Inhibitory Effect of Tritiated Thymidine on Regeneration of the Liver in the Young Rat.*‡ (26174)

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Since its introduction, tritiated thymidine (H^3T) has had wide application in the study of proliferating tissue(1). Because it is concentrated in the cell nucleus during synthesis of deoxyribose nucleic acid (DNA) and because most of the radiant energy which it emits is absorbed by the nucleus(2), it is possible that the latter could be adversely affected under some conditions. Cytologic changes attributed to radiation have been noted in HeLa cells grown in fluid containing H^3T(3) and recently in spermatogonia of mice injected with H^3T(4). Evidence is reported here that regeneration of the liver of the young rat is transiently depressed, as indicated by rate of increase of residual liver weight, time sequence of mitotic rate, and presence of abnormal mitoses, by doses of H^3T that are considered to be within the generally accepted tracer range.

Methods. It was determined that after a 12-hour fast the livers of a group of 20 male, Wistar albino rats (60 to 90 g) constituted 4.26 ± 0.10% of their total body weight. Partial hepatectomy(5) was performed on litter mates after a similar fast and the portion of liver removed was blotted on filter paper and weighed. From the ratio of liver weight to body weight previously determined, amount of residual liver was calculated. Times of operation were adjusted so that all animals were killed between 7 and 9 a.m. when mitotic rate is relatively high and constant(6). After operation animals were individually caged and fed Purina chow *ad lib.* H^3T§ (specific activity 360 mc/mM) was given intraperitoneally in doses of 0.25 to 1.0 μc/g body weight (diluted in normal saline solution to a volume of 2.0 ml) at 20 hours following hepatectomy, a time when the liver is maximally synthesizing DNA(7). Control animals were injected with equivalent amounts of nonradioactive thymidine. Groups of animals (6 to 10 per group) were killed at 6, 12, 18, 24, 36, 48 and 72 hours after receiving thymidine. Two additional groups received 1.5 and 2.0 μc H^3T/g body weight at 20 hours after hepatectomy and were killed 24 hours later. A third group was given 1.0 μc H^3T/g body weight every 12 hours, beginning 20 hours after hepatectomy and ending 60 hours later when they were killed. Residual liver was removed, blotted on filter paper, and weighed. Portions of the liver were fixed in 10% neutral buffered formalin for histological preparations. Amount of hepatic regeneration was determined from amount of calculated residual liver at time of operation and amount of liver present at time of death and expressed as per cent increase of residual liver. Corrections were made for changes in body weight. Histological sections were stained by the Feulgen method and with hematoxylin and eosin. Mitotic figures were counted in 6 μ thick, Feulgen-stained sections and expressed as mitoses/100 parenchymal cells. At least 200 mitotic figures or 10,000 nuclei were counted for each animal. Abnormal mitotic figures were defined as bridged anaphase figures(8) and expressed as per cent of total anaphase figures. At least 50 figures were counted for each animal. Autoradiographs were prepared by the dipping technic with melted nuclear track emulsion.‖

Results. Animals receiving nonradioactive thymidine showed an approximately straight-line rate of regeneration for the entire time period studied when corrections were made for changes in body weight (Table I). The mitotic rate reached a peak 26 hours after

* Supported by U.S.P.H.S. grant C-2548.

† Life Insurance Medical Research Fund Fellow.

‡ Portions of this work were read before the Am. Assoc. for Study of Liver Disease, Chicago, Ill., Nov. 5, 1958.

§ Schwarz BioResearch, Mt. Vernon, N. Y.

‖ Eastman Kodak Co., Rochester, N. Y. Nuclear Track Emulsion, NTB-3.

TABLE I. Per Cent Increase in Residual Liver as a Function of Amount of Tritiated Thymidine Given.

Time (hr) after hepatectomy	μc H^3T/g body wt						
	0	.25	.5	1.0	1.5	2.0	5.0*
6†	5.9 ± 1.0‡	—	—	—	—	—	—
12	10.1 ± 1.3	—	—	—	—	—	—
20	18.4 ± 1.6	—	—	—	—	—	—
26	22.0 ± 1.4	23.2 ± 1.6	20.2 ± 1.5	17.4 ± 1.5	—	—	—
32	26.0 ± 1.2	24.6 ± 1.7	22.4 ± 1.8	18.0 ± 1.6§	—	—	—
38	30.4 ± 1.3	31.5 ± 1.7	27.6 ± 1.9	22.2 ± 1.8§	—	—	—
44	39.0 ± 1.5	42.3 ± 1.8	35.4 ± 1.9	26.2 ± 2.0§	22.8 ± 2.2§	21.5 ± 2.5§	—
56	43.0 ± 2.0	45.1 ± 2.2	39.5 ± 2.0	34.2 ± 2.4	—	—	—
68	52.1 ± 2.6	53.0 ± 3.1	48.7 ± 2.7	43.5 ± 2.5	—	—	—
80	59.6 ± 2.5	62.2 ± 3.2	54.6 ± 3.4	52.1 ± 2.8	—	—	40.8 ± 3.4§
92	69.7 ± 3.0	71.6 ± 3.4	64.2 ± 3.2	65.6 ± 2.4	—	—	—

