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Synthesis of RNA and DNA at Various Intervals After Erythropoietin Injection in Transfused Mice. (31919)

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Erythropoietin injection in hypertransfused mice induces a wave of erythropoiesis in the spleen(1). ^{59}Fe incorporation into Hem does not occur until 24 hours after erythropoietin (EP) injection in this assay system(2), while immediate increase of ^{59}Fe uptake is observed in marrow cultures on addition of EP(3). Increase of ^{14}C-uridine incorporation into cultured marrow RNA is seen within 20 minutes of EP addition(4) and of ^{14}C formate in rat marrow RNA *in vivo,* within 4 hours of EP injection(5). Since there is marked difference in time of response between the transfused mouse spleen and rat marrow "in culture" and *in situ* in relation to hemoglobin synthesis, it was considered of interest to establish the time course of the RNA, DNA synthesis in the spleen after EP injection in hypertransfused mice.

Methods. Three-months-old 20 g female CBA mice were used. Transfusion erythrocytosis, with Hb concentrations greater than 18 g/100 ml, was produced by intraperitoneal injection of 1 ml of whole CBA blood, per 20 g on days zero and one. Erythropoietin 1 mg of a 60-80% ethanol fraction of anemic rabbit plasma(6) equivalent to 5 U St.B, was injected on day 6 and the following tracers obtained from the Radiochemical Center, Amersham, were injected at times indicated in *Results:* uridine $5-^{3}H$ 8 μc/mouse, iv., thymidine $-^{3}H$ (methyl) 8 μc/mouse, i.v.

Groups of at least 5 mice were killed 5 minutes after ^{3}H uridine and 60 minutes after ^{3}H thymidine injection. Spleens were removed and immediately frozen in dry ice-ethanol. The nucleic acid fraction was prepared from a water homogenate by the method described in(7). The dry powder obtained was dissolved in 1 ml hyamine and counted in POP, POPOP toluene phosphor in a TriCarb liquid scintillation spectrometer. An internal ^{3}H toluene standard was used for quench corrections. Samples were kept in the dark and cold for 48 hours before counting (8).

Results. Uridine uptake in rapidly labelled RNA increases 2 hours after EP injection and remains high for 60 hours (Fig. 1). Thymidine uptake does not rise until after 12 hours and shows 2 maxima at 24 and 48 hours separated by a dip at 36 hours. By 60 hours it is back to control levels. Control experiments with EP inactivated by mild acid treatment(9) showed no increase of thymidine uptake at 24 hours and of uridine at 6 hours after EP (Table I).

Discussion. It is known that $5-^{3}H$-uridine

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TABLE I. Uptake of ^{59}Fe into RBC's and of $5^{3}H$-Uridine and ^{3}H-Thymidine into Nucleic Acids of Spleen. ^{59}Fe injected 48 hours after EP blood withdrawn 24 hours after tracer injection.

Group	% ^{59}Fe/ml	dpm/spleen ^{3}H-uridine (6 hr)	dpm/spleen ^{3}H-thymidine (24 hr)
Active EP	15.6 ±1.4 (5)	6990 ± 760 (5)	107,000 ±16,750 (4)
Inactive EP	1.4 ± .3 (2)	4382 ± 702 (4)	51,300 ± 3260 (3)
Transfusion control	1.8 ± .7 (4)	4317 ± 262 (9)	41,900 ± 2370 (6)

No. of animals in parentheses. ± one standard error.

specifically labels RNA(10). The results of studies with 5-minute pulses indicate that rapidly labelled RNA synthesis increases significantly in polycythemic mouse spleen within 2 hours after EP injection. This indicates that, as in the case of rat marrow *in vitro*(4) and of normal rat injected with EP(5), increase of precursor incorporation into RNA is also the earliest observable effect of EP injection in the polycythemic mouse. That this effect is specific to EP and not a reaction to foreign protein in the extract is suggested by the control experiments using EP inactivated by mild acid hydrolysis. Since rapidly labelled RNA is a heteregenous mixture(11) elucidation of the types of RNA synthesized and the possible presence of messenger activity in the newly synthesized RNA requires further investigation. Krantz and Goldwasser's(4) data indicate that uptake is predominantly in the fractions 4 to 16 S.

