lin. In common duct stone additional activity in the albumin fraction was a consistent finding. In early pregnancy the electrophoretic pattern of LAP was similar to that observed in normal serum, whereas during the third trimester LAP activity was demonstrable only in the α_2-globulin.

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Further Observations on Induction of Tumors in Mice with Radioactive Thymidine.* (27618)

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We have reported(1) preliminary results of an experiment on induction of tumors in mice with radioactive thymidine. It was shown that tumors could be induced in mice with a single injection of tritiated thymidine at a dose level of 1.0 μc/g of body weight. These studies have been continued and the earlier results confirmed and extended. We wish to summarize here all of the presently available data, including observations on mice injected with thymidine labeled with carbon-14 and other data not mentioned earlier.

Four aspects of tumor induction and life shortening by radioactive thymidine were studied as follows (Table I): 1. Effect of tritium labeled thymidine as a function of age of the animal at the time of injection (Groups 1-3); 2. Effect of tritium labeled thymidine as a function of dose and dose-fractionation (Groups 5-8); 3. Effect of carbon-14 labeled thymidine as a function of dose and dose-fractionation (Groups 11-14); 4. Effect in offspring of mice injected with tritium labeled thymidine during pregnancy (Group 10).

Methods. F_1 hybrids of C and A mice, whose lines have been maintained at Northwestern University for more than 5 years, were housed in plastic cages, in air-conditioned quarters, and fed Rockland Mouse Diet and water. The H^3-thymidine (New England Nuclear Co., Boston, Mass.) had a specific activity of 360 mc/mM, while the thymidine-2-C^{14} (New England Nuclear Co.) had a specific activity of 3.0 mc/mM. Non-radioactive thymidine, M.W. 242.2, was obtained from the California Corp. for Biochemical Research, Los Angeles. The required amount of thymidine injected was dissolved in 0.1 ml of sterile water per mouse. Adult mice were injected intraperitoneally, and newborn mice subcutaneously in the suboccipital region, at the dose-levels of thymidine indicated in Table I. An additional group of mice consisted of the offspring of pregnant mice injected with 250 μc of H^3-thymidine between the 15th and 17th day of pregnancy. All animals received a single injection of radioactive thymidine, with the exception of Groups 8 and 14, which received thymidine in 6 fractionated doses spread over a period of 8 days. Control mice were in-

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TABLE I. Number of C × A F_1 Mice in Each Experimental Group, Treatment and Age at Time of Treatment.

Group No.	No. of mice	Treatment (μc/g body wt)		Age at time of treatment
1	100	H^3-thymidine	1.0	12-14 mo
2	99	"	1.0	6 "
3	100	"	1.0	2 "
4	100	Controls*		—
5	85	H^3-thymidine	.1	newborn
6	101	"	1.0	"
7	85	"	10.0	"
8	113	"	10.0†	"
9	383	Controls*		—
10	176	H^3-thymidine	1.0‡	embryos§
11	65	C^{14}-thymidine	.02	newborn
12	63	"	.2	"
13	74	"	2.0	"
14	75	"	2.0†	"
15	464	Controls*		—

* Inj. either with saline or with non-radioactive thymidine.
† In 6 injections over a period of 8 days.
‡ Estimated.
§ Offspring of pregnant mice inj. with 250 μc of H^3-thymidine during pregnancy.

jected either with saline or with non-radioactive thymidine. No difference was noted between the 2 control groups and they are therefore combined in the results.

The animals were allowed to live out their life span. They were weighed once weekly and examined for the presence of tumors. At autopsy, tissue samples were taken of all relevant lesions, especially of the tumors. All final diagnoses were based on histologic examination.

Results. It was not feasible to begin all phases of this work at the same time. This accounts for the difference in the length of observation periods for the various groups as seen in Tables II-III. The tables give the number of surviving mice and tumor incidences in the various groups at various intervals from the beginning of experiment. In calculating the tumor incidence only malignant tumors were considered. There were no appreciable differences in mortality rate or in incidence of tumors between males and females.

