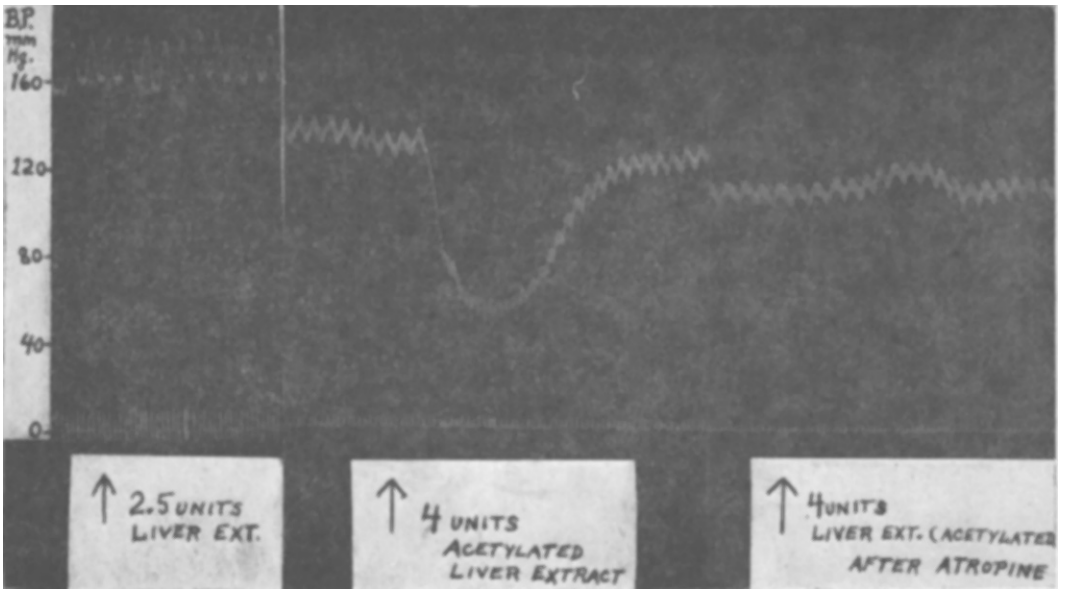

FIG. 1.
Typical Blood Pressure Record Following the Intravenous Injection of Untreated and Acetylated Liver Extracts.

Tracing at left shows the effect of 0.25 cc of a brand of purified solution of liver upon the blood pressure of a dog. The same brand of liver extract (Number III; Table I) was acetylated and injected into another dog both before and after atropinization. Time is recorded in seconds.

increased vasodepressor action has been shown by Hunt[6] and by Dale.[7] However, few such substances are known to be present in the animal body, most of them being products of laboratory synthesis.

Conclusion. Six injectable liver extracts were found to contain choline or a choline-like substance, since their acetylation produced vasodepressor activity which was antagonized by atropine.

[6] Hunt, R., *J. Pharm. and Exp. Therap.*, 1914, **6**, 477.

[7] Dale, H. H., *J. Pharm. and Exp. Therap.*, 1914, **6**, 147.

14398 P

Phosphatase Activity of Human Erythrocytes.

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Evidence has accumulated which indicates that phosphatases occur in the erythrocytes of dog, beef, horse, rabbit, rat, and man. To provide further information about phosphomonoesterase occurring in the normal human red blood cells the enzymatic activity of red cell hemolysates was studied as to (1) the presence of alkaline and acid phosphatase, (2) the effect of fluoride and magnesium upon the enzymatic activity, (3) the relation of erythrocyte phosphatase to plasma phosphatase.

