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Delayed Incorporation of Tritiated Thymidine into DNA

Abstract. *Delayed utilization of tritiated thymidine by regenerating mouse liver can be almost completely suppressed by a continuous infusion of nonradioactive thymidine. In addition, as shown earlier for lymphocytes, labeled granulocytes are a potential source of the tritium marker. These observations suggest that the delayed incorporation of label into DNA must be due to the transfer of labeled nucleoside, which may be derived either from the degradation of DNA or from a long-lived intracellular pool. In either case, the transferred material probably originates from all tissues that have a high rate of cell turnover.*

Tritiated thymidine has been widely used in the study of DNA synthesis and cell kinetics. Interpretation of work in vivo has been based on the assumption that, once administered, thymidine is available for incorporation into DNA for only a brief period, and that there is no reutilization of label after such incorporation has taken place. Results of a number of recent studies, however, indicate that labeling of DNA may continue to occur for some time after the administration of H^3-thymidine (*1–4*). Delayed tritium labeling has usually been ascribed to the reutilization of DNA, and pathways which have been considered include the transfer of large fragments of nucleic acids from initially labeled cells, or a specific "trephocytic" function of lymphocytes, whereby whole DNA molecules or DNA fragments are passed on to dividing tissues as part of some nutritive process. This communication presents evidence that the substance transferred is thymidine itself, and that it is probably derived from all tissues in which there is a high rate of cell turnover.

In the experimental procedure, a high concentration of nonlabeled competitor was made continuously available in a system in which delayed transfer of label is known to occur. Such a system is regenerating liver following partial hepatectomy, as described by Bryant (*2*).

Using his experimental method, male CDF_1 mice were given three intraperitoneal injections of H^3-thymidine at 12-hour intervals, and 28 hours after the last injection a subtotal hepatectomy was performed with removal of an average of 68 percent of the original liver mass. DNA synthesis in regenerating mouse liver is maximal on the second day after hepatectomy (*5*) or at least 48 hours after the final administration of the thymidine tracer. Since a tracer dose of H^3-thymidine is available for immediate DNA synthesis for only 1 to 2 hours (*6*), labeling of regenerating liver which exceeds the baseline activity of the excised tissue is presumably due to reutilization of the original tritium marker.

Nine animals that were treated as described received, in addition, continuous intravenous infusions (*7*) of nonradioactive thymidine, beginning just prior to hepatectomy and continuing until the time of autopsy. A 0.9-ml volume of isotonic fluid, containing 31.5 mg of thymidine, was delivered each day into the inferior vena cava through a catheter inserted in a tail vein. A "swivel-conduit" allowed the recipient almost free movement within its cage, although the tail was necessarily maintained in a vertical position. Three other mice received infusions of saline instead of thymidine; four had no infusions; and three had sham operations, with manipulation of the liver, to provide controls for the degree of liver radioactivity at the time of autopsy.

All the animals were killed 72 hours after operation, and radioautographs were prepared from touch preparations and histologic sections of the residual right lateral and caudate lobes. Kodak N.T.B.-3 emulsion was used and the preparations were exposed for a period of 30 days.

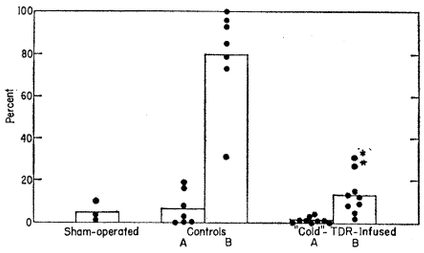

Fig. 1. Percentages of labeled liver cells of mice given tritiated thymidine and then sham operated or partially hepatectomized. *A*, specimens removed at hepatectomy; *B*, samples of regenerated liver removed 3 days after hepatectomy. Controls include three animals which received saline infusions and four which had no infusions. Note the high percentage of labeling in the regenerated liver of the controls and the suppression of labeling in animals receiving cold thymidine (TDR) after hepatectomy. Asterisks denote two animals that had unintentional interruption of continued infusion.

The mean weight of the excised livers in the experimental animals was 1.09 g. In nine controls, the mean weight of excised livers was 1.05 g; these animals were killed immediately after hepatectomy, and the weight of the residual liver was found to average 0.5 g. Regenerated liver at autopsy in the experimental animals averaged 0.96 g. The mean loss in weight of the hepatectomized mice was 4.6 g. There were no significant differences in any of these measurements in mice treated with thymidine or saline, or in those which did not receive infusions.

The results obtained from the radioautographs are summarized in Fig. 1. Less than 3 percent of parenchymal cells were labeled in livers removed at hepatectomy, or at autopsy in sham-operated controls. However, in four animals aged less than 6 weeks, which were used in an early experiment, up to 19 percent of the cells were labeled; consequently, only animals aged 3 to 6 months were used in subsequent experiments.

In control mice which received either no infusions or saline, the majority of regenerating liver cells were labeled 72 hours after hepatectomy, as described by Bryant (*2*). In contrast, animals which received cold thymidine infusions showed only a minimal increase over the baseline in the percentage of labeled liver cells, with the exception of two mice which are indicated by asterisks

in Fig. 1. In these two animals, interruptions of flow lasting approximately 8 hours occurred during the 24- to 48-hour post-operative period, when DNA synthesis is at a maximum. These chance observations emphasize the importance of making the competing agent available continuously, as was true for the other seven animals in this group.