Table III shows that the 3 groups of experimental animals injected with H^3-thymidine as adults have a higher incidence of tumors than control animals. The differences, at 27 months of age, were statistically significant at the 1% level for each experimental group. Furthermore, mice that were 2 months old at time of injection have a higher incidence of tumors than animals injected when 6 or 12 months old. Tables II and III show that mice injected as newborn with 1.0 and 10.0 μc of H^3-thymidine per gram of body weight have, at 24 months of age, a higher mortality rate and a higher incidence of tumors than control mice. The differences between each experimental group and the control group are statistically significant at the 1% level. A dose-level of 10.0 μc is more effective in reducing the life span or increasing the incidence of tumors than a dose level

TABLE II. Number of Surviving C × A F_1 Mice at Various Intervals after Beginning of Experiment.

Group No.	No. of mice	Survivors at age (in mo) 2	4	6	9	12	15	18	21	24	27
1	100						100	95	89	76	38
2	99			99	99	98	96	92	78	55	35
3	100		100	100	100	97	95	91	76	61	38
4*	100		100	100	100	100	98	94	77	59	29
5	85	85	85	85	84	83	79	77	67	49	
6	101	100	99	99	96	95	91	87	80	46	
7	85	82	82	82	82	80	78	70	53	36	
8	113	110	110	108	106	105	98	74	52	47	
9*	383	382	382	381	380	376	367	354	300	220	
10	176	176	175	175	174	167	162	155	133		
11	65	63	63	63	63	63	59	49			
12	63	62	62	61	61	59	59	52			
13	74	74	74	74	74	74	72	63			
14	75	75	75	75	72	71	67	63			
15*	464	463	463	462	460	455	442	421			

* Controls.

TABLE III. Number of C × A F_1 Mice with Tumors at Various Intervals after Beginning of Experiment (Cumulative Incidence).

Group No.	No. of mice	No. of animals with tumors at age (in mo) 2	4	6	9	12	15	18	21	24	27
1	100						0	2	4	12	35
2	99			0	0	0	0	5	11	22	35
3	100		0	0	0	0	1	3	13	25	44
4*	100		0	0	0	0	0	0	5	10	20
5	85	0	0	0	0	0	0	0	4	10	
6	101	0	0	0	1	2	2	2	6	28	
7	85	0	0	0	0	1	2	5	13	19	
8	113	0	0	0	3	3	3	10	21	29	
9*	383	0	0	0	1	2	2	3	20	50	
10	176	0	0	0	2	5	6	9	18		
11	65	0	0	0	0	0	0	1			
12	63	0	0	0	0	0	0	2			
13	74	0	0	0	0	0	2	3			
14	75	0	0	0	2	3	3	4			
15*	464	0	0	0	1	2	3	6			

* Controls.

of 1.0 μc. Moreover, 10.0 μc is more effective when given in 6 doses over a period of 8 days than when given as a single dose. The animals injected with a dose of 0.1 μc of H^3-thymidine per g of body weight have so far failed to show an increased mortality rate or tumor incidence.

Tables II and III show that mice injected as newborn with 0.2 or 2.0 μc of C^{14}-thymidine per gram of body weight have, at 18 months of age, a higher mortality rate and higher incidence of tumors than control mice. The differences are statistically significant at the 1% level. A dose-level of 0.02 μc of C^{14}-thymidine per g of body weight has, thus far, failed to increase the incidence of tumors above the level observed in control mice, although the death rate has been noticeably high. In the fourth part of the experiment, (Group 10), there were 176 male and female mice, the offspring of pregnant mice injected with 250 μc of H^3-thymidine between the 15th and 17th day of pregnancy. Of these animals 24.5% are dead at 21 months and tumor incidence is 10%. This is significantly higher than the tumor incidence of control mice at the corresponding age.

