Specificity of Tritiated Thymidine as a Precursor of DNA Under Conditions of Prolonged Administration[1] (34936)

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Since its introduction by Taylor *et al.* (1) and Verly and Hunebelle (2), tritium-labeled thymidine has been used extensively as a precursor of DNA in autoradiographic as well as in biochemical studies. Several observations, however, have indicated that the specificity of thymidine-^{3}H as a precursor of DNA is limited, and that chemical cell components other than DNA (3–5) or extranuclear materials (4, 6), respectively, may also become labeled to some extent after administration of this radioactive precursor. Such unspecific labeling should be taken into consideration, particularly in experiments of long duration.

In order to obtain quantitative information on the degree of specificity of tritiated thymidine as a precursor of DNA under conditions of prolonged administration, the distribution of radioactivity in several biochemical fractions of various organs of the mouse was determined after 90 injections of thymidine-methyl-^{3}H at intervals of 8 hr. The results of these studies are presented in this communication, while autoradiographic studies on the same material will be reported elsewhere.

Materials and Methods. Thymidine-^{3}H, labeled in the methyl group, with a specific activity of 1.9 Ci/mmole, was obtained from Schwarz Bioresearch Inc. and stored at 4° under sterile conditions until used. It was diluted with sterile isotonic saline containing 100 mg of Na-Penicillin G per liter to obtain an activity of 16.7 μCi/ml. This solution was injected intraperitoneally, at a dose of 0.02 ml per g of body weight, into four female Swiss albino mice of the Hale-Stoner strain which at the beginning of the experiment were approximately 4 weeks old; each mouse received 90 injections at intervals of 8 hr, *i.e.*, a total of 30 μCi per g of body weight. Each animal was kept in an individual cage and had free access to food and water.

One hour after the last injection the animals were injected intraperitoneally with Nembutal (0.1 mg per g of body weight). For subsequent biochemical fractionation, the spleen, liver, kidneys, gastrointestinal tract (rinsed with isotonic saline), peritoneal fat, heart, striated muscle from thigh, brain, and skin were removed and each organ was weighed, placed in 2 ml of isotonic phosphate-buffered saline and frozen at —25° until being further processed. For biochemical fractionation, the samples were thawed, homogenized at 0°, and the homogenates were mixed with an equal volume of cold 10% trichloroacetic acid (TCA); the precipitate was centrifuged and suspended in 5% cold TCA and further fractionated according to the procedure of Schmidt and Thannhauser (7) modified by Schneider (8). This permitted the separation of acid-soluble materials, lipids, RNA, DNA, and protein. The proteins were dissolved by heating with a 2 *M* NaOH solution during 6 hr. The radioactivities of these fractions were measured in a liquid scintillation counter, and background as well as quenching effects of individual fractions were corrected for.

Results. Radiochemical purity of thymidine-^{3}H. In order to test its purity, the thy-

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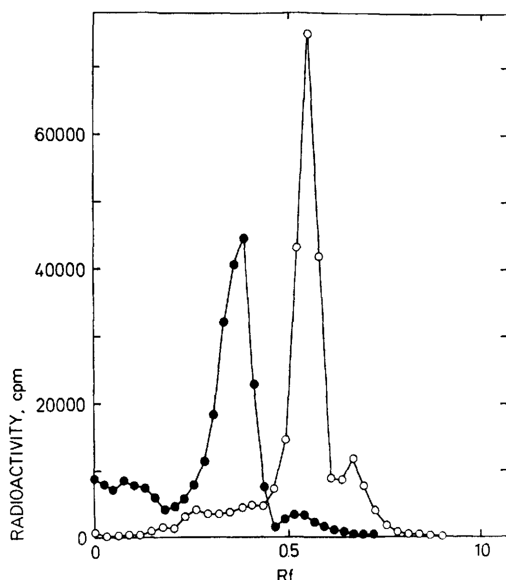

FIG. 1. Paper chromatography of thymidine-^{3}H. Solvent systems: *n*-butanol–acetic acid–water 30:6:15 (open symbols); *n*-butanol–0.6 N NH_4OH 6:1 (closed symbols).

midine-^{3}H preparation used for injecting the mice was mixed with unlabeled thymidine (to obtain 0.8 mg/ml) and subjected to paper chromatography in two different solvent systems. The results, as presented in Fig. 1, indicate that most of the radioactivity was in thymidine, while a second peak, according to known R_F values, appears to be due to free thymine.

Radioactivity of biochemical fractions in different organs. The results of these measurements are presented in Table I.

Characterization of TCA-soluble radioactivity. In order to determine what proportion of the radioactivity found in the cold TCA extracts is attributable to tritiated water, a small amount of water was distilled from the acid-soluble fraction of kidney and liver. With both organs the radioactivity (per ml) of the distillate was 71% of that of the total cold TCA extract.

Next, a cold TCA extract was repeatedly evaporated to dryness at 40° and redissolved in water to determine the proportion of nonvolatile radioactivity which does not exchange with water. A value of approximately 27% of the total cold TCA-soluble radioactivity was obtained.