* Given in 5 divided doses, 1 μc/g body wt every 12 hr beginning at 20 hr after hepatectomy.
† Each group contains 6-10 animals.
‡ Mean ± stand. error of mean.
§ Indicates P equal to or greater than .01 when compared to control value.

hepatectomy, then declined quickly (Table II). In control animals at 26 hours after hepatectomy, 4.7 ± 1.0% of anaphase mitotic figures were abnormal (Table III).

In animals receiving 1.0 μc H^3T/g body weight, replacement of liver mass at 12, 18 and 24 hours after injection was significantly depressed. The inhibition was transitory and by 36 hours after injection the weights of residual liver were not significantly different from the controls (Table I). In animals receiving 1.5 and 2.0 μc H^3T/g body weight, replacement of liver mass was also depressed 24 hours after injection, the only time interval measured. Six hours after injection of 1.0 μc H^3T/g body weight the mitotic rate was no greater in hepatectomized animals than in nonhepatectomized controls. A rebound peak was reached 24 hours after injection and the number of mitoses continued to be significantly elevated above control values for the entire period under study (Table II). At 44 hours after hepatectomy, 28.2 ± 4.8% to 33.4 ± 7.9% of the anaphase mitotic figures were abnormal in animals receiving 1.0 to 2.0 μc H^3T/g body weight (Table III). Autoradiography showed 70 to 90% of the abnormal mitotic figures to be radioactive.

In animals receiving 1.0 μc H^3T/g body weight every 12 hours between 20 and 80 hours after hepatectomy, the residual liver was significantly smaller than in the controls,

TABLE II. Mitotic Rate in Residual Liver as a Function of Amount of Tritiated Thymidine Given.

Time (hr) after hepatectomy	μc H^3T/g body wt						
	0	.25	.5	1.0	1.5	2.0	5.0*
0†	.15 ± .10‡	—	—	—	—	—	—
6	.20 ± .07	—	—	—	—	—	—
12	.40 ± .15	—	—	—	—	—	—
20	.90 ± .20	—	—	—	—	—	—
26	3.31 ± .41	3.10 ± .34	2.44 ± .32	.80 ± .20§	—	—	—
32	2.40 ± .30	2.56 ± .40	2.85 ± .44	.99 ± .23§	—	—	—
38	1.52 ± .31	1.82 ± .31	2.20 ± .40	1.92 ± .32	—	—	—
44	1.10 ± .15	1.43 ± .22	1.60 ± .34	3.79 ± .45§	3.20 ± .55§	3.00 ± .60§	—
56	.80 ± .13	1.02 ± .20	1.20 ± .25	2.60 ± .32§	—	—	—
68	.70 ± .15	.85 ± .16	.90 ± .20	2.23 ± .25§	—	—	—
80	.60 ± .12	.70 ± .10	.75 ± .15	2.02 ± .22§	—	—	2.40 ± .42§
92	.50 ± .15	.55 ± .12	.70 ± .13	1.65 ± .20§	—	—	—

* Given in 5 divided doses, 1 μc/g body wt every 12 hr beginning at 20 hr after hepatectomy.
† Each group contains 6-10 animals.
‡ Mean ± stand. error of mean.
§ Indicates P equal to or greater than .01 when compared to control value.

TABLE III. Per Cent Abnormal Anaphase Figures as a Function of Amount of Tritiated Thymidine Given.

Time (hr) after hepatectomy when anaphase figures counted	μc H^3T/g body wt						
	0	.25	.5	1.0	1.5	2.0	5.0*
26†	4.7 ± 1.0‡	5.6 ± 1.4	9.6 ± 3.7	—	—	—	—
44	—	—	—	28.2 ± 4.8§	30.8 ± 5.2§	33.4 ± 7.9§	—
80	—	—	—	—	—	—	43.5 ± 7.8§

* Given in 5 divided doses, 1 μc/g body wt beginning at 20 hr after hepatectomy.
† Each group contains 6-10 animals.
‡ Mean ± stand. error of mean.
§ Indicates P equal to or greater than .01 when compared to control value.

the mitotic rate was much higher, and 43.5 ± 7.8% of the anaphase figures were abnormal.

Discussion. The regenerating liver of the rat subjected to partial hepatectomy is a mitotically active tissue, surpassing at certain stages in its growth any other normal adult tissue, embryonic tissue, and many neoplasms (9). Cater, *et al.*(10) have shown the regenerating liver to be very sensitive to radiations. They detected mitotic abnormalities with the smallest dose of x-rays used in their study (150 r).