Increase of thymidine uptake into DNA is not observed until 12 hours after EP injection; this is similar to what occurs in rat spleen(12). The effect seems to be specific for EP, as shown by control experiments (Table I) and suggests that the cell produced by differentiation of a stem cell does not enter S phase for some time after exposure to EP. It is thus conceivable that the early increase in RNA synthesis may be preparatory to the cell entering S phase, rather than to making RNAS concerned with building up the machinery for hemoglobin synthesis which does not begin until much later. The situation of the cell produced by the action of EP on the stem cell in an hypertransfused mouse is analogous to a liver cell after partial hepatectomy; in the latter case one has a cell that does not start DNA synthesis until 15 hours after the triggering event, and which shows as its first response an increase in RNA synthesis preparatory to entering S phase(7). The oscillatory behaviour of DNA synthesis suggests that EP produces a rather synchronized population of cells going through S period for DNA at about 24 and 48 hours after EP, and that in mice as in dogs(13), the number of divisions occurring from entry of a cell into the erythropoietic series to delivery of the erythrocyte is about 2. Further studies with thymidine injections separated by shorter time intervals will be needed to substantiate this hypothesis.

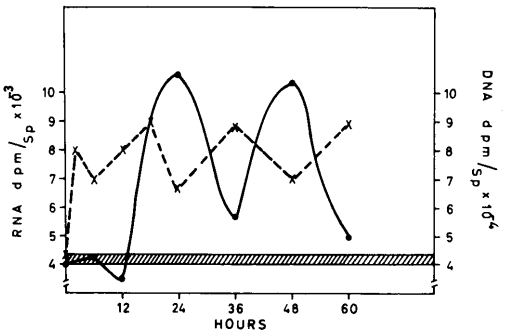

FIG. 1. Ordinate: Uptake of uridine-$5^{3}H$ in RNA, ×- - -×, and thymidine ^{3}H (methyl) in DNA, ●———●, in spleen (Sp) of transfused mice. Abscissa: Time of tracer injection after EP. Mice were killed 5 min after ^{3}H uridine, and one hour after ^{3}H thymidine. Hatched area corresponds to uptake in control, non EP injected transfused mice.

Summary. Uptake of $5^{3}H$ uridine in 5 minutes into spleen RNA increases within 2 hours after EP injection and remains high for 60 hours. ^{3}H thymidine uptake in spleen DNA begins 12 hours after EP injection, shows 2 maxima at 24 and 48 hours and drops to control levels by 60 hours. Control experiments using EP inactivated by mild acid hydrolysis suggest that these effects are specific for EP.

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Polyoma Virus-Induced "Complement-Fixing Antigen" in Tumors and Infected Cells as Detected by Immunofluorescence.* (31920)

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The cellular response to infection with DNA oncogenic viruses may be expressed in two different ways. In one case the cells lyse as a result of virus multiplication while in the other the intervention of the viral genome brings about changes leading to malignant transformation of the cells. There is as yet no evidence available on the persistence of the complete viral genome in the transformed cells; however, the virus leaves traits in the host cells in the form of specific antigenic substances by which the cell-virus interaction can be identified. One of these antigens, "tumor" antigen(1), detectable by the complement-fixation test, will be referred to as induced complement-fixing antigen (ICFA)(2). The presence of ICFA has been demonstrated during lytic interactions in cells infected by SV40 and adenoviruses, as well as in the tumors that these viruses induce and that are free of infectious activity(1-8). As shown by immunofluorescent technique ICFA has nuclear localization, and, at least in the case of SV40, there is evidence that the antigens demonstrated by the complement-fixation and immunofluorescence tests are identical(2). Polyoma ICFA has been demonstrated by the complement-fixation test in tumors produced by polyoma(9-11) as well as in primary infected cultures(10,11), but until very recently(12-13), only in the latter case could the antigen be shown by immunofluorescence.

From the results reported here it is evident that the polyoma virus (PV) ICFA can be demonstrated at a cellular level in polyoma-induced rat, mouse, and hamster tumors and that in embryonic tissue cultures of the same species infected with PV, the proportion of ICFA-containing cells greatly exceeds that of cells positive for viral antigens.

Materials and methods. Viruses. Two different pools of polyoma virus were used: One (P-178) was derived from a wild strain ob-

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