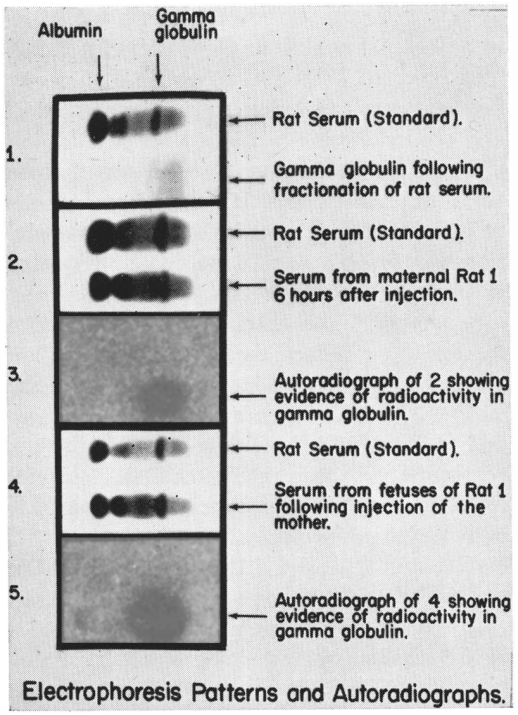

FIG. 1. Electrophoretic and autoradiographic patterns of maternal and fetal serum following administration of I-131 rat gamma globulin.

forming a total of 1.08% of the original I-131 gamma globulin recovered from these feti and placentae.

The percentage of original I-131 gamma globulin recovered from the maternal serum following intraperitoneal injection of the feti in 2 pregnant rats is shown in Table III. The percentage recovered per ml maternal serum increased during the 6 hours of the experiment in direct relation to the length of time following injection. T.C.A. precipitation showed that 96.9% of the I-131 was bound to gamma globulin in the sera of the injected feti, between 70.8 and 87.7% remained bound in the mothers' sera and 54.3% was bound in the sera of the non-injected feti. These figures indicate that the degree of dissociation of I-131 from gamma globulin increased with passage from injected feti to mother to non-injected feti.

The results indicate that in rats there is a complex interaction between the circulations of mother and fetus at the sites of closest approximation.

Summary. I-131 rat gamma globulin was shown to pass from the mother to the fetuses *in utero,* during the 24 hour period following injection of 3 maternal rats. I-131 gamma globulin was also shown to pass from the fetuses *in utero* into the maternal circulation, and thence into the circulation of the non-injected fetuses, following injection of fetuses *in utero* in 2 pregnant rats.

1. Bangham, D. R., Hobbs, K. R., Terry, R. J., *Lancet,* 1958, v2, 351.

2. DuPan, R. M., Wenger, P., Koechli, S., Schneidegger, J. J., Roux, J., *Clin. Chem. alta.,* 1959, v4, 110.

3. Edsall, S., *Ann. N. Y. Acad. Sci.,* 1956, v66, 32.

4. Brambell, F. W. R., Hemmings, W. A., in *The Placenta and Fetal Membranes,* Williams and Wilkins Co., 1st Ed., 1960, p.71.

5. Peterson, E. A., Sober, H. A., *J. Am. Chem. Soc.,* 1956, v78, 751.

6. Masouredis, S. P., Melcher, L. R., Koblick, D. C., *J. Immunol.,* 1951, v66, 297.

7. Grodsky, G. M., Contopoulos, A. N., Fanska, R., Carbone, J. V., *Am. J. Physiol.,* 1963, v204, 837.

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Uptake of Tritiated Thymidine by Leukemic Cells: Effect of Various Leukocyte Preparations. (28828)

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Recently, Craddock has suggested that normal mature granulocytes contain a potent inhibitor of DNA synthesis which is absent from leukemic cells(1). Using dog thoracic duct lymphocytes as test cells, he demonstrated that DNA-P^{32} incorporation and thymidine-H^3 uptake were inhibited by intact or homogenized normal human granulocytes but

not significantly decreased by leukemic leukocytes. The inhibitory substance was not identified. Gray *et al* reported(2) that normal rat liver extracts markedly decreased DNA synthesis in extracts of rabbit bone marrow and in extracts of other proliferating cell systems.

