Effect of Erythropoiesis on P^{32} Uptake by Deoxyribonucleic Acid of Spleen in the Rat.* (24580)

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Erythropoiesis has been a subject that has commanded great interst for many years. A number of parameters have become "classical" methods for measuring erythropoiesis. Some of these parameters are: red cell counts, packed red cell volume, circulating hemoglobin per cent, and reticulocyte per cent. More recently, various radioactive isotopes have been used to measure erythropoiesis, among these are C^{14} and Fe^{59}. The method for measuring increased erythropoiesis to be described utilizes differences in radioactive phosphorus (P^{32}) uptake by the deoxyribonucleic acid (DNA) fraction of the rat spleen(1) of erythropoietically stimulated and non-stimulated animals. Rats were stimulated by standard hemorrhage, anoxic anoxia, and injections of anemic rabbit or hypoxic rabbit plasma.

Chemical methods. The method used for isolating DNA from rat spleen was a modification of the Schmidt-Tannhauser procedure (2). Experimental and control animals were injected intraperitoneally with 2 μc P^{32} per 100 g of body weight. Exactly 4 hours after injection the animals were sacrificed and the spleens removed. Three or 4 spleens were pooled, immersed in 3 or 4 volumes of 10% NaCl, and homogenized for one minute in a micro Waring Blendor. The homogenates were heated in a water bath at 85°C for 4 hours. After heating, the homogenates were filtered through medium sintered glass with the aid of a small amount of Celite to remove splenic residue. Three volumes of 95% ethanol were added to the filtrate. The resulting suspension was centrifuged and the supernatant discarded. Fifteen ml of 1 *N* KOH were added to dissolve the precipitate. The solution was incubated at 37°C for 18 hours. After incubation 1.5 ml of 6 *N* HCl and 3.0 ml of 10% trichloracetic acid were added. This step precipitates the DNA and separates it from the RNA. The DNA precipitate was washed with 95% ethanol and ethyl ether. 0.1 *N* NaOH was added to the dried material and a small amount of heat applied to insure solution. One ml of this solution was placed on a planchet and dried for gas-flow counting. A 0.2 ml aliquot was taken for total phosphorus determination by the method of Gomori(3). *Standard hemorrhage.* Male Wistar strain rats weighing 180-200 g were bled 2% of the body weight by cardiac puncture under light ether anesthesia. Prior to the standard hemorrhage, hematocrit and reticulocyte percentages were determined to evaluate the hematologic condition of the rat. Only rats within normal limits were used. At one, 2, 3, 4, 5 and 8 days after hemorrhage, the hematocrit and reticulocyte percentages were determined. Immediately after sampling 2 μc of P^{32} were injected and 4 hours later the rats were sacrificed for splenic DNA isolation as described above. *Anoxic anoxia.* Male rats weighing 180-200 g were placed in a low pressure chamber for 8 hours per day from zero to 4 days at an ambient pressure of 260 mm Hg. Rat pellets and water were supplied *ad lib.* At the designated intervals blood samples were obtained, the animals were sacrificed, and splenic DNA isolated. *Production of anemic rabbit plasma.* Male New Zealand rabbits were made severely anemic by daily injections of phenylhydrazine hydrochloride. After 5 to 7 days of repeated injections, the hematocrit percentage was 8 to 12%. At this time the animals were bled out by cardiac puncture. The plasma from the anemic rabbits was frozen until used. *Production of anoxic rabbit plasma.* Male New Zealand rabbits were placed in a low pressure chamber for 8 hours for 4 days at an ambient pressure of 260 mm Hg. At the end of the fourth day the animals were bled out by cardiac puncture. The plasma from the anoxic rabbits was frozen until used. Male Wistar strain rats weighing 180-200 g were the receptor animals of the "anemic" and "anoxic plasma."

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TABLE I. Effect of Hemorrhage on DNAP of Spleen in 7 Experiments, 3 Rats/Exp.

Time between hemorrhage and sacrifice (days)	Hematocrit, %	Reticulocyte, %	P^{32} uptake* by DNA of spleen
	at time of sacrifice		
0	50.0	.9	152
1	35.3	4.6	426
2	40.7	4.3	520
3	38.3	5.2	936
4	40.7	9.0	580
5	42.0	5.7	349
8	46.2	.9	236

* Expressed as cpm/0.1 mg P.

The animals were injected intraperitoneally with 2 or 4 ml of plasma or saline. Sixteen to 18 hours later 2 μc P^{32} per 100 g body weight was administered by intraperitoneal injection. Four hours after P^{32} injection the animals were sacrificed. The spleens of 3 or 4 rats were pooled for DNA isolation as previously described.

Results. Standard Hemorrhage. The P^{32} uptake by the DNA of the spleen increased to a maximum 3 days after a standard hemorrhage, Table I. Thereafter the values decreased toward normal through the eighth day after hemorrhage. Reticulocyte percentage attained maximum values by the fourth day after hemorrhage, then decreased to normal. Hematocrit percentage was lowest on the first day after hemorrhage, gradually increasing toward normal through the eighth day post-hemorrhage.