Irradiation of the cell during certain stages of the mitotic cycle dissociates DNA synthesis from mitosis by blocking the latter(11). In our animals, the radioactive material was incorporated during synthesis and labeled cells were apparently prevented from entering mitosis for a variable period of time. The depression of cell division was also associated with some slowing of regeneration of liver mass as compared to controls. About 12 to 24 hours after the rats received the injection of H^3T, the cells began to recover their mitotic ability but did not reach a peak near that of the controls until some 12 hours later. The mitotic rate remained elevated at a time when that in control animals had already returned to normal. This rebound phenomenon is typical of mitotically active tissue in response to sublethal doses of radiation(8). Associated with the reappearance of mitoses were an increased number of mitotic abnormalities as indicated by anaphase bridges. Brues(8) has discussed the difficulty of using bridged figures as an indication of radiation damage in liver since normally there is an appreciable incidence of mitotic aberrations associated with regeneration. This normal incidence we found in the controls, but the level in the treated animals was clearly higher and significantly elevated, as has been found by Albert(12) in radiation depression of hepatic regeneration in mice and by Cater, *et al.* (10) in rats. Between 70 and 90% of the abnormal mitoses were radioactive, therefore the nonradioactive abnormal mitoses were similar in number in both groups.

After reestablishment of mitoses in the treated group, liver mass increased until 72 hours after injection when it reached control levels. Animals receiving 1.0 μc H^3T/g body weight have been followed for long intervals and no further abnormality has been noted. Apparently the damage is transitory and the irradiated hepatic cells either recover completely or die and are replaced by normal cells.

Such changes are extremely important in attempting to study temporal relationships of DNA synthesis and mitosis. For instance, we have observed then when 0.2 to 0.3 μc H^3T/g body weight is given to rats at intervals after hepatectomy and shortly before they are killed, rate of appearance of labeled cells and mitotic rate parallel each other with the former preceding the latter by about 6 to 8 hours. However, when 1 μc H^3T/g body weight or more is given the rate of appearance of mitoses is delayed for 6 to 12 hours and the curve is skewed to the right. Obviously, it is necessary to use a dose of H^3T that does not cause mitotic inhibition for a study of this type to have validity. Doses of 0.3 μc H^3T/g body weight in rats or mice give adequate labeling for autoradiography, and to

prevent the complication of possible radiation damage, larger amounts should not be used. This is especially important in studies in which subsequent development or movement of the labeled cells is to be followed.

Summary. Tritiated thymidine, when given to young male rats 20 hours following partial hepatectomy in doses of 1.0 μc/g body weight or greater, causes a transient inhibition of regeneration as mirrored in rate of increase of residual liver mass, mitotic rate, and incidence of abnormal mitotic figures. The importance of this effect in tracer studies is obvious.

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Received October 5, 1960. P.S.E.B.M., 1960, v105.

Action of Angiotensin on Isolated Guinea Pig Ileum. (26175)

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Earlier studies(1,2) with angiotensin (hypertensin, angiotonin) indicated a direct spasmogenic action on smooth muscle. A recent report(3) suggests that on rabbit and guinea pig ileal strips, angiotensin may have an indirect action in producing contraction. The present study attempts to delineate the site of action of angiotensin in guinea pig ileum by use of pharmacologic blocking agents. The results suggest that angiotensin produces contraction by acting on the post-ganglionic cholinergic mechanism. They also indicate that care should be taken in interpreting such results and that a number of blocking agents of each class should be examined over a wide range of concentrations before conclusions are reached.

Materials and methods. Freshly prepared strips of guinea pig ileum were suspended in duplicate 10 ml baths of aerated Tyrode's solution maintained at 37°C. The responses were recorded on soot-paper by frontal writing levers. Maximal contractions were induced at 4-minute intervals by exposing the tissues to 0.05 γ/ml of angiotensin* for 60 seconds and to 1 γ/ml of nicotine for 30 seconds. The antagonists were added midway between contractions allowing 2-minute exposure of the tissue. Three or more concentrations of each antagonist were studied to obtain dose-response curves. The EC_{50}'s, concentration effecting a 50% inhibition of the contractions, were obtained from graphic plots. Each of the antagonists was studied on 6 ileal strips. The results are presented in Table I.

Results and discussion. The data establish that the spasmogenic actions of angiotensin and nicotine on the ileum are effectively blocked by low concentrations of atropine and morphine. Emmelin and Feldberg (4) have shown that atropine effectively blocked the action of nicotine on the gut while Schaumann(5) and Paton(6) have

* We are indebted to Dr. Frederic Tausig, Research Laboratory Divison, Merck and Co., Inc., Rahway, N. J., for preparing and supplying angiotensin octapeptide.