be interpreted as due to the dicoumarin administration. This pathological material is still under investigation. We have administered the drug to 20 other patients not included in this series and have not observed bleeding or other manifestations of toxicity. However, bleeding has been observed after the first week of major surgical procedures, as when a packing is removed. On this account, dicoumarin should be administered with caution to such patients.

Discussion. These observations indicate that the action of a single dose of 3,3′-methylenebis (4-hydroxycoumarin) is prolonged, and suggest the possibility that a few properly spaced doses may be sufficient to lengthen the coagulation time in humans for 2 weeks or longer. The therapeutic possibilities of this procedure are being investigated. During the latent period, 1 to 3 days before the action of the drug is apparent, heparin by continuous intravenous drip may be used. The heparin is discontinued as soon as the dicoumarin effect is seen. Further experimentation with these drugs in humans is desirable before they can be recommended for general use in thromboembolic states.

Summary. A prolongation in the coagulation time and a lowering of the prothrombin index of the blood for approximately 6 days beginning between 24 and 72 hours after the drug has been ingested will usually be produced by oral administration of a single dose of 400 mg of 3,3′-methylenebis (4-hydroxycoumarin) to a patient weighing 130 lb or less, or 600 mg to a patient weighing over 160 lb.

13701

Toxic Effects of Some Basic Proteins.

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Proteins which have an isoelectric point on the basic side of neutrality (protamine, histone), or near neutrality (globin) have recently been used in combination with insulin as depository therapeutic preparations. Their toxicities, however, have not been extensively studied. Vartiainen and Marble[1] reviewed the literature on the toxicity of protamine and determined its acute toxicity by

[1] Vartiainen, I., and Marble, A., *J. Lab. Clin. Med.*, 1941, **26**, 1416.

subcutaneous injection in mice and rabbits and found the LD 50 to be about 250 mg/kg. Kossel[2] notes that the toxicity of histones is similar to that of protamines. Thompson[3] studied the pharmacology of protamines and thymus histone and Annau, *et al.*,[4, 5] studied more extensively the pharmacology of histone prepared from avian red cells. The action was found to be similar to that of shock-producing substances. Obviously, the acute toxic dose of these proteins is much larger than their average daily doses given together with insulin. Local reactions, however, are not infrequent after the injection of protamine zinc insulin, but they have been attributed to sensitivity to protamine.

In view of the wide and repeated application of these proteins, it seemed of interest to investigate their general and tissue toxicity.

Acute Toxicity. The monoprotamine sulfate solutions used contained 5 mg/cc and were injected intraperitoneally into mice in graded doses. Deaths occurred within a few hours. The symptoms were depression, cyanosis, and short, convulsive hops. The LD 50 was 94 mg/kg. The slope of the regression line, when the logarithm of the dose was plotted against probit units, was 9.

Histone sulfate was injected intraperitoneally in a solution containing 20 mg/cc, using graded doses. Deaths occurred within 2 days. The symptoms were depression followed by coma. The LD 50 was 255 mg/kg. The slope of the regression line was 12.5.

Globin was injected as a solution containing 30 mg of globin per cc. One cc per mouse was well tolerated by all 10 mice used.

Tissue Toxicity. In order to obtain some evidence with regard to the toxicity of these proteins to mammalian tissue, the oxygen consumption of minced rat liver was determined in buffer solutions (0.10 molar phosphate, pH 7.3) containing 0.3% glucose and 10% fresh rat serum. To 1 cc of liver suspension (25 mg dried liver) in this medium, 0.35 cc of 1.7% of the various protein solutions was added from the side arm, after a short initial period of respiration. The oxygen consumption was measured in the Barcroft-Warburg vessel at 37°C with air as the gas mixture.

The relative rates of oxygen consumption are illustrated in Graph 1. Monoprotamine sulfate and histone sulfate showed a definite inhibition of the oxygen consumption, whereas globin did not.