Anoxic anoxia. P^{32} uptake of splenic DNA of rats exposed to varying periods of anoxic anoxia is greater than control values (Table II). However, variation is considerable and follows no general pattern. The reticulocyte per cent and hematocrit per cent increases with increase in duration of exposure to anoxia. The latter results agree with previous observations of others and in this laboratory.

TABLE II. Effect of Anoxia on DNAP of Spleen, 3 Rats/Experiment.

Days exposure in low pressure chamber	Hematocrit, %	Reticulocyte, %	P^{32} uptake by DNA of spleen
	at time of sacrifice		
0	45.3	1.1	328
1	44.2	1.8	1180
2	48.0	2.7	600
3	50.2	3.7	1125
4	52.0	8.1	912

Anemia rabbit plasma. Table III compares P^{32} uptake by splenic DNA of normal rats injected with 4 ml of anemic rabbit plasma or saline. Of the 10 individual experiments with 3 or 4 rats per group, with one exception, P^{32} uptake values of the anemic rabbit plasma injected rats are 2 to 5 times greater than the saline injected controls. This difference is statistically significant, ($p < 0.01$).

Hypoxic rabbit plasma. Plasma from anoxic rabbits was injected into normal rats, followed by P^{32} as previously described. The data (Table IV) suggest erythropoietic stimulation in rats following a 4 ml injection of hypoxic rabbit plasma. Erythropoietic stimulation of anoxic rabbit plasma does not appear as great as that following injection of anemic rabbit plasma. This is in general agreement with other observations in this laboratory using other methods.

TABLE III. Effect of Anemic Rabbit Plasma on P^{32} Uptake by Spleen DNA, in 10 Exp.

Saline	Anemic rabbit plasma
cpm/0.1 mg P	
262	446
240	710
210	446
216	369
158	445
158	588
322	262
280	469
308	284
90	140

$p < .01$.

TABLE IV. Effect of Anoxic Rabbit Plasma on P^{32} Uptake by Spleen DNA, in 5 Exp.

Saline	Anoxic rabbit plasma
cpm/0.1 mg P	
353	614
210	945
87	174
87	102
87	121

Discussion. Current opinions in the field of cell division generally agree that when a cell divides mitotically, there is a doubling of DNA. When an animal is erythropoietically stimulated (*i.e.* hemorrhage, anoxic anoxia) a greater number of cells divide in the erythropoietic organs to fulfill the demand for new cells created by the stimulus; therefore, quantity of DNA is increased. Since the spleen is an erythropoietic organ under certain conditions, it was reasonable to assume an increase in splenic DNA in stimulated animals. This increase was measured by the P^{32} uptake

by the DNA of the spleen after standard hemorrhage or anoxic anoxia. Carnot(4) first postulated a humoral mechanism for regulation of erythropoiesis. It is generally accepted that in the plasma of erythropoietically stimulated animals there is a factor that will increase red cell formation in normal animals. Using a different method here described, the presence of such a factor in the plasma of phenylhydrazine anemic or hypoxic rabbits has been confirmed.

Summary. 1. A method for evaluating erythropoietic stimulation has been described. 2. Following a standard hemorrhage there is a 2- to 5-fold increase in P^{32} uptake by splenic DNA. 3. Exposure to anoxic anoxia increases P^{32} uptake by the DNA of spleen. 4. Injection of anemic rabbit or hypoxic rabbit plasma increased P^{32} uptake by splenic DNA in normal receptor rats.

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Relation Between Platelet Integrity and Sulfhydryl Groups.* (24581)

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During the last few years platelets have been shown to contain a number of "factors" which are concerned with different phases of clotting mechanism(1). Since it is known that, in formation of thrombin, platelets together with their plasma cofactors produce a powerful intrinsic thromboplastin, it has been postulated that intravascular coagulation may be initiated once appreciable amounts of certain materials are derived from platelets(2). Whether or not release of platelet factors requires actual breakdown of platelets or only changes in their "membrane" permeability characteristics is not as yet known. What is known is that retraction of the fibrin gel requires presence of intact platelets(3) whereas antiheparin and antifibrinolytic activities of platelets are enhanced markedly as result of lysis(4,5). Furthermore, bleeding tendency associated with thrombocytopathy A is related to functional platelet abnormality resulting in increased resistance to disintegration(6). It would thus appear that variations in rate of normal platelet breakdown may have important bearing on several aspects of coagulation mechanism. This paper describes experimental findings suggesting a relationship between platelet integrity and platelet sulfhydryl groups.

Methods and materials. Platelet suspensions were prepared from fresh human blood collected with versene (ethylenediamine tetraacetate Na_2-salt) according to the method of Minor and Burnett(7). Platelets were washed 3 times in physiological saline containing 0.1% versene and 2% Triton WR-1339 (Rohm & Haas), the latter substance used to facilitate resuspension following centrifugation. They were finally suspended in physiological saline containing 2% Triton WR-1339 to give "standard suspension" containing approximately 2.5 x 10^6 platelets/mm^3. Sulfhydryl inhibitors, studied for effects on platelet stability, were dissolved in de-ionized distilled water, adjusted to pH 6.5, and added to "standard platelet suspension" in small volumes to preserve isotonicity. Reduced glutathione and cysteine ethyl ester were dissolved in de-ionized distilled water immediately prior to use, and adjusted to pH

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