(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

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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-

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

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

TABLE I.
Average Doses in Million r Producing Complete Hemolysis of Rabbit Erythrocytes.

Medium	Dilutions			
	0.1%	0.5%	1%	5%
Saline	No hemolysis to 7×10^6 r	2.0	3.5	3.5
Glucose	1.25	2.0	3.5	7.0
Saline plus 25% serum	1.5			

duced at 2×10^6 r. It could be expected, therefore, that at 0.1% erythrocyte concentration an even smaller dose would cause hemolysis. In fact, hemolysis could not be induced at this cell concentration even by 7×10^6 r. Nor could hemolysis be induced, furthermore, by repetition of the irradiation after an interval of 24 hours. The impression was obtained that erythrocytes in suspensions of 0.1% in saline, irradiated with 2×10^6 r, became "stable" and could tolerate 7×10^6 r and more.

The phenomenon of hemolysis-stability (H-St.) does not occur under the conditions mentioned above, if glucose or saline plus serum is used as medium. In saline it does not occur if an erythrocyte concentration of 0.5% or more is employed. The noted influence in our experiments with erythrocytes of the concentration of the irradiated object and of the nature of the medium find an analogy in results obtained with other test objects. W. M. Dale[4,5] demonstrated the influence of the concentration of the irradiated object ("dilution effect") and the inhibitory influence of certain medium constituents ("protective effect") for the case of enzymes and other substances. Similar observations have been recorded with enzymes by Goldhaber and Leibowitz[6] and with virus by Lacassagne and Nyka[7], Friedewald and Anderson[8] and Luria and Exner[9]. Recently, Evans, Slaughter, Little and Failla[10] showed in experiments on Arbacia sperm and eggs that irradiation of the sperm can prevent fertilization and that the dose effective in this respect depends on the concentration of the sperm as well as on the medium. To explain such phenomena Dale and other investigators assume that the primary effect of radiation is an action on the milieu and that the reaction of the irradiated object is a secondary result. A similar relation appears to hold for irradiated erythrocytes.

Summary. The doses of X-rays which produce immediate hemolysis *in vitro* have been determined for different conditions. The X-ray dose producing complete hemolysis is dependent on the concentration of the irradiated erythrocyte suspension and on the medium employed. Erythrocytes suitably irradiated at high dilutions (0.1%) in saline become stable to X-ray hemolysis.

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8 Friedewald, D. F., and Anderson, R. S., *J. Exp. Med.*, 1941, **74**, 463.

9 Luria, S. E., and Exner, F. M., *Proc. Nat. Acad. Sc.*, 1941, **27**, 370.

10 Evans, T. C., Slaughter, J. C., Little, E. P., and Failla, G., *Radiology*, 1942, **39**, 663.