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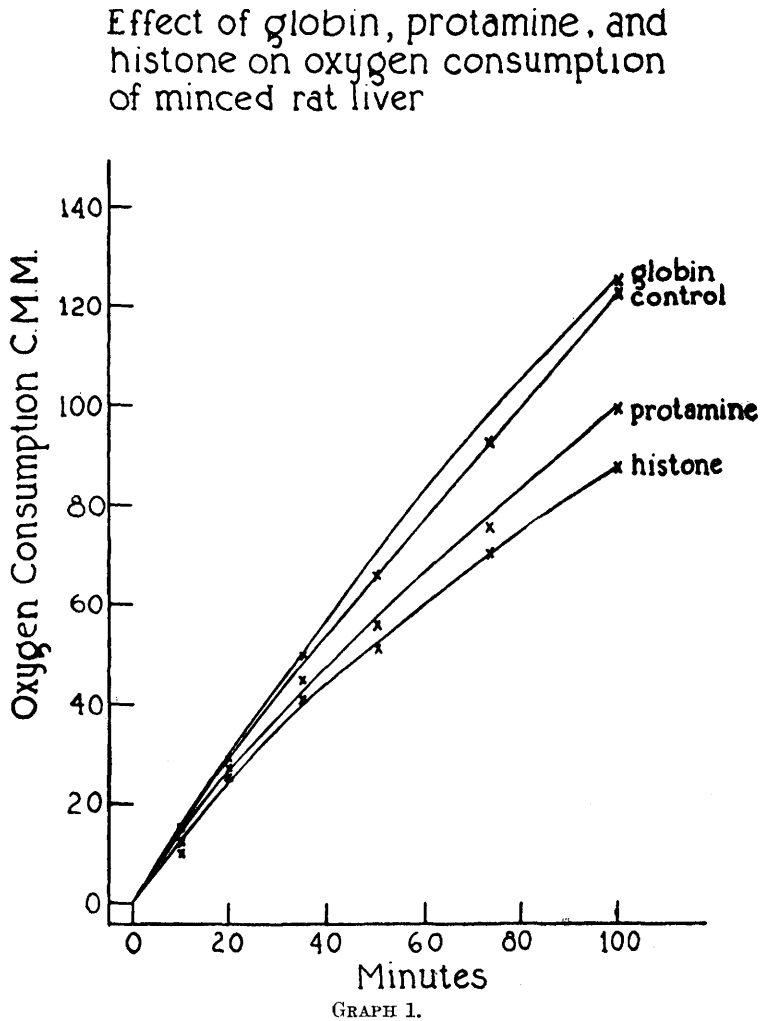
2 Kossel, A., *The Protamines and Histones*, Longmans, Green and Co., Ltd., 1928.

3 Thompson, W., *Z. physiol. Chem.*, 1900, **29**, 1.

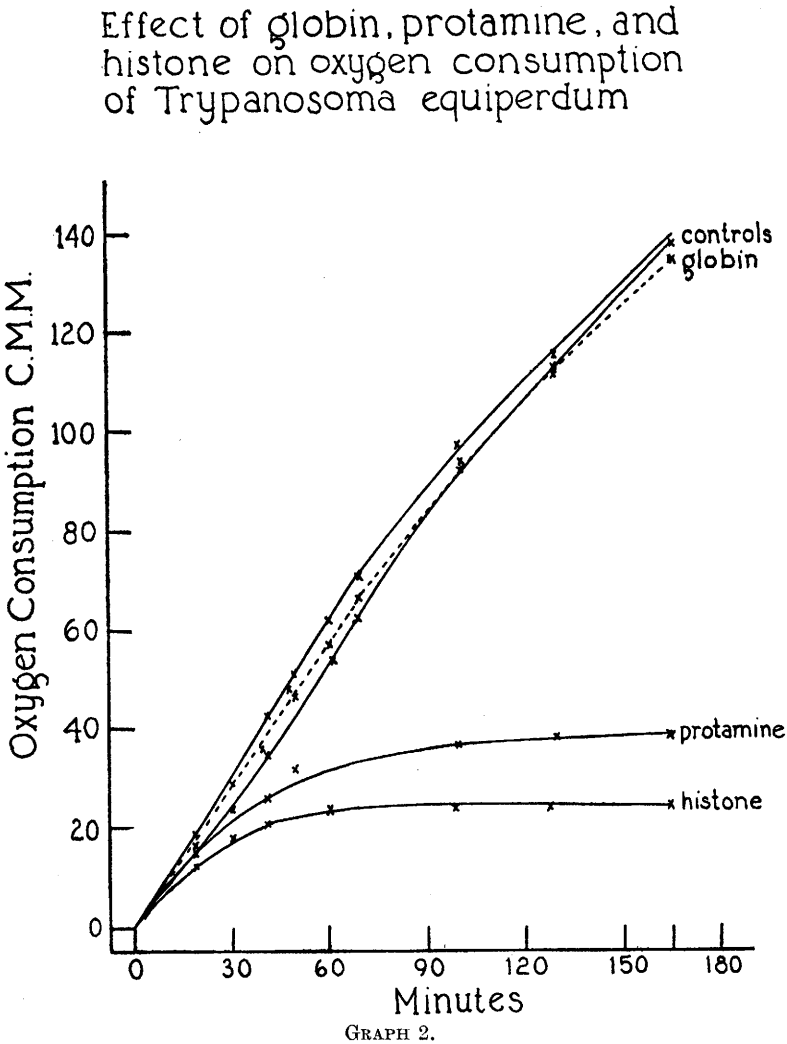
4 Annau, E., and Augustin, V., *Arch. exp. Path. Pharm.*, 1931, **161**, 337.

