microsomes was not washed in these experiments and the small amounts of adhering supernatant could be expected to increase the glutathione values for the precipitates above their true levels, especially with normal rat livers. Nevertheless, the results were clear in showing that at least 85-90% of the glutathione in rat liver was present in the soluble supernatant fraction, and it was from this fraction that the labile glutathione was lost during protein depletion.

Summary. A protein-free diet reduced liver glutathione in rats to about 40% of its normal value within 1 to 2 days, but did not deplete blood glutathione. The labile 60% of the liver glutathione was lost completely before the liver xanthine oxidase was reduced more than 50%; the residual 40% of the liver glutathione was then retained while the remainder of the xanthine oxidase was lost. 85-90% of the glutathione in rat liver was present in the soluble supernatant fraction obtained by centrifuging an homogenate at 30000 x g.

1. Meikleham, V., Wells, I. C., Richert, D. A. and Westerfeld, W. W., *J. Biol. Chem.*, 1951, v192, 651.

2. Leaf, G. and Neuberger, A., *Biochem. J.*, 1947, v41, 280.

3. Westerfeld, W. W. and Richert, D. A., *Science*, 1949, v109, 68.

4.———, *J. Biol. Chem.*, 1951, v192, 35.

5. Woodward, G. and Fry, E. G., *J. Biol. Chem*, 1932, v97, 465.

6. Benedict, S. R., and Gottschall, G., *J. Biol. Chem.*, 1933, v99, 729.

7. Bass, A. D., Tepperman, J., Richert, D. A. and Westerfeld, W. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 687.

8. Westerfeld, W. W., Richert, D. A. and Hilfinger, M. F., *Cancer Research*, 1950, v10, 486.

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Nucleic Acid Metabolism of Bone Marrow and Spleen. I. Normal Values and Effect of Sodium Pentobarbital.* (19272)

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The nucleic acid metabolism of various organs and tumor tissue has been studied extensively(1-4) but only a few isolated observations have been reported on the intact bone marrow(5-7). This organ is of particular interest because of its very active cell division and cell maturation. We have studied the concentration and the relative rate of formation of DNA and RNA in the bone marrow and spleen of the rat. After a tracer dose of radiophosphorus (P^{32}), the DNA and RNA fractions were isolated and determinations made of the phosphorus and P^{32}. The amount of phosphorus in each fraction is a measure of the nucleic acid content, and the % of injected P^{32} per mg phosphorus, or specific activity (SA), an index of the nucleic acid newly formed during the interval studied. Thus the desoxyribose nucleic acid phosphorus (DNAP) is related to the cellularity of the bone marrow and the SA_{DNAP} an index of the rate of cell division. Further, the ribose nucleic acid phosphorus (RNAP) is related to the cytoplasmic content, while the SA_{RNAP} reflects in part the process of cell maturation. This concept is supported by experiments on irradiated rats(8), which show a close correlation between the nucleic acid determinations and the morphologic changes in the bone marrow.

In this paper, normal nucleic acid values are presented and it is shown that anesthetic doses of sodium pentobarbital produce a significant depression of DNA formation in both the bone marrow and spleen.

Methods. White male rats of the Sprague-Dawley strain, approximately 3 to 4 months old, were employed. Their weights varied

