodium chloride animals at the lower limit of the normal and somewhat exceeding it.

The remarkable therapeutic effect of *simultaneous* administration of ammonium chloride and sodium bicarbonate (Group E) in exactly the same concentrations, which, when given separately, had such deleterious effects (Groups C and D), demonstrates that the mixture of an acidifying and an alkalinizing salt apparently prevents dangerous changes in the pH of the blood, while the increased crystalloid concentration enhances the diuretic effect of the solution, possibly by inhibiting the reabsorption of water in the renal tubules. This contention would also explain the therapeutic action of a hypertonic sodium chloride solution (Group B), despite the fact that sodium chloride is a slightly acidifying agent.

Since all animals had free access to water, the thirst stimulus caused by the administration of hypertonic salt solutions resulted in a considerably increased water intake in the saline and salt mixture group, particularly after the establishment of a pronounced diuresis. This observation makes the administration of hypertonic salt solutions comparable to the "forcing" or much larger volumes of isotonic solutions. Preliminary re-

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-

* This study was aided by a grant from the Josiah Macy, Jr., Foundation.

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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.