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Skeletal Cell Proliferative Rates: Studied Autoradiographically in Mice With Increasing Doses of Tritiated Thymidine (H^3TDR).* (32437)

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Several studies have shown that the β-radiation emitted from tritiated thymidine is capable of suppressing DNA synthesis, and producing mutagenesis and possibly carcinogenesis. Tritiated thymidine (H^3TDR) has been useful in evaluating the proliferative potentials of skeletal compartments during growth, aging, and trauma(24,25). The autoradiographic analysis depends on the assessment of the number of grains appearing in the emulsion over labeled cells, and this is in part related to the dose of radioactive isotope used, duration of exposure of the emulsion, as well as rate of uptake and proliferative rate. In our laboratory, as in many others, the usual H^3TDR dose of 0.5 μc/g body weight (Sp. Act. = 1.9 C/mM) and an emulsion exposure of 16 or 20 days is used to determine skeletal cell labeling indices (2,24). This low level of H^3TDR is preferred to avoid the possible depressive effect of the radioactive compound on cell proliferation (6). However, due to the lower autoradiographic efficiency, the labeling indices reported in the literature appear to be lower than the actual cell proliferative activity of a given skeletal tissue. The significance of this parameter in the assessment of labeling indices prompted us to study the possible acute dose effects of H^3TDR exposure on skeletal cell proliferation.

Materials and methods. A total of fifteen 4-to-5-weeks-old female mice of a Brookhaven National Laboratory inbred strain of Swiss albino were used in this experiment. The animals were divided into 5 groups which received the following doses of H^3TDR per gram body weight: 0.1 μc, 0.5 μc, 1.0 μc, 5.0 μc and 10.0 μc (Sp. Act. = 1.9 C/mM). All the animals were sacrificed one hour after subcutaneous injection of H^3TDR. Both femora were removed, fixed in 10% neutral buffered formalin, washed in water, and decalcified in versene prior to paraffin embedding. Five

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TABLE I. Tritiated Thymidine-Labeled Cellular Population of the Osteogenic Layer (Including Perichondrial Zone) of Distal Half of Mouse Femoral Periosteum.

Dose	0.1 μc of H^3TDR	0.5 μc of H^3TDR	1.0 μc of H^3TDR	5.0 μc of H^3TDR	10.0 μc of H^3TDR
Distal-half					
Labeling index	.0005	.022	.029	.045	.065
% Labeling	.05	2.2	2.9	4.5	6.5
No. of cells counted	1896	2475	4145	2599	3484

micron tissue sections were cut and prepared for autoradiography using NTB_3 Kodak liquid emulsion. The emulsions were exposed for 16 days in a cold, dry, atmosphere. Labeling indices of the periosteal and distal epiphyseal plate cell populations were determined. Cells of the perichondrial region were counted separately from the diaphyseal periosteal region, whereas cells of the proliferative and hypertrophic zones constituted the epiphyseal plate count. The labeling index represents the ratio of the number of labeled cells to the total cell population counted. Per cent labeling values are obtained by multiplying the labeling indices by 100.

Results. The data (Table I) show a progressive increase in the per cent labeling of the osteogenic layer of the periosteum (0.05 to 6.5%) as the dose of H^3TDR was increased from 0.1 μc to 10 μc. Similar findings are observed in Table II, with an expected higher per cent labeling seen over periosteal osteogenic cells at the perichondrial zone than over cells at the diaphysis. Levels of 1.0% (diaphyseal periosteum) and 3.3% (perichondrial zone) were obtained at a dose of 0.5 μc H^3TDR, while 4.0% and 9.0%, respectively, were obtained with a dose of 10 μc H^3TDR. The proliferative rate of the epiphyseal plate appears to be almost twice as high (15.8% and 16.6%) after an injected dose of 10 μc H^3TDR as the value (9.0%) obtained with a standard dose of 0.5 μc H^3TDR. A graphic representation of the data of Tables I, II, and III given in Fig. 1 shows that the largest increments in the labeling indices occur when the dose is increased from 0.1 μc to 1.0 μc H^3TDR per gram body weight (Figs. 2 and 3). Increasing the dose levels beyond this value produces further increases in labeling

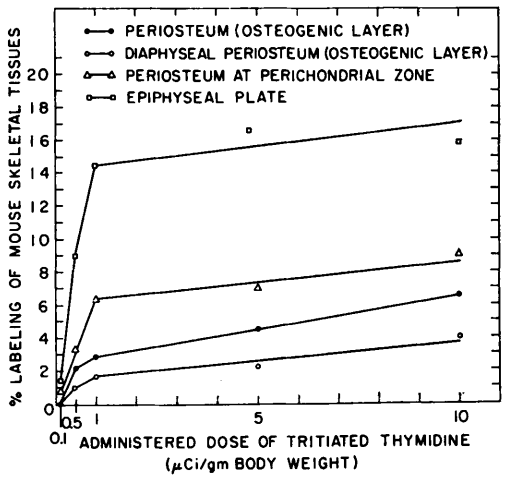

FIG. 1. Autoradiographic data consisting of the % labeling of mouse skeletal tissues 1 hr following administration of various doses of tritiated thymidine plotted for comparison.