These findings are consistent with suggestions by several investigators(3,4) that human leukocyte production is regulated by a "feedback" control mechanism. The study reported here explores this possibility further, comparing the effects produced by normal and leukemic leukocyte preparations on *in vitro* uptake of thymidine-H^3 by leukemic cells.

Methods. Blood was obtained from normal individuals and patients with chronic myelocytic leukemia (CML) not on therapy and with white blood counts above 60,000 per cu mm. Leukocytes were separated in the cold using a modified dextran sedimentation technique(5).

Leukocyte homogenates were prepared by homogenizing 2.7×10^8 cells per ml in a total volume of 3 ml of 0.1 M phosphate buffer pH 7.2 with 2 g of glass beads in the cold (MSE Homogenizer, Measuring and Scientific Equipment, Ltd., London). The beads were allowed to settle out and the homogenate was centrifuged at $37,000 \times g$ for 30 minutes in the cold. The residue was discarded.

"Incubation supernates" were obtained by incubating normal or CML leukocytes in autologous plasma or in 0.1 M phosphate buffer pH 7.2 at a concentration of 10^9 cells per ml for 2 hours at 37°C. The cells were then removed by centrifugation in the cold at $14,000 \times g$ for 30 minutes. Cellular metabolism and viability in the incubated cells did not appear to be seriously impaired as determined by staining reactions in both types of cells and thymidine-H^3 uptake and C^{14}-valine uptake as an indication of protein synthesis in the CML cells. The test system used consisted of CML leukocytes (2×10^7) in 0.2 ml 5% dextrose in water, 1.2 ml of modified Robinson's medium(8), 0.3 ml of autologous plasma, and normal leukocytes (4×10^7) in 0.2 ml dextrose or 0.3 ml of the "incubation supernate" or homogenate described above. After one hour's incubation in a Dubnoff shaker at 37°C, 1 μc of thymidine-H^3 (sp. act. 0.36 c per mM, Schwarz BioResearch, Orangeburg, N. J.) was added and the incubation continued for an additional hour. To study the effect on uptake after the leukocyte preparations had been allowed to act directly on the thymidine, pre-incubation experiments were done. The thymidine-H^3 was incubated at 37°C with the incubation supernate or homogenate and medium for one hour before the CML test cells and plasma were added for another hour's incubation. Controls included incubation of thymidine-H^3 alone, thymidine-H^3 with the test cells only, with the supernates alone and with the homogenates. The test cells were washed with saline, hydrolyzed with 0.5 ml of 5% NaOH and 0.1 ml of the resulting solution was added to 18 ml of a scintillation mixture (70% toluene, 30% methanol containing 3 g of 2,5 diphenyloxazole and 0.050 g of 1,4-bis-2(4-methyl-5 phenyloxazolyl)-benzene per liter. The samples were then counted in duplicate in a liquid scintillation spectrometer. Variable degrees of isotope dilution were excluded by using carefully measured constant volumes and hydrolyzing the entire sediment in each tube after centrifugation.

Distribution of cellular radioactivity in the test cells was studied with a modification of the method of Williams and Schilling(6). The residual media was chromatographed in a descending system with ethyl acetate, water and formic acid as the solvent(7).

Results. Normal intact leukocytes as well as normal homogenized leukocytes markedly inhibited thymidine-H^3 uptake by CML leukocytes when these were incubated together for an hour as shown in Fig. 1. Average inhibition by 4 normal leukocyte homogenates was 67% as compared to 51% by 4 CML homogenates. The effect was more striking when the thymidine-H^3 was pre-incubated with the homogenates before addition of the test cells. Under such conditions, there was essentially no uptake of the isotope.

The most striking difference between normal and CML leukocytes, however, was found in the effect of the respective incubation supernates on thymidine-H^3 uptake (Fig.