Finally, the nonvolatile residue of the cold TCA extract was dissolved in a small amount of water and subjected to paper chromatography, using *n*-butanol–acetic acid–water (40:6:15) as the solvent system. The R_F value of thymidine-^{3}H (added to an aliquot of the cold TCA extract) was determined in the same chromatogram. No significant peak of radioactivity at the R_F value of thymidine was observed. This indicates that the radioactivity found in the TCA-soluble fraction was almost entirely due to metabolic products of the injected thymidine, and that thymidine-^{3}H itself was completely metabolized or excreted within the interval of 1 hr between the last injection and sacrifice of the animals. The time of disappearance after

TABLE I. Radioactivity of Different Biochemical Fractions in Various Organs of Mice After 90 Injections of Thymidine-^{3}H at 8-hr Intervals.

	Radioactivity (μCi/kg wet weight, ± standard deviation)				
Organ	Acid-soluble fraction	Lipids	RNA	DNA	Protein
Spleen	1306 ± 159	62 ± 6	57 ± 8	3465 ± 1054	59 ± 26
Liver	1550 ± 608	140 ± 34	70 ± 14	331 ± 89	108 ± 23
Kidney	1688 ± 885	73 ± 11	47 ± 6	496 ± 146	25 ± 5
Gastrointestinal tract	574 ± 176	59 ± 13	41 ± 10	2435 ± 217	115 ± 7
Fat tissue	1030 ± 375	181 ± 89	55 ± 17	1357 ± 547	29 ± 10
Heart	1186 ± 236	76 ± 26	31 ± 11	101 ± 18	12 ± 5
Striated muscle	1278 ± 237	70 ± 40	31 ± 2	80 ± 72	10 ± 4
Brain	1420 ± 429	60 ± 15	32 ± 4	34 ± 5	15 ± 3
Skin	706 ± 297	84 ± 36	26 ± 14	319 ± 91	18 ± 6

repeated injections does not, therefore, markedly differ from what is observed after a single systemic injection of the labeled precursor (9, 10).

Discussion. Chromatography of the tritiated thymidine used in our studies has shown it to be of reasonably good purity, although some impurities were detected. These impurities may have been present in the original preparation or, more probably, formed due to self-radiolysis during storage (11). It is difficult, therefore, to decide whether the incorporation of radioactivity into biochemical fractions other than DNA is due primarily to these impurities or to metabolic conversion of thymidine-^{3}H.

The amounts of radioactivity (per gram of wet weight) in the acid-soluble fraction are very similar in the different organs studied. This is not surprising in view of the finding that over 70% of the activity represents tritiated water. The somewhat lower activity found in the gastrointestinal tract may be attributed to the rinsing of stomach and intestine with saline which probably resulted in an erroneously high wet weight and elution of some tritiated water; on the other hand, the low activity of skin may be explained by its low water content.

The high radioactivities found in the DNA fraction of spleen and the gastrointestinal tract are in good agreement with the intense cell proliferation observed in these organs. On the other hand, the high activity found in DNA of fat tissue is rather surprising. The low activity of this fraction in skin might well be related to the intermittence of proliferative activity in the hair follicle of the adult mouse, which is characterized by bursts of intense activity lasting 2.5 weeks, separated by long periods of quiescence (12).

In general, the activities found in the lipid, RNA, and protein fractions are low. However, in the organs exhibiting low activities in DNA, *i.e.*, in brain, striated muscle, heart, skin, and liver, the relatively small amounts of label incorporated into non-DNA fractions represent a significant proportion of the total TCA-insoluble radioactivity. With the possible exception of liver and the gastrointestinal tract, the absolute radioactivities in RNA and protein, as expressed per gram wet weight, are of the same order of magnitude in all organs tested.

In conclusion, a high degree of specificity of thymidine-^{3}H is observed in cell systems with high proliferative activity. On the other hand, in systems with a low rate of cell renewal, a significant proportion of radioactivity is present in tissue components other than DNA after long-term administration of thymidine-^{3}H. Whereas, a high proportion of label incorporated into the acid-soluble fraction and into lipids is removed during routine histological procedures, this is usually not the case with RNA and protein. Since thymidine-^{3}H exhibits a high specificity as a precursor of DNA in systems with intense proliferative activity, it appears likely that incorporation of label from this precursor into DNA is highly specific in the vast majority of individual DNA-synthesizing cells. In autoradiographic studies, therefore, the relatively high uptake of thymidine-^{3}H by individual DNA-synthesizing cells may, in general, easily be distinguished from the low amounts of radioactivity incorporated into cellular constituents other than DNA by cells not engaged in DNA synthesis. In evaluation of cellular grain counts at low levels which are near background values will, however, be rather difficult after prolonged thymidine-^{3}H administration.

Summary. After 90 injections of thymidine-methyl-^{3}H into adult mice at intervals of 8 hr, the radioactivities of several biochemical fractions (acid-soluble material, lipids, RNA, DNA, and protein) were determined in homogenates of various organs. A high specificity of incorporation of labeled thymidine into DNA was observed in tissues characterized by a high rate of cell proliferation, while this specificity was of a lower degree in tissues exhibiting a low rate of cell proliferation.

With few exceptions, the amounts of radioactivity (per gram wet weight) in the fractions other than DNA were similar for all organs tested. Approximately 70% of label contained in the acid-soluble fraction were in

tritiated water, whereas no free thymidine-^{3}H could be detected 1 hr after the last injection of this precursor.

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