TABLE II. Tritiated Thymidine-Labeled Cellular Population of the Osteogenic Layer of Diaphyseal Periosteum and the Perichondrial Zone of the Distal Half of Mouse Femora.

Dose	0.1 μc of H^3TDR	0.5 μc of H^3TDR	1.0 μc of H^3TDR	5.0 μc of H^3TDR	10.0 μc of H^3TDR
Diaphyseal periosteum					
Labeling index	.001	.010	.016	.022	.040
% Labeling	.1	1.0	1.6	2.2	4.0
No. of cells counted	2720	2241	2702	2206	3780
Perichondrial zone					
Labeling index	.008	.033	.63	.070	.090
% Labeling	.8	3.3	6.3	7.0	9.0
No. of cells counted	794	1218	1579	1226	1496

TABLE III. Tritiated Thymidine-Labeled Cellular Population of the Distal Epiphyseal Cartilage (Including Hypertrophic and Proliferative Zones).

Dose	0.1 μc of H^3TDR	0.5 μc of H^3TDR	1.0 μc of H^3TDR	5.0 μc of H^3TDR	10.0 μc of H^3TDR
Labeling index	.014	.090	.145	.166	.158
% Labeling	1.4	9.0	14.5	16.6	15.8
No. of cells counted	1442	1226	1825	1804	1751

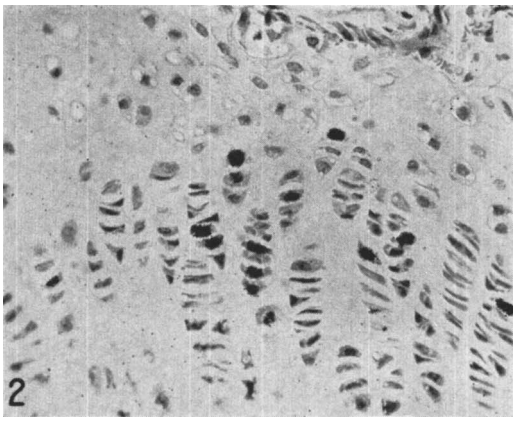

FIG. 2. An autoradiograph of the epiphyseal plate of the distal femur of a mouse treated 1 hr prior to sacrifice with 10 μc of tritiated thymidine and the emulsion exposed for 16 days. Note heavy labeling over chondrocytes. The low magnification allows for assessment of the geographic distribution of labeled cells. Hematoxylin stained. × 133.

indices; however, the increments are much smaller. Obviously, in cell populations whose labeling indices are normally low (diaphyseal periosteum) a change in the labeling index is more substantial in comparison to changes occurring in cell populations (epiphyseal plate cartilage cells) with normally higher labeling indices.

Discussion. The desirability of tritium (H^3), a radioisotope of hydrogen, for use in autoradiography stems from the unique physical properties of the element. Because of the pure low energy beta emission and consequently short path length in absorbers of low density, excellent autoradiographic localization is generally obtained. The beta spectrum of tritium exhibits a maximum energy of 18 KeV, and the particles have a maximum range in water of 6 to 7 μ. However, few β-particles exhibit this maximum energy. A larger percentage of particles fall into the average energy range of 5.6 KeV; having a range of only 1 μ in water (21). Thymidine, a precursor of DNA is localized within the cell

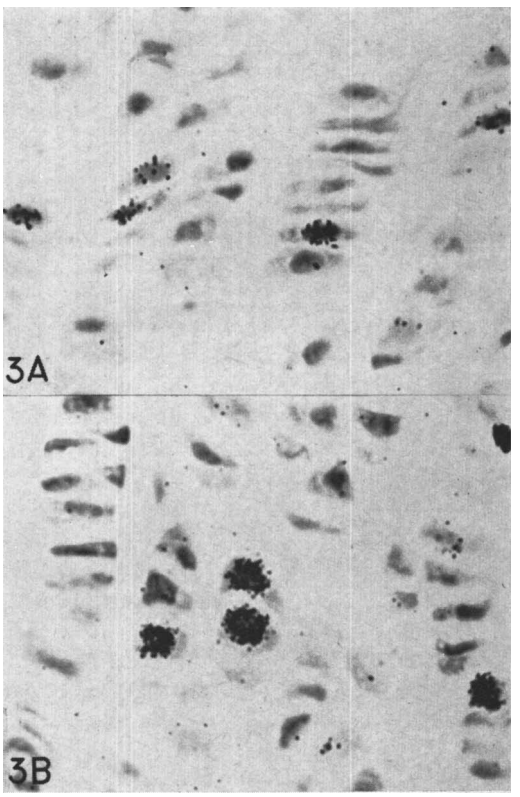

FIG. 3. Autoradiographs of the epiphyseal plate 16 days after exposure and illustrating the degree of labeling following administration of (A) 0.5 μc and (B) 10 μc of tritiated thymidine. Hematoxylin stained. × 398.

