TABLE II. Distribution of Mice and Mitotic Rates of Mice that Died or Survived.

															Total
No. of mice dying	5	5	2	5	5	3	7	1	2	6	1	2	0	1	46
Mitotic count	0-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	
No. of mice surviving	10	5	4	1	0	2	1	1	0	0	0	0	0	0	24

tage might well be taken of the influence of diurnal variation in therapeutic utilization of X-radiation, for it is generally known that neoplasms do not undergo the degree of diurnal variation in mitotic activity that normal tissues do, remaining instead at a relatively high level.

Summary. A correlation is demonstrated between mitotic rate of individual mice at time of exposure and lethal effect of X-irradiation. The significance of this finding is discussed.

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Deposition of C^{14}-Labelled Cholesterol* in the Atheromatous Aorta.† (26068)

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This laboratory has been concerned with the relationship between cholesterol metabolism(1,2,3,4) and development of atheromatous conditions in patients. During investigation of the "biological halflife" of C^{14}-cholesterol in various patients, it was felt that the aorta might be permeable to circulating tagged cholesterol. For this reason 5 terminal patients received intravenous C^{14} tagged cholesterol and the aortas were assayed post-mortem to determine amount of C^{14}-cholesterol in various parts of the aorta, especially near the atheromatous plaques.

Methods. Radioactive labelled cholesterol-4-C^{14} (1-3 mg) obtained from a commercial source was dissolved in a minimal quantity of ethanol (0.3 ml). This cholesterol was introduced by use of a micro-syringe into a sterile ampule containing 2-3 ml of human serum albumin solution and a sterile glass-covered magnetic stirrer. While the stirrer was rotated by means of an external magnet, the alcoholic-cholesterol solution was slowly added. The sterile suspension of cholesterol in human serum albumin was used immediately after preparation(5). (The preparation has a tendency to settle after long standing.) This preparation containing the cholesterol (with 15 to 20 μc C^{14} radioactivity) was injected slowly (10 to 15 minