pattern was found in a pair of monozygotic twins and their mother indicating that the observed variation is under genetic control.

It has now been demonstrated that band A_4 is composed of two distinct bands. In the genetically determined variation the slower band of this doublet migrates at the same rate as in the normal pattern whereas the advanced band migrates faster than normal. A new band has also been located between bands A_4 and A_5 which shows decreased activity in the atypical condition under genetic control.

I am very much indebted to Drs. Margery W. Shaw, Robert L. Hunter, and Donald L. Rucknagel for prompt and valuable help and advice during this study. The excellent research assistance of Mrs. Dorothy P. Douglas is especially acknowledged.

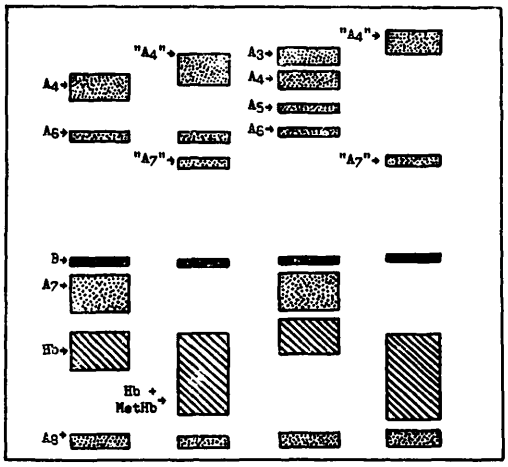

FIG. 3. Diagram of Fig. 2. Location of band B is indicated even though it is not well defined.

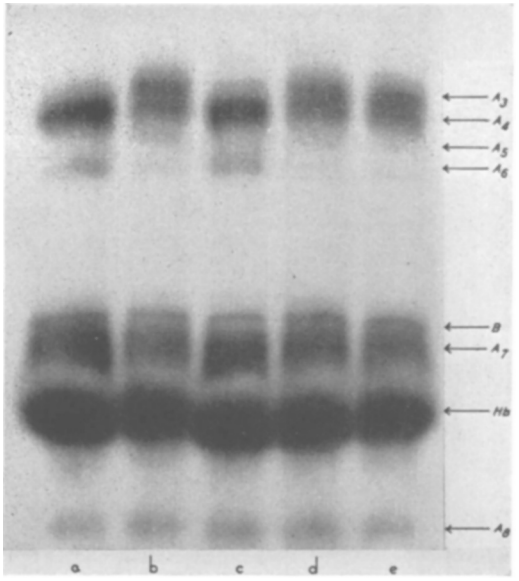

FIG. 4. Zymograms exhibiting genetic segregation of normal and atypical esterase forms from the family study: father (a); mother (b); daughter (c); and identical twin sons (d, e).

1. Augustinsson, K.-B., *Acta Chem. Scand.*, 1959, v13, 571.

2. ———, *Ann. N. Y. Acad. Sci.*, 1961, v94, 844.

3. Hunter, R. L., Denuce, J. M., Strachan, D. S., *Proc. First Intern. Congr. Histochem. Cytochem.*, in press.

4. Hunter, R. L., Strachan, D. S., *Ann. N. Y. Acad. Sci.*, 1961, v94, 861.

5. Smithies, O., *Biochem. J.*, 1959, v71, 585.

6. Markert, C. L., Hunter, R. L., *J. Histochem. Cytochem.*, 1959, v7, 42.

Received July 13, 1961. P.S.E.B.M., 1961, v108.

Effects of 5-Br-Deoxyuridine and 5-F-Deoxyuridine on Nucleic Acid Metabolism by Rabbit Bone Marrow Cells.* (26940)

JACK C. WILSON† AND JAMES S. DINNING

Department of Biochemistry, University of Arkansas School of Medicine, Little Rock

A series of investigations has demonstrated that 5-bromodeoxyuridine (5-Br-deoxyuridine) may be utilized in lieu of thymidine for deoxyribonucleic acid (DNA) biosynthesis in animal and microbial systems(1-5). The anti-metabolite is not only incorporated into DNA but also blocks the incorporation of thymidine and thymidine precursors into DNA(6-10). 5-fluorodeoxyuridine (5-F-deoxyuridine) is known to inhibit formation of DNA thymine and specifically interferes with

* Supported by research grant from U.S.P.H.S.

† U.S.P.H.S. post-sophomore research fellow.

methylation of deoxyuridylic acid to thymidylic acid(6). In view of the high mitotic rate in bone marrow it seemed desirable to study the effects of these antimetabolites on nucleic acid metabolism in marrow cells.

Methods. New Zealand and California rabbits of both sexes weighing 500 to 1500 g were used. The rabbits were fed commercial chow and as prophylaxis for coccidiosis were given 0.04% sodium sulfaquinoxaline in the drinking water for 4-day periods alternating with 3-day periods of tap water.

The marrow from both tibias and femurs was suspended in Robinson's medium(11) and filtered through several layers of gauze. The final volume of the marrow suspension was adjusted to the amount needed. Two milliliters of the marrow cell suspension containing approximately 40 μg of DNAP, were incubated in 20 ml beakers with 13.5 mg of sodium succinate as an energy source, 0.05 mg of heparin to prevent clotting, labeled nucleic acid precursors, varying concentrations of 5-Br-deoxyuridine or 5-F-deoxyuridine, and other additions as indicated to a final volume of 3 ml. The radioactive nucleic acid precursors employed were: thymidine-2-C^{14}, 1.43 μcuries per μmole and sodium formate-C^{14}, 9.5 μcuries per μmole. Different batches of P^{32} (as Na_2HPO_4) of varying specific activities were used in different experiments. In the experiments with 5-Br-deoxyuridine and P^{32} unlabeled thymidine was added to a concentration of 0.05 μmole/ml. After 1 hour of incubation in air at 37°C in a Dubnoff shaker, the marrow was subjected to a combined Schneider(12) and Schmidt-Thannhauser(13) fractionation. When thymidine-2-C^{14} was used as the precursor the lipid extraction was omitted and the nucleic acids were extracted as one fraction with hot 5% trichloroacetic acid (TCA). The nucleic acid fractions were extracted with ether to remove the TCA and aliquots of the various fractions were counted in an end-window Geiger counter.

DNA P and RNA P concentrations were determined by the diphenylamine and orcinol procedures, respectively(12).

Results and discussion. 5-Br-deoxyuridine greatly inhibited the incorporation of thymidine-2-C^{14} into bone marrow DNA while 5-F-deoxyuridine was much less inhibitory (Table I). Degree of inhibition exhibited by 5-Br-deoxyuridine increased linearly with concentration. A 50% inhibition was obtained with a 5-Br-deoxyuridine/thymidine concentration ratio of 1.6. To determine if the inhibition was competitive the effect of 5-Br-deoxyuridine at varying concentrations of thymidine on the Lineweaver-Burk(14) plot was determined. The results (Fig. 1), demonstrate that 5-Br-deoxyuridine acts as a competitive inhibitor of incorporation of thymidine into the DNA of bone marrow cells.

TABLE I. Effects of 5-Br-deoxyuridine and 5-F-deoxyuridine on Thymidine-2-C^{14} Incorporation into Marrow DNA. The concentration of thymidine-2-C^{14} was 0.16 μmole/ml.

Exp. No.	5-Br-deoxyuridine, μmoles/ml	5-F-deoxyuridine, μmoles/ml	cpm/μg DNA P	Inhibition, %
1	0	0	5.7	—
	.16	0	3.6	37
	.48	0	1.9	67
	.96	0	1.2	79
2	0	0	3.5	—
	0	.16	3.4	3
	0	.48	2.9	17
	0	.96	2.3	34

The data in Table II show that 5-Br-deoxyuridine does not inhibit incorporation of