The tumors in the control animals were almost entirely lung tumors, and a few disseminated non-thymic lymphomas. For example, of the 50 tumors in the controls of Group 9, 31 were lung tumors, 16 non-thymic lymphomas, 1 thymic lymphoma and 2 subcutaneous sarcomas. Among the experimental animals, a greater variety of tumors was observed. For example, among the 76 tumors in Groups 6-8, 41 were lung tumors, 19 non-thymic lymphomas, 4 thymic lymphomas, and 12 other tumors including sarcomas of the subcutaneous tissue, carcinomas of the salivary glands, of the skin and of the liver. The present data give evidence that administration of radioactive thymidine has not only accelerated the appearance of tumors occurring spontaneously in this strain of animals, but has, in fact, induced some types of tumor *de novo*.

Discussion. The data can be summarized by stating that: both thymidine labeled with tritium and with carbon-14 have been found to shorten the lifespan and to produce tumors in mice; incidence of tumors is greater in animals injected as young adults than in animals injected later in life; incidence of tumors increases with dose; and finally, that labeling of embryos *in utero* with tritiated thymidine likewise increases the incidence of tumors.

It was pointed out earlier(1) that the aim of our study has been to explore the possibility of inducing tumors in mice with a mode of irradiation that affects primarily the cell nucleus (tritiated thymidine) or whole cells (carbon-14 labeled thymidine). It has now been demonstrated that both materials are effective in doing this under a variety of

experimental conditions.

Thymidine, as a precursor of DNA, is exclusively incorporated into the nucleus of cells synthesizing DNA prior to mitosis(2). At least 50% of the injected thymidine is incorporated into new DNA of proliferating cells, while the remainder is promptly catabolized to tritiated water and other non-volatile compounds(3). The biological half-life of the tritiated water in mice is from 24 to 36 hours(4), while the thymidine incorporated into DNA remains there until the death of the cell or its descendants. Because of the short range of the tritium beta particles (in tissues, an average of 1 μ and a maximum of 6 μ) 90% of the energy of the beta particles from H^3-thymidine is dissipated within the nucleus. On the other hand, beta particles from C^{14} have a longer range (in tissues, an average of 10 μ and a maximum of 60 μ) and most of their energy will be dissipated within the volume of a cell. Unless the carcinogenic action of radioactive thymidine is ascribed to one of its catabolites that are eliminated from the body in a few days, it would seem that radiation dissipated within the volume of a cell and perhaps even in the nucleus of a cell is sufficient to induce tumors.

Summary. Thymidine labeled with tritium or with carbon-14, when incorporated into DNA of proliferating cells, produces a selective mode of irradiation of cells and cell nuclei. Single and multiple injections of radioactive thymidine have been found to shorten the life span and to produce an increased incidence of tumors in CaF1 mice. The incidence of tumors increased with dose and it was greater in animals injected as young adults than in animals injected later in life. Labeling of embryos *in utero* with tritiated thymidine likewise increased the incidence of malignant tumors.

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A Preliminary Investigation of the Source of the Creatinuria Following Transection of the Spinal Cord. (27619)

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In our studies on the metabolic changes following spinal cord transection in dogs, it was observed that creatine was excreted in large amounts for periods of from 2 to 3 weeks after the cervical cord had been transected(1). This was in marked contrast to the slight creatinuria which occurred for a short period only in animals immobilized by removal of femurs and humeri or by cutting muscle tendons. Creatinine excretion was not altered immediately following cord transection, but fell slowly over a period of a month as the muscles affected by the cord transection atrophied. The high creatinuria set in before creatinine excretion showed any reduction. One of the many questions raised in connection with these studies was whether the creatine in the urine came from the muscles affected by the cord transection or whether it was newly synthesized creatine which the affected muscles could no longer use. To answer this question, labeled creatine was fed to a dog a few days before cervical cord transection and excretion of creatine and creatinine followed over a period of 6 weeks. Since the classical studies of Bloch

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