2 to 8 mg doses had no effect upon milk ejection. Atropine tended to increase blood flow slightly and completely prevented any response to acetylcholine and acetyl-β-methylcholine but had no effect upon the action of Pitocin or pitressin.

From these observations it would appear that the mammary gland is innervated with the parasympathetics as well as the sympathetics.

The acetylcholine, Mecholyl and Pitocin used in these experiments were furnished by Merck and Company through the courtesy of Doctor D. F. Green.

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Studies on the Toxicity of Protamine.

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The parenteral administration of protamine has been suggested by Jorpes[1] and Ravdin[2] as a clinical method of terminating the action of administered heparin. Since the toxic effects of the protamines have not been extensively studied, further investigations seemed desirable.

Thompson[3] and later workers have shown that relatively large doses of protamines produce dyspnea, a marked fall in the blood pressure, diminished blood coagulability, and in sufficient dosage, death. These symptoms were observed in several animal species. Thompson felt that the action of protamines could best be explained on the basis of a general toxic effect on the cardiovascular and respiratory systems. Postmortem studies on rabbits and mice, killed by subcutaneous injection of protamine, have been performed by Vartiainen and Marble.[4] Hemorrhages in the thymus, lungs, and kidneys were the most constant findings.

Although Kern and Langner[5] and earlier workers[6] had failed to produce anaphylaxis with protamine, Walther and Ammon[7] recently claimed to have produced fatal anaphylactic shock in guinea pigs

1 Jorpes, E., Edman, P., and Thaning, T., *Lancet*, 1939, **237**, 975.

2 Ravdin, I. S., *Am. J. Med. Sc.*, 1941, **201**, 299.

3 Thompson, W. H., *Trans. Roy. Acad. Med. in Ireland*, 1900, **18**, 426.

4 Vartiainen, I., and Marble, A., *J. Lab. and Clin. Med.*, 1941, **26**, 1416.

5 Kern, R. A., Langner, P. H., *J. A. M. A.*, 1939, **113**, 198.

6 Wells, H. G., *Physiol. Rev.*, 1921, **1**, 44.

7 Walther, G., and Ammon, R., *Klin. Wschr.*, 1939, **18**, 288.

with this substance. Their work, however, does not rule out the possibility that their animals died from direct toxicity of the shock dose (anaphylactoid shock) rather than from anaphylactic shock. Accordingly, we have investigated the effect in previously untreated animals of the shock dose employed by Walther and Ammon (120 mg/kg).

Method and Results. One to 3% aqueous solutions of salmine sulfate* were employed. Injections were made into the heart, saphenous vein, and peritoneal cavity of 23 guinea pigs and 3 rats. The results are summarized in Table I. The animals showed typical anaphylactoid symptoms,[6] including weakness, dyspnea, and convulsions, leading to death. The heart beat continued after respiration had ceased. Hence it is probable that Walther and Ammon did not produce anaphylactic shock since their only proof rests on the

TABLE I.
Effect of Saline Sulfate on the Normal Animal.

Animal	Dose, mg/100 g body wt	Survival period (min.)
	A. Intracardiac Injection.	
Guinea Pig	12	3.0
" "	12	3.0
" "	12	3.5
" "	12	2.0
" "	12	2.0
" "	12	5.0
" "	12	2.0
" "	12	3.0
" "	10	3.0
" "	10	2.5
" "	10	3.5
" "	6	18.0
" "	5	Survival
" "	3	"
Rat	36	0.2
"	12	3.5
"	12	3.0
	B. Intravenous Injection.	
Guinea Pig	12	2.0
" "	12	3.5
" "	12	2.5
" "	12	4.0
" "	12	3.5
	C. Intraperitoneal Injection.	
Guinea Pig	12	2 hr 15 min
" "	12	1 " 55 "
" "	12	2 " 20 "
" "	12	17 "

* Kindly placed at our disposal by Eli Lilly and Company, Indianapolis, Indiana.

symptomatology which we have duplicated entirely in the normal non-sensitized animal.

In an attempt to explain these anaphylactoid symptoms, further experiments were conducted. It was found that the addition of salmine to the whole blood of human beings, dogs, rabbits, cats, guinea pigs, rats, and mice produced a thready precipitate. In the human, dog, cat, and rat blood, gross agglutination of the blood corpuscles occurred. However, in rabbit, guinea pig, and mouse blood this phenomenon did not take place. Upon the addition of salmine to plasma a heavy white flocculent precipitate appeared. Serum, when treated similarly, exhibited only turbidity. These effects occurred in the whole blood, plasma, and serum in both the presence and absence of such anticoagulants as heparin, citrate and oxalate.

Ten mg of salmine per ml of plasma were required to produce maximum precipitation. Analysis of the total protein before and after such treatment with salmine showed removal of a quantity somewhat larger than the determined fibrinogen content. It was found that the precipitate could be redissolved in Ringer's solution or plasma by the addition of polyvalent ion salts or by a change in pH. After treatment of plasma with 10 mg of salmine per ml, 25% saturation with $(NH_4)_2SO_4$ yielded no precipitate. Upon increasing the concentration to 50% and 100%, however, voluminous precipitates resulted.

Since the addition of salmine to blood produced a precipitate, and

TABLE II.
Effects of Salmine Sulfate upon Perfusion Flow through Organs of Guinea Pig.