nucleus. Tritiated thymidine is taken up exclusively during the cell DNA synthesis phase and used extensively in cellular biology today(26). The accumulation of intranuclear label poses a serious problem to radio-sensitive nuclear elements. This problem has long been realized by investigators and numerous studies have been done to show that the radiation emitted from tritium will in fact suppress DNA synthesis, produce mutagenesis, and possibly carcinogenesis(3-6,8,9,14-20,23,30, 31). Johnson and Cronkite(8) demonstrated morphological injury to mouse spermatogonia

4 days post-injection with doses of 1.0 μc of H^3TDR/g of body weight (Sp. Act. = 1.0 C/mM) and larger. Grisham(6) also used H^3TDR (Sp. Act. = 360 mc/mM) injected in doses of 0.25 to 2.5 μc/g body weight. Animals whose livers had been traumatized prior to being injected revealed a depression of liver regeneration with dose levels of 1.0 μc and larger. Lisco(14) reported an increase in malignant tumors in 1-2-day-old and a few month-old CAF mice after a single intraperitoneal injection of 1.0 μc/g body weight (Sp. Act. = 360 mc/mM). The animals were followed over a period of 17 to 24 months.

Although all these authors point up the dangers of high doses of H^3TDR, the usual practice in our laboratory as elsewhere is to use a dose of 0.5 μc/g body weight for short periods of time prior to sacrifice. As indicated by Painter and Drew(17), HeLa cells which ordinarily show radiation damage at 0.02 μc/cc of H^3TDR after long exposure will show no damage when the limit of exposure is 30 minutes. Similarly, a one-hour "labeling index" does not give the cell which is in active DNA synthesis and which will presumably take up tritiated thymidine enough time to pass through mitosis, when apparently the effects of radiation damage can influence the data(18). Tonna(28) reported that in the periosteum the first labeled mitotic cells are seen as early as 1.5 hours post-injection. Therefore, using a "flash labeling" technique one can increase the dose of H^3TDR to 1.0 μc/g body weight in order to obtain a more realistic value for the labeling index of a given cell compartment without the fear of possible radiation damage affecting the data. The realization of higher labeling indices in skeletal cells concomitant with higher doses of H^3TDR occurs because of the improved autoradiographic efficiency. It must be kept in mind, however, that because of the short range of tritium β-particles, the overall geometric efficiency is low and variable(12). Even with high resolution methods only an overall efficiency of 2% is claimed for tissue sections, 5% for smears, and 20% for microorganisms(1,5,10,11,13,22).

Studies of skeletal growth, development, fracture repair and aging have been reported from our laboratory using a "flash labeling" technique with a 0.5 μc H^3TDR/g body weight and a 16 to 20 day emulsion exposure (20,26-29). Our present study has shown that the proliferative rate is higher than usually reported in skeletal tissues; namely, 6.5% *vs* 2.2% for the periosteum and 15.8% *vs* 9.0% for the cells of the epiphyseal plate. Previous studies have assumed a periosteal labeling index of about 2.9% in calculating the generation time of the osteogenic cells (7 to 19.4 days)(28). The present study would indicate a higher proliferative rate and therefore a shorter generation time in the order of 3.2 to 8.7 days. Kisieleski(11) demonstrated that labeling indices were constant in studies where increasing doses of H^3TDR were used in a highly proliferative system such as the testis. Similar findings were seen in the present study where labeling indices of the epiphyseal cartilage did not increase appreciably beyond a dose of 1.0 μc of H^3TDR. However, in the periosteum where there is a lower proliferative rate small changes reflect a larger per cent variation in the labeling index. The observed rapid initial rise in labeling indices with increasing doses of H^3TDR, followed by a much slower increase, is believed to indicate existing wide time-variations in the cell cycle of a given cell population. It can be seen from the present study that a 1.0 μc dose of H^3TDR can be used in autoradiographic studies employing "flash labeling" techniques to determine labeling indices. However, we do not recommend the use of doses of 1.0 μc and above in studying cell migration or cell kinetics because of the inherent dangers of intranuclear irradiation. Where the use of lower doses of tritiated thymidine is expedient, one may increase the autoradiographic exposure time until the 1.0 μc dose "flash labeling" level is reached, provided background levels remain insignificant.

Summary. An autoradiographic study of mouse skeletal cell proliferation rates using increasing doses of H^3TDR were reported. The data showed that in both the osteogenic cells of the femoral periosteum and epiphyseal plate the labeling indices increased rapidly with increasing doses of H^3TDR to 1.0 μc/g

body weight and less rapidly with larger doses. It was concluded from this study that previously reported estimates of labeling indices based on lower doses of administered H^3TDR are lower than the actual values. A dose of 1.0 μc H^3TDR/g body weight is recommended for the estimates of labeling indices using the "flash labeling" technique. However, doses of 1.0 μc and above are not to be used for cell migration or cell kinetic studies.

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Influence of Positive Inotropic Agents on the Action of a Myocardial Depressant Factor in the Plasma of Cats in Postoligemic Shock.* (32438)

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Plasma of cats in irreversible postoligemic shock contains a myocardial depressant factor (MDF) which depresses the developed tension of isolated cat papillary muscles(1,2,3). The myocardial depression produced by MDF was completely reversed when the shock

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