Mean grain counts for regenerating liver were calculated for those animals which did, and which did not, receive cold thymidine. Only the 3- to 6-month old mice were analyzed because, in these, baseline labeling was virtually nil. The mean for three animals not receiving cold thymidine was 14.7 radioautographic grains per cell nucleus, with a standard error of 2.1. In four animals which did receive thymidine infusions the mean grain count was 7.4 $\pm$ 0.4. Some of the tritium activity after thymidine treatment may be accounted for by division of the few liver cells labeled prior to hepatectomy. Incomplete suppression of reutilization from extrahepatic sources may also play a role.

That secondary tritium labeling of regenerating liver can be almost completely suppressed by an excess of cold thymidine indicates that the transfer of the tritium label occurs at the nucleoside level. The molecule transferred may be thymidine itself or it may be a related substance which is readily derived from the competing dose of cold thymidine.

In a preliminary experiment concerning the origin of the transferred marker, heavily labeled white cells from mice bearing an A280 tumor, which elicits in the host a granulocytic leukemoid response (*8*), were found to serve as a source of radioactivity for regenerating liver cells. This observation would exclude a specific "trephocyte" function of the lymphocyte which might have been inferred from studies in which transfer of a tritium label was effected by lymphocyte transfusions (see *3* and *4*). Furthermore, it suggests that any tissue in which there is rapid cell turnover can serve as a source of the transferred nucleoside, whether this is derived from an intracellular pool or from the products of DNA catabolism.

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Polypeptide Chains of Rabbit Gamma Globulin

Abstract. *Rabbit gamma globulin has been extensively reduced, and its polypeptide chains separated by gel filtration on Sephadex G-200 in 5M guanidine hydrochloride. The larger H (or A) chains make up two-thirds of the molecule and have a molecular weight of approximately 55,000 each. The smaller L (or B) chains account for the other one-third and have a molecular weight of approximately 25,000 each. The data are consistent with a model of the gamma globulin molecule that has two H and two L chains.*

Reduction of γ-globulin in the presence of denaturing agents was first reported by Edelman (*1*) who showed that human γ-globulin has polypeptide subunits. He and Poulik (*2*) partially separated and characterized these chains. Franek (*3*) demonstrated that a multichained structure is common to the γ-globulin of several animal species and therefore that analyses of *N*-terminal amino acids are unreliable as indicators of the number of polypeptide subunits per molecule because the number of moles of *N*-terminal amino acid per mole of γ-globulin varies from about 0.5 to 3 for different species (*4*). More recently, on the basis of studies on reduction in the absence of denaturing agents, a four-chain model for the structure of γ-globulin has been proposed (*4, 5*). Conclusive proof for this structure has been lacking, however, because of uncertainties concerning the completeness of reduction, the adequacy of fractionation, and the mass of subunits. In our study, rabbit γ-globulin has been extensively reduced in the presence of a denaturing agent, and the polypeptide chains have been separated. The yields of the two classes of subunits and their molecular weights are reported.

Fraction II of rabbit sera (*6*) was chromatographed on DEAE (diethylaminoethyl) cellulose (*7*) and the eluted protein showed, upon immunoelectrophoresis, a single line characteristic of 7*S* γ-globulin. In all experiments the γ-globulin was then reduced for 1 hour with 0.5*M* β-mercaptoethanol in the presence of a denaturing agent (see Table 1) and 0.1*M* tris buffer, *p*H 8.2. The resulting material was applied to a column of Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride. Fig-

Table 1. Gel filtration of reduced γ-globulin on Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride.

Reduction conditions		S-H "blocking" method*	Recovery (%)	γ-Globulin in peak (%)			
Molarity of guanidine for denaturation	Temperature (°C)			1st	H peak	L peak	$\frac{H}{H+L}$
5	56	Cysteine	95.4	7.0	58.6	34.4	63.0
5	56	HCl	103	8.1	61.5	30.4	66.9
5	56	ICH_2CONH_2	80	9.6	61.0	29.4	67.5
5	56	ICH_2CONH_2	79.3	8.3	63.8	27.9	69.7
6	40	HCl	87.9	2.7	64.8	32.5	66.6
7	25	HCl	95.6	7.3	65.8	26.9	70.9
Sat.	25	HCl	73.7	0	66.0	34.0	66.0
Urea †	45	HCl	95.1	23.0	48.0	29.0	(62.3)
						Average =	67.2

* Three procedures were used to prevent disulfide bonds from forming again after reduction. In some experiments the Sephadex column was equilibrated with 0.1*M* cysteine or 0.1*M* HCl in addition to 5*M* guanidine hydrochloride. For other experiments, the reduced γ-globulin was alkylated by addition of 2 moles of iodoacetamide for each mole of β-mercaptoethanol present during the reduction, and the *p*H was maintained at approximately 8 by addition of 25 percent trimethylamine in water. The resulting solution was then added to the column and the effluent was used for molecular weight determinations. † 11*M* urea was used instead of guanidine.