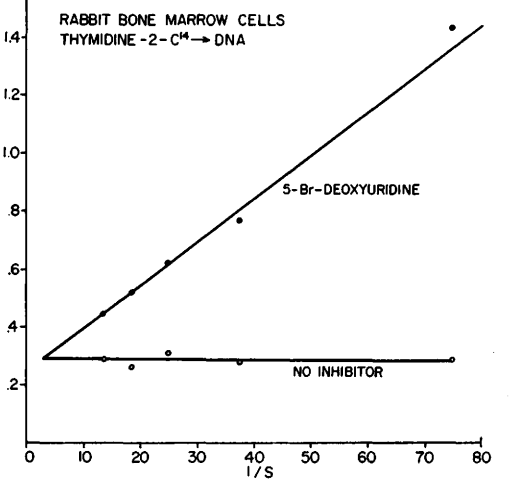

FIG. 1. Lineweaver-Burk plot(14) of inhibition by 5-Br-deoxyuridine of thymidine-2-C^{14} incorporation into bone marrow DNA. Concentration of 5-Br-deoxyuridine was .053 μmole/ml. S = μmoles thymidine-2-C^{14}/ml. V = cpm/μg DNA P.

TABLE II. Effects of 5-Br-deoxyuridine and 5-F-deoxyuridine on the Incorporation of Formate-C^{14} and P^{32} into Marrow Nucleic Acids. The concentration of formate-C^{14} was 0.77 μmole per ml.

Radioactive precursor	Exp. No.	5-Br-deoxyuridine, μmoles/ml	5-F-deoxyuridine, μmoles/ml	Nucleic acid specific activity, cpm/μmole P	
				DNA	RNA
Formate-C^{14}	1	0	0	7.9	10.2
"	1	.77	0	.7	11.4
"	1	0	.77	1.1	11.6
P^{32}	2	0	0	1.8	8.7
"	2	.32	0	2.3	14.4
"	3	0	0	31.8	129.0
"	3	0	.37	27.0	92.7

phosphate into bone marrow DNA. The competitive inhibition of thymidine incorporation by 5-Br-deoxyuridine without concurrent reduction in phosphorylation indicates that 5-Br-deoxyuridine does not inhibit thymidine kinase and that the anti-metabolite is used in lieu of thymidine for DNA biosynthesis by bone marrow cells.

5-F-deoxyuridine inhibited phosphate incorporation into DNA to the same degree that it inhibited thymidine-2-C^{14} (Table II).

When formate-C^{14} was used as the DNA precursor (Table II), specific activities of DNA were drastically reduced by both 5-Br-deoxyuridine and 5-F-deoxyuridine, but specific activity of RNA was slightly increased. This indicates a specific effect of 5-F-deoxyuridine on formate utilization for thymidine synthesis and reflects the inhibition by 5-Br-deoxyuridine of thymidine utilization.

The results of these experiments demonstrate that in bone marrow cells 5-Br-deoxyuridine is incorporated into DNA in lieu of thymidine while 5-F-deoxyuridine inhibits thymidine formation. Such an effect may be related to the relative sizes of the fluorine and bromine atoms. It has been pointed out that the atomic radius of the fluorine atom is quite similar to that of hydrogen(6) while the atomic radius of the bromine atom is similar to that of the methyl group(15). Thus fluorine substituted uracil nucleosides might be expected to act as deoxyuridine inhibitors while the corresponding bromine substituted analog would act as a thymidine inhibitor.

Summary. The effects of 5-Br-deoxyuridine and 5-F-deoxyuridine on thymidine-2-C^{14}, formate-C^{14}, and P^{32} incorporation into rabbit marrow DNA *in vitro* were studied. 5-Br-deoxyuridine competitively inhibits incorporation of thymidine-2-C^{14} into rabbit marrow DNA, and is apparently used in lieu of thymidine for DNA biosynthesis. The bone marrow is quite sensitive to the action of 5-Br-deoxyuridine. 5-F-deoxyuridine does not appreciably affect incorporation of thymidine-2-C^{14} or P^{32} into marrow DNA or formate-C^{14} into RNA but greatly inhibits utilization of labeled formate for DNA synthesis indicating an interference with thymidine formation.

1. Eidinoff, M., Cheong, L., Rich, M., *Science,* 1959, v129, 1550.

2. Djordjevic, B., Szybalski, W., *J. Exp. Med.,* 1960, v112, 509.

3. Hakala, M., *J. Biol. Chem.,* 1959, v234, 3072.

4. Littlefield, J., Gould, E., *ibid.,* 1960, v235, 1129.

5. Bessman, M., Lehman, I., Adler, J., Zimmerman, S., Simms, E., Kornberg, A., *Proc. Nat. Acad. Sci.,* 1958, v44, 633.

6. Handschumacher, R., Welch, A., in *The Nucleic Acids.* Agents which influence nucleic acid metabolism. E. Chargaff and J. Davidson, Eds., Academic Press, New York and London, 1960, v3, p453.