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from 265 g to 330 g with an average of 297 g. There was no difference between the average weights of the control and experimental series. The animals received a diet of Rockland Dog Blocks and were fasted for 12 hours before the injection of P^{32}. The control animals were injected intraperitoneally with 1 microcurie carrier free P^{32}† per 100 g of body weight and sacrificed 4 hours later. This tracer dose apparently does not cause a depression of metabolic activity by irradiation, since the P^{32} uptake of the nucleic acid fractions of the bone marrow and spleen after the injection of 1 microcurie and 2 microcuries per 100 g have agreed within the experimental error of the method. The experimental rats were anesthetized by the intraperitoneal injection of 5 mg of sodium pentobarbital per 100 g weight. Radiophosphorus was given 1½ or 24 hours after anesthetization and the animals were sacrificed 4 hours after the tracer injection. The animals were killed by a sharp blow to the occiput. The femurs and tibias of both hind legs were removed immediately and the bones opened transversely through the cancellous portion with a small circular saw. The remainder of the cancellous bone was curretted out with a scalpel and the marrow expressed intact from the shaft by air pressure from a dry syringe. The marrow content, averaging 120 mg in weight, from the four bones was collected on a clean aluminum foil contained in an ice cold moist chamber. It was weighed on a 500 mg capacity torsion balance with a sensitivity of 1 mg. The entire procedure of removing the marrow and weighing was never longer than 10 minutes. The spleen was removed and a portion from the midsection, weighing approximately 150 mg, was placed on an aluminum foil and weighed with the torsion balance. To obtain total weight, the remainder of the spleen was weighed on an analytical balance with a sensitivity of 0.1 mg. The tissues were homogenized in ice water using a Potter-Elvehjem homogenizer(9). The isolation of DNA, RNA and acid soluble phosphorus (ASP) followed the method of Schmidt and Thannhauser(10) as modified in this laboratory for use with small samples. The phosphorus content of these fractions was estimated by the method of Gomori(11). Using standard counting procedures the P^{32} content was determined on aliquots dried in cup planchets. The methods for fractionation of tissue phosphorus compounds were examined by P and P^{32} analyses during the extraction procedures. The ASP and phospholipid were found to be completely extracted without appreciable decrease of RNA and DNA. The separation of RNA and DNA from the total nucleic acid fraction was found to be satisfactory. Davidson(4) has stated that in the Schmidt and Thannhauser procedure there is contamination of the RNA fraction by phosphoprotein which has a higher specific activity than the RNAP and would give falsely high values for the specific activity of the latter. Precipitation of the inorganic phosphorus in the RNA fraction by the method of Delory(12) did not appreciably change the specific activity. It would appear that the quantity of phosphoprotein or its specific activity does not appreciably affect the SA_{RNAP} in the spleen and bone marrow. The analyses of small spleen sections were compared to values obtained with the entire spleen and were shown to be entirely comparable.

Results. The phosphorus content of the fractions isolated from the bone marrow and spleen is given in Table I. Results are reported in terms of milligrams of phosphorus per gram of wet weight of tissue sample. The P^{32} uptake in each fraction is expressed in terms of specific activity. Specific activity represents the percentage of the injected dose of P^{32} present per milligram of phosphorus in the fraction.

Spleen weights expressed both as total weights and percentage of the body weight of the rat are recorded in Table II. Since the spleens were not weighed in some of the earlier experiments, additional animals were studied for spleen weights only. The periods of 5½ and 28 hours after injection of sodium pentobarbital are the same intervals at which the animals were sacrificed in the nucleic acid experiments.

All the results have been statistically evalu-

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TABLE I. Phosphorus Fractions in the Bone Marrow and Spleen. Normal values and the effect of sodium pentobarbital.

Group	No. of animals	Phosphorus content, mg/g ASP	RNAP	DNAP	Specific activity ASP	RNAP	DNAP
			Bone marrow				
Control	8	1.003	1.261	1.296	.462	.158	.165
σ		±.181	±.324	±.239	±.064	±.064	±.021
1½ hr*	8	1.011	1.311	1.287	.478	.150	.102
σ		±.192	±.464	±.271	±.104	±.064	±.030
P		<.50	<.50	<.50	<.50	<.50	>.99
24 hr*	7	1.132	1.149	1.255	.434	.170	.157
σ		±.217	±.201	±.088	±.065	±.012	±.020
P		<.50	.70	<.50	<.50	.50	.50
			Spleen				
Control	8	.939	.864	.841	.475	.107	.068
σ		±.082	±.149	±.124	±.093	±.023	±.029
1½ hr*	8	1.177	1.103	1.070	.364	.091	.025
σ		±.357	±.587	±.185	±.059	±.023	±.018
P		.90	.80	.50	.98	.90	>.99
24 hr*	7	1.170	.993	1.147	.415	.120	.043
σ		±.140	±.144	±.217	±.021	±.017	±.028
P		>.99	.90	.40	.90	.70	.80

* Interval between administration of sodium pentobarbital and P^{32}. All animals sacrificed 4 hr after P^{32} injection.

ated. The significance of changes in the experimental animals as compared to control animals were subjected to "t" analysis by the method of Fisher(13). Changes were considered significant if the probability was 0.95 or greater. Standard deviations (σ) and probability values (P) are given in each table.

Discussion. In these experiments the specific activity values are considered to be a measure of the relative renewal rate of nucleic acid fractions. The absolute renewal rate cannot be determined because the immediate precursor of the nucleic acid phosphorus and its specific activity is unknown. The precursor is probably in the ASP fraction(4,14).

In a preliminary report from this laboratory (6) values for the DNAP and RNAP content of the intact bone marrow and the rate of incorporation of radiophosphorus into these fractions were presented. The high values of the nucleic acid content and specific activities of the fractions in the bone marrow reported here parallel the findings of other authors (5,7). Our results in general confirm previous studies on the spleen(1-4) and indicate a lower DNAP and RNAP content, and a slower renewal rate for these fractions in the spleen than in the bone marrow.