5 Annau, E., and Huszák, I., *Arch. exp. Path. Pharm.*, 1932, **163**, 541.



GRAPH 1.

Toxicity to Trypanosomes. Trypanosoma equiperdum, obtained from infected rats by fractional centrifugation of the blood, were suspended in a medium consisting of 10 parts of 0.11 molar phosphate buffer, pH 7.3, 9 parts of 0.85% sodium chloride, and 1 part of 6% glucose. The suspension contained 4×10^7 trypanosomes per cc. 0.2 cc of the 1.7% protein solutions was added from the side arm, but otherwise experimental conditions were the same as above. The results given in Graph 2 show that both monoprotamine sulfate and histone sulfate were more toxic to trypanosomes than to rat liver, and that no toxic effects were observed when globin was added.



GRAPH 2.

Discussion. Our findings confirm in general the qualitative information discussed by Kossel. These results can be considered as evidence from a physiologic point of view for the necessity of distinguishing globins, histones, and protamines from each other.

It is of interest to note in this connection that insulin was found to be well precipitated by various capillary-active, strongly basic compounds, among others, by the quaternary ammonium salt type of disinfectant.[6] We now find that proteins containing a relatively large amount of basic amino acids with a long chain such as arginine and lysine are toxic to tissues and to trypanosomes. Bacteriostatic

[6] Lang, E. H., Buck, J. S., and Reiner, L., *Pharm. Arch.*, 1941, **12**, 81.

activity of protamines was also observed by McClean.[7] Annau, *et al.*, believe that the pharmacological effects of histones are associated with the arginine radical present in the molecule in a peptide linkage. Free arginine having a free carboxyl group is entirely ineffective pharmacologically. It seems probable that the presence of arginine radicals in peptide combination conveys properties to the histone and protamine molecules which are to some extent similar to those of cationic detergents. The mechanism by which these substances exert their toxic effects on cells is not clearly understood. Whether or not the reactivity of these substances with insulin can be taken as suggestive of the mechanism of their toxic action remains to be investigated.

Summary. The order of the toxicity of the proteins investigated is as follows: protamine > histone > globin. Globin was found to be devoid of appreciable toxicity when injected into animals or tested on tissues and on monocellular organisms *in vitro*. Protamine and histone were found to be highly toxic to trypanosomes. Factors which may contribute to the toxicity of these proteins were discussed.

13702

Origin of Ionized Iron after Action of Acids on Blood and Influence of Carbon Monoxide.

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When dilute acids are added to hemolyzed blood, a small amount of iron is split off in ionized form,[1-4] varying from 14 to 18 mg per liter of human blood.[5, 6, 7] It was assumed that this easily split-off

[7] McClean, F., *J. Path. Bact.*, 1931, **34**, 459.

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[1] Barkan, G., *Z. physiol. Chem.*, 1925, **148**, 124.

[2] Lintzel, W., *Z. Biol.*, 1925, **83**, 289.

[3] Barkan, G., *Z. physiol. Chem.*, 1927, **171**, 179.

[4] Barkan, G., *Z. physiol. Chem.* 1927, **171**, 194.

[5] Schwarz, L., and Deckert, W., *Klin. Woch.*, 1935, **14**, 601, 900.

[6] Olesk, J., *Klin. Woch.*, 1935, **14**, 1006.

[7] Rooks, G., *Deutsch. Z. gerichtl. Med.*, 1936, **27**, 47.