Procedure. The erythrocytes were washed 3 times with normal saline. The washed cells obtained from x cc of oxalated or heparinized blood were hemolyzed by adding distilled

water to bring the total volume of the hemolysate up to 1.5 times x cc. If the hematocrit value is known the phosphatase activity of the erythrocytes can be calculated from the activity of the hemolysates. The phosphatase activity of the hemolysate as well as of the plasma was tested by determining the amount of phosphorus (according to Fiske and Subbarow[1]) liberated from a sodium glycerophosphate substrate, or by measuring the amount of phenol freed from a disodium phenylphosphate substrate, according to King and Armstrong.[2] Michaelis'[3] veronal-acetate buffer was used preferably, since certain buffer salts interfere with the colorimetric determination of phenol. In a few of the experiments with glycerophosphate as substrate, citrate and veronal buffers were used. The results are expressed in mg of phosphate or phenol liberated per 100 cc of hemolysate or plasma during one hour of incubation at 37°C.

Results. (1) Phosphatase activity of red cell hemolysates was found to be constantly present (Fig. 1). Strong activity was observed between pH 4.8 and pH 6.1. Below and above this zone of acidity hydrolysis drops abruptly. The optimum pH of the phosphatase of human erythrocytes was found around pH 5.3. Its action upon β-glycerophosphate is slow compared to the strong action upon disodium monophenylphosphate. A second zone of optimal activity at alkaline reaction as reported by Roche and Bullinger[4] for horse blood cells, was missing. If confirmed, this result would preclude the occurrence of an "alkaline phosphatase" in human erythrocytes.

(2) The inhibiting effect of sodium fluoride upon the activity of the intracellular phosphatase was found to be only very slight. If the concentration of fluoride during incubation was M/100 to M/200, the greatest decrease of activity, observed at pH 5.3, was not more than 10.2%. The average decrease was 6.2%. The strong inhibition by fluoride of the plasma phosphatase at pH 5.0 (Gutman[5]) could be confirmed constantly. Experiments failed to show a definite effect of magnesium chloride upon the phosphomonoesterase activity of red cell hemolysates. The action of magnesium chloride was tested—

(a) In final concentrations ranging from M/100 to M/800, at the optimal pH of 5.3. Stronger Mg-solutions caused a precipitation in the substrate-buffer mixture.

(b) With magnesium chloride concentrations kept between M/250 and M/300, but the acidity varying from pH 3.4 to pH 8.9. If changes took place they were slight increases of hydrolysis at the optimal pH of 5.3. In no instance was an increase of enzymatic activity observed as recorded by Jenner and Kay[6] for red cells of rat, dog, beef, and rabbit.

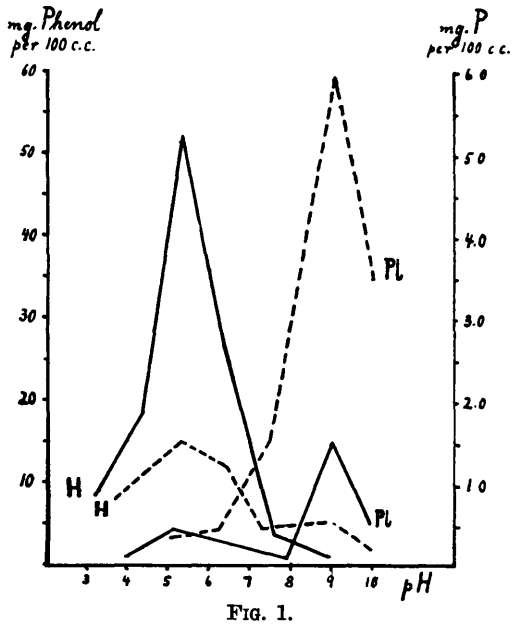

FIG. 1.

Phosphatase activity per 100 cc of human red cell hemolysate (H) and of plasma (Pl) with relation to [H+]-concentration. Incubation at 37°C for one hour.

Broken lines: Phosphorus, liberated from buffered β-glycerophosphate. Buffer: veronal or citrate.

Solid lines: Phenol, liberated from buffered phenylphosphate. Buffer: veronal-acetate.