TABLE II. Spleen Weights. Normal values and the effect of sodium pentobarbital.

Group	No. of animals	Avg wt (g)	Avg % of body wt
Control	14	.794	.25
σ			±.06
5½ hr*	12	1.013	.34
σ			±.05
P			>.99
28 hr*	12	.650	.24
σ			±.05
P			<.50

* Time after administration of sodium pentobarbital.

The SA_{DNAP} and SA_{RNAP} of the bone marrow are essentially equal while in the spleen the SA_{RNAP} exceeds the SA_{DNAP}. This probably reflects a similar degree of cell division and maturation in the bone marrow and a relatively lesser degree of cell division in the spleen.

During the 5½-hour-period following the administration of an anesthetic dose of sodium pentobarbital, there is a statistically valid decrease in the SA_{DNAP} of both the bone marrow and the spleen. The SA_{DNAP} returns to normal in 24 hours. There is no significant change in the nucleic acid concentration or in the SA_{RNAP}.

The increase in spleen weight observed has been reported in other animals(15,16). Since

the DNAP and RNAP per gram tissue do not change significantly, it would appear that the total nucleic acid content of the spleen increases after anesthesia. Recalculation of the per cent of injected P^{32} present in the total nucleic acid in the spleen still shows a depression of the SA_{DNAP} and no significant change in SA_{RNAP}. Thus, it is suggested that an anesthetic dose of sodium pentobarbital temporarily inhibits the rate of cell division in the bone marrow and the spleen. The increase in size of the spleen along with an elevation of the total nucleic acid content are compatible with the sequestration of nucleated cells from the peripheral blood.

Other workers(17,18) have reported reduction in nucleic acid synthesis by the barbiturates. The mechanism by which barbiturates affect cellular metabolism is obscure. Quastel and his associates(19) were the first to demonstrate that barbiturates were capable of inhibiting enzyme systems. Grieg(20) subsequently offered indirect evidence that barbiturates interfere with the mechanism of the hydrogen transport system. More recently Persky, *et al.*(21) and Brody and Bain(22) have suggested that the barbiturates interfere with the normal formation of ATP. This latter compound may be the precursor of DNAP.

Summary. (1) Normal values are presented for the desoxyribose nucleic acid phosphorus and ribose nucleic acid phosphorus as well as the rate of uptake of P^{32} by the DNA and RNA in the bone marrow and spleen of adult rats. (2) Anesthetic doses of sodium pentobarbital were found to produce a significant decrease in the relative rate of formation of DNA in the bone marrow and spleen during a period of 5½ hours. (3) The significance of these observations is discussed.

1. *Cold Spring Harbor Symp. Quant. Biol.*, 1947, v12.

2. *Symposia Soc. Exp. Biol.*, 1947, v1.

3. Hevesy, G., *Radioactive Indicators,* Interscience, New York, 1948.

4. Davidson, J. N., *The Biochemistry of the Nucleic Acids,* Wiley and Sons, New York, 1951.

5. Andreasen, E., and Ottesen, J., *Acta. Physiol. Scandinav.*, 1945, v10, 258.

6. Martin, T. S., Alt, H. L., and Cooper, J. A. D., *J. Lab. Clin. Med.*, 1950, v36, 961.

7. Abrams, R., *Arch. Biochem.*, 1951, v30, 90.

8. To be published.

9. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, v114, 495.

10. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, 1945, v161, 83.

11. Gomori, G., *J. Lab. Clin. Med.*, 1942, v27, 955.

12. Delory, J., *Biochem. J.*, 1938, v32, 1161.

13. Snedecor, G. W., *Statistical Methods,* Collegiate Press, Ames, Iowa, 1946.

14. Marshak, A., and Calvet, F., *J. Cell. and Comp. Physiol.*, 1949, v34, 451.

15. Hausner, E., Essex, H. E., and Mann, F. E., *Am. J. Physiol.*, 1938, v121, 387.

16. Hahn, P. F., Bale, W. F., and Bonner, J. F., Jr., *Am. J. Physiol.*, 1943, v138, 415.

17. Holmes, B. E., *Brit. J. Radiol.*, 1947, v20, 450.

18. Brachet, J., *Compt. rend. Soc. biol.*, 1945, v139, 193.

19. Quastel, J. H., *Physiol. Rev.*, 1939, v19, 135.

20. Grieg, M. E., *J. Pharm. and Exp. Therap.*, 1946, v87, 185.

21. Persky, H., Goldstein, M. S., and Levine, R., *J. Pharm. and Exp. Therap.*, 1950, v100, 273.

22. Brody, T. M., and Bain, J. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 50.

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