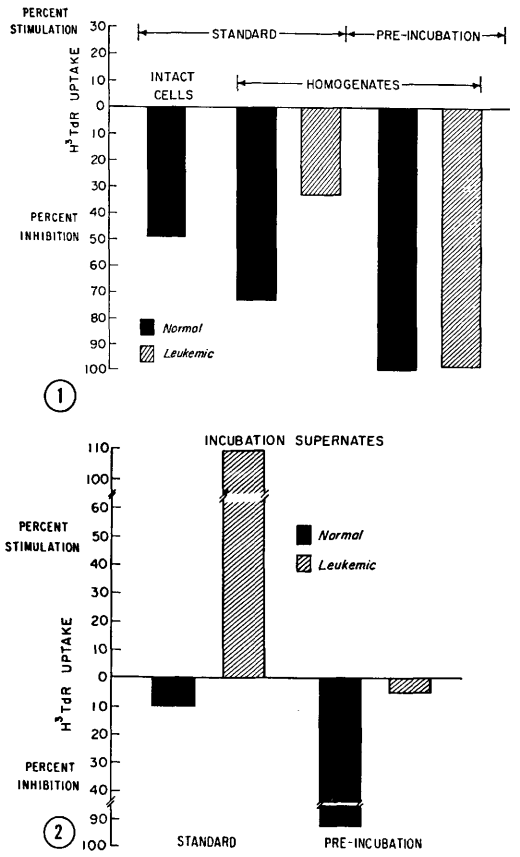

FIG. 1. Inhibition of thymidine-H^3 uptake in CML leukocytes by intact normal cells and homogenates of normal and CML leukocytes.

FIG. 2. The effect of incubation supernates from normal and CML leukocytes on the uptake of thymidine-H^3 by CML leukocytes.

2). The incubation supernates obtained from CML leukocytes increased the uptake of the radioisotope but the normal leukocyte incubation supernates were inhibitory. The enhancement was consistently demonstrable when the CML incubation supernates were prepared in either plasma or 0.1 M phosphate buffer and was observed with homologous or autologous CML leukocytes as the test cells. The average enhancement by 6 preparations in plasma was 61% and by 4 in phosphate buffer, 79%. In contrast, normal incubation supernates inhibited thymidine-H^3 uptake. This effect was regularly observed if both autologous plasma and 0.1 M phosphate buffer were present. Results in 4 experiments ranged from 10 to 72% inhibition in the standard test with an average of 35%. Preincubation of these supernates with thymidine-H^3 decreased thymidine-H^3 uptake by approximately 100% in each experiment. With the normal incubation supernates, if either autologous plasma or 0.1 M phosphate buffer were omitted from the test system, the inhibitory effect was not regularly obtained. In 7 such experiments, inhibition was observed in 4 and enhancement in 3 experiments.

It is not yet possible to obtain morphologically uniform leukocyte populations from peripheral blood but preliminary data using human thoracic duct lymphocytes suggest that granulocytes are primarily responsible for the inhibitory effect seen with the normal leukocyte preparations.

Chromatographic analysis of the residual media revealed that more than 96% of the thymidine-H^3 had been catabolized, largely to thymine, but also to dihydrothymine by the homogenates. CML supernates and the normal supernates catabolized approximately 35% and 20% respectively. Details of these studies will be reported separately. Thymidine-H^3 in the control tubes did not become converted to thymine-H^3 during the incubation in the absence of a leukocyte preparation.

Analysis of the CML test cells showed that 90 to 95% of the radioactivity was present in the DNA.

Discussion. This study has demonstrated that under certain experimental conditions, normal leukocytes inhibit the uptake of thymidine-H^3 by leukocytes from patients with CML and that CML leukocytes increase this uptake. Homogenates from both normal and CML cells inhibit the uptake of thymidine-H^3. Work now in progress suggests that the inhibition by these homogenates as well as by supernates from normal leukocytes is related to the conversion of thymidine to thymine by thymidine phosphorylase in the leukocyte preparations. Stimulatory substances for various types of mammalian cells in culture have been described(9-11), but there have been no studies to our knowledge in which intact leukemic leukocytes have been used. The exact nature of the substance responsible is not yet known but from preliminary work, it does not appear to be thy-

midine kinase, recently described in leukemic white cells(12). It is relatively stable on storage at –20°C, to heat at 60°C for 30 minutes and is dialyzable. The mechanism through which uptake is enhanced also is not known at present.