[1] Fiske, C. H., and Subbarow, V., *J. Biol. Chem.*, 1925, **66**, 375.

[2] King, E. J., and Armstrong, A. R., *Can. Med. Assn. J.*, 1934, **31**, 376.

[3] Michaelis, L., *Bioch. Z.*, 1931, **234**, 139.

[4] Roche, J., and Bullinger, E., *Enzymologica*, 1939, **7**, 278.

[5] Gutman, A. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 513.

[6] Jenner, H. D., and Kay, H. D., *J. Biol. Chem.*, 1931, **93**, 733.

(3) By drawing the curves of activity obtained by intracellular and extracellular blood phosphatases it is possible to demonstrate the relation of these enzymes to each other (Fig. 1). At alkaline reaction the plasma phosphatase activity is far superior to the erythrocyte phosphatase, whereas on the acid side of neutrality the red cell phosphatase activity is predominant. When acting on β-glycerophosphate the "alkaline" plasma phosphatase, at the optimal pH, will liberate four times as much phosphate as the "acid" erythrocyte phosphatase at its optimal pH. But when allowed to act upon phenylphosphate the erythrocyte phosphatase will free about four times as much phenol as plasma phosphatase, both acting as their respective optimal pH

14399 P

Effect of X-Rays on Erythrocytes.

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Earlier attempts to elicit hemolysis *in vitro* by means of X-ray radiation have been almost uniformly negative. Rare positive results relate to delayed hemolysis beginning 15-20 hours (Ting and Zirckle[1]) or more (Holthusen[2]) after irradiation. Levin and Piffault[3] alone observed immediate hemolysis with doses of 1.5×10^6 r. The experiments reported here were undertaken in order to ascertain the X-ray doses which induce immediate hemolysis under different conditions.

Fresh rabbit blood was defibrinated, washed twice in saline and irradiated at different degrees of dilution in saline and other vehicles. Small hanging drops under mica slips were irradiated and the effects observed with the microscope.

Irradiation was carried out by means of a demountable X-ray tube with copper anode at a tension of 35 K.V. and a current of 40 M.A. The window was aluminium foil 30 μ in thickness; the mica slip for the hanging drop was 0.02-0.025 mm thick. The X-ray intensity at the distance of the hanging drop—3.5 mm—was about 95,000 r/min.

In a series of tests to determine the doses inducing immediate hemolysis, *i.e.*, hemolysis completed within the time of the irradiation, erythrocytes in saline or physiological glucose solution were irradiated in concentrations of 0.1%, 0.5%, 1%, and 5%. At 1% erythrocyte concentration, serum was also added to the saline in some experiments. The results obtained were satisfactorily constant. The averages are set down in Table I.

The experiments show that the doses necessary in order to induce immediate hemolysis are very high. A noteworthy feature is the importance of the erythrocyte concentration in glucose. Within the erythrocyte concentration range of 0.1% to 5%, the effect of radiation in this medium is augmented by decrease in concentration. At 5% erythrocyte concentration in physiological glucose solution the dose producing immediate hemolysis is 6 times that necessary for an erythrocyte concentration of 0.1%. The dose which produces hemolysis at 5% erythrocyte concentration in glucose, furthermore, is twice that producing this effect at the same erythrocyte concentration in saline.

An unexpected and hitherto unknown effect was observed on erythrocytes irradiated in saline at erythrocyte concentrations less than 0.5%. As may be seen in Table I, the immediate hemolysis dose for 1% and 5% of erythrocytes in saline is 3.5×10^6 r. At 0.5% concentration the same effect is pro-

[1] Ting, T. P., and Zirckle, R. E., *J. Cell. and Comp. Physiol.*, 1940, **16**, 189.

[2] Holthusen, H., *Strahlentherap.*, 1922, **14**, 561.

[3] Levin, B., and Piffault, C., *C. R. soc. biol., Paris*, 1934, **116**, 1324.