7. Kit, S., Beck, C., Graham, O., Gross, A., *Cancer Res.,* 1958, v18, 598.

8. Eidinoff, M., Knoll, J., Marano, B., *Proc. Am. Assn. Can. Res.,* 1957, v2, 198.

9. Eidinoff, M., Knoll, J., Marano, B., Klein, D., *Cancer Res.,* 1959, v19, 738.

10. Prusoff, W., *ibid.,* 1960, v20, 92.

11. Robinson, J., *Biochem. J.,* 1949, v45, 68.

12. Schneider, W., *J. Biol. Chem.*, 1945, v161, 293.
13. Schmidt, G., Thannhauser, S., *ibid.*, 1945, v161, 83.
14. Lineweaver, H., Burk, D., *J. Am. Chem. Soc.*, 1934, v56, 658.
15. Zamenhof, S., Reiner, B., De Giovanni, R., Rich, K., *J. Biol. Chem.*, 1956, v219, 165.

Received July 14, 1961. P.S.E.B.M., 1961, v108.

Thromboplastic and Fibrinolytic Activities of Large Human Vessels.* (26941)

S. COCCHERI AND TAGE ASTRUP

James F. Mitchell Foundation, Washington Clinic, Washington, D.C.,† Biological Inst., Carlsberg Foundation, Copenhagen, Denmark and Instituto di Patologia Speciale Medica, University of Bologna, Italy.‡

Intima and media of the human aorta are highly thromboplastic with little or no fibrinolytic activity, while adventitia is less thromboplastic and highly fibrinolytic(1). Hence, after tissue injury fibrin can be easily deposited on the intimal surface. Its removal, however, depends upon a fibrinolytically active system in blood. We have now investigated some large human vessels to elucidate further the physiological role of the hemostatic balance in the organism.

Experimental. Materials and methods were similar to those previously described (1). Thromboplastic activity was estimated on freshly drawn citrated rabbit plasma and compared with a suspension of human brain used for prothrombin estimations. Fibrinolytic activity was estimated after extraction with KSCN and expressed in units of a standard preparation of plasminogen activator prepared from pig heart. The fibrin plate method with 0.12% bovine fibrinogen clotted with bovine thrombin (Leo Pharmaceutical Prod., Copenhagen) was used. All estimations (thromboplastic and fibrinolytic) were made on serial dilutions of the samples.

Samples of the vessels were obtained from autopsies (21) at the Inst. of Forensic Medicine, Univ. of Copenhagen (with kind permission of Prof. H. Gormsen). Most of the samples came from subjects who had died from accidents. As in our previous report apparently normal or only slightly pathological specimens were compared. Complete separation of layers (under water) was obtained for arteries only. In veins media and adventitia could usually not be separated and the activities had to be estimated together. No effort was made to separate the endothelium from the intima proper, since nearly identical activities had been found in our previous study.

Results. The thromboplastic activity in the different layers of the vessels appears in Table I. In all vessels thromboplastic activity was low in adventitia. In the pulmonary vein and inferior vena cava it was also low in the intima. It reached medium values in the media and intima of the pulmonary artery. As before aortic intima was nearly always highly active. None of the curves indicated presence of antithromboplastic material. The activities of aortic and coronary intima in the same individuals were estimated in 5 subjects (Table II). The distribution follows identical patterns and the coronary intima has even higher thromboplastic activity than the aortic intima.

Estimations of plasminogen activator concentrations in 14 subjects are presented in Table III. Fibrinolytic activity was found in the aortic intima in only 4 cases, and in only one was there more than a trace of activity. It is perhaps significant that the sample with highest intimal activity (44.4

* This work was supported by the Josiah Macy Jr. Foundation and by a grant from U. S. Public Health Service, Nat. Heart Inst. The participation of S. C. was made possible by a Danish-Italian Exchange Fellowship.

† Present address of Dr. Astrup.

‡ Present address of Dr. Coccheri.