Organ perfused	Perfusion fluid	Injection dose (mg)	Control rate of flow (ml/min)	Min. rate of flow (ml/min)	Interval between inj. and min. flow (min)
I. Lung	Ringer's Solution	60	20.0	20.0	
"	Dog whole blood (oxalated)	60	10.4	0	2.5
"	"	10	12.0	0	4.0
"	"	20	10.0	0	7.0
"	"	20	15.0	0	3.0
"	"	20	9.0	0	0.25
"	"	30	8.0	0	0.5
"	"	20	6.4	0	1.0
II. Liver	Dog Serum	20	19.2	18.2	
"	" "	40	20.0	20.0	
"	Guinea pig whole blood (citrated)	20	8.2	0.6	3.0
"	"	20	7.2	0	8.0

in some animals cell agglutination, it seemed possible that these changes would have vascular occlusive effects. To test this hypothesis, salmine was added to blood flowing into perfused organs. The results are presented in Table II. Dog blood was used in most of these experiments because it was difficult to secure a sufficient quantity of guinea pig blood. It is seen that the addition of 10 mg or more of salmine to either dog or guinea pig whole blood resulted in rapid decline or cessation of blood flow. On the other hand no such effect was obtained upon the addition of salmine to dog serum or Ringer's solution.

Discussion. The data in Table I demonstrate that the dose of salmine which Walther and Ammon interpreted as causing death by anaphylaxis actually produced death in previously untreated guinea pigs. The *in vitro* experiments conducted on the blood suggest a more reasonable explanation of the toxicity of protamines than those proposed by previous authors.[3, 4, 7] The data indicate that salmine exerts a toxic effect by the formation of multiple emboli. The precipitate noted when salmine was added to whole blood, as well as the agglutination of cells observed in some species, probably caused the vascular occlusive effects found in the perfusion experiments.

The studies of the salmine precipitate lead to the view that, in suitable concentrations, salmine will precipitate certain blood proteins, essentially fibrinogen.† By virtue of its high isoelectric point, salmine at blood pH has a positive charge while the plasma proteins are negatively charged. Hence, it is likely that salmine combines with these oppositely charged colloids. The peptizing effect of pH changes and of the addition of polyvalent ion salts on the salmine precipitate lend further support to this view. Abramson[8] has pointed out that "mutual precipitation of charged colloids is observed only when at least one of the substances is easily precipitated by electrolytes." On this basis it is not surprising that fibrinogen, which is most easily salted out, is precipitated by salmine.

Of equal importance is the effect of salmine upon the erythrocytes. It is probably not a coincidence that the animal species shown by Jaques[9] and Jorpes[1] to be most sensitive to protamine are also those whose red blood cells are agglutinated by this agent. On the con-

† Since the writing of this report Mylon, Winternitz, and de Suto-Nagy have published a method for determination of fibrinogen with protamine (*J. Biol. Chem.*, 1942, **143**, 21).

8 Abramson, H. A., Moore, D. H., *J. Lab. Clin. Med.*, 1940, **26**, 174.

9 Jaques, L. B., Charles, A. F., and Best, C. H., *Acta Med. Scand.*, 1938, Supp., **90**, 190.

trary, the species which are more resistant to protamine show absence of red cell agglutination by this agent.

On the basis of these findings, the intravenous use of large doses of protamine in clinical practice would seem not to be without danger.

Summary. 1. Intravenous administration of the protamine, salmine sulfate, to untreated guinea pigs and rats produced death at dose levels of 6-12 mg/100 g. Intraperitoneal injection delayed these effects. 2. The addition of salmine sulfate to whole blood or plasma resulted in the formation of a flocculent protein precipitate. Addition to whole blood caused hemagglutination in the human, dog, cat, and rat. 3. The addition of salmine sulfate to whole blood perfusing isolated organs caused cessation of flow. Addition of comparable amounts to Ringer's fluid and serum perfusing organs was without effect upon the rate of flow. 4. These experiments suggest the possibility that protamines exert toxic effects through embolic vascular phenomena.

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Studies on Peptic Inhibition.

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This report is a study of peptic activity as affected by the various substances used clinically in the treatment of gastro-duodenal ulcerative disease as well as of pepsin inactivators. Our specific objective was to learn whether these substances act directly on the pepsin or indirectly through the medium of a pH change.

Hammarsten[1] and Helmar, *et al.*,[2] showed that peptic activity decreases as the pH increases from 1 to 7, being completely inactivated at pH 7. Recently Komarov[3] and Schiffrin[4] reported that various aluminum and magnesium compounds inhibit peptic activity at the optimum pH range, from 1 to 2. Their use of Mett tubes to determine peptic activity plus their procedure of centrifuging

1 Hammarsten, O., *Hoppe Seyler's Z.*, 1922, **121**, 261.

2 Helmer, O. M., Fonts, P. J., and Zerfas, L. G., *Am. J. Dig. Dis.*, 1934, **1**, 120.

3 Komarov, S. A., and Komarov, O., *Am. J. Dig. Dis.*, 1940, **7**, 166.

4 Schiffrin, M. J., and Komarov, S. A., *Am. J. Dig. Dis.*, 1941, **8**, 215.