Although thymidine is not thought to be in the normal intracellular pathway for DNA synthesis, the suggestion has been made that the enzymes involved in thymidine metabolism may be important in initiating and regulating DNA synthesis(13). There have been a number of reports on the levels of thymidine phosphorylase and thymidine kinase in various mammalian tissues and the alterations in their content with tissue proliferation(14-18).

There is evidence that the peripheral blood leukocytes in CML may be merely an "extension" of the bone marrow leukocytes in that disease(19,20). Hence, it is felt that the CML leukocytes obtained for testing in this study may well be representative of the relationships present in the CML bone marrow. Nevertheless, a more definitive interpretation of the results will be possible only when normal and leukemic marrows are studied. Marrow cells in sufficient quantity and free from peripheral blood contamination are technically difficult to obtain. This problem is being investigated.

The observation that leukocytes, under certain, even though artificial, circumstances, elaborate substances which influence thymidine uptake and possibly DNA synthesis may have significant implications in explaining normal and leukemic leukopoiesis. It is conceivable, although unproven, that the relative local concentration of inhibitory and stimulatory substances has a regulating effect on leukocyte DNA synthesis and subsequent cell division in the proliferating pool in the bone marrow.

Summary. Homogenates of normal and chronic myelocytic leukemic leukocytes inhibit the uptake of tritiated thymidine by leukocytes from patients with chronic myelocytic leukemia. However, intact leukocytes from patients with chronic myelocytic leukemia elaborate a factor which increases the uptake of the labeled thymidine whereas normal leukocytes inhibit thymidine uptake under these conditions. The observations suggest that local bone marrow concentrations of stimulatory and inhibitory substances may influence leukopoiesis.

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1. Craddock, C. G., Jr., *Am. J. Med.,* 1960, v28, 711.

2. Gray, E. D., Weissman, S. M., Richards, J., Bell, D., Keir, H. M., Smellie, R. M. S., Davidson, J. N., *Biochem. Biophys. Acta,* 1960, v45, 111.

3. Osgood, E. E., *J. Nat. Cancer Inst.,* 1957, v18, 155.

4. Lajtha, L. G., *Cancro.,* 1962, v15, 139.

5. Fallon, H. J., Frei, E., III, Davidson, J. D., Trier, J. S., Burk, D., *J. Lab. and Clin. Med.,* 1962, v59, 779.

6. Williams, A. M., Schilling, R. F., *ibid.,* 1961, v58, 76.

7. Fink, E., Cline, R. E., Henderson, R. B., Fink, R. M., *J. Biol. Chem.,* 1956, v221, 425.

8. Robinson, J. R., *Biochem. J.,* 1949, v45, 68.

9. Foley, J. F., Kennedy, B. J., Ross, J. D., *Cancer Res.,* 1963, v23, 368.

10. Alfred, L. J., Pumper, R. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 688.

11. Kutsky, R. J., *Science,* 1950, v219, 1489.

12. Bianchi, P. A., *Biochem. Biophys. Acta,* 1962, v53, 547.

13. Bianchi, P. A., Crathorn, A. R., Shooter, K. V., *ibid.,* 1962, v61, 728.

14. Friedkin, M., Roberts, DeW., *J. Biol. Chem.,* 1954, v207, 245.

15. Canellakis, E. S., Jaffe, J. J., Mantsavinos, R., Krakow, J. S., *ibid.,* 1959, v234, 2096.

16. Beltz, R. E., *Arch. Biochem.,* 1962, v99, 304.

17. Weissman, S. M., Smellie, R. M. S., Paul, J., *Biochem. Biophys. Acta,* 1960, v45, 101.

18. Bollum, F. J., Potter, Van R., Cancer Res., 1959, v19, 561.

19. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G., Schork, P. K., *Acta Haemat.,* 1959, v21, 1.

20. Craddock, C. G., *J. Clin. Invest.,* 1962, v41, 360.

Received July 11, 1963. P.S.E.B.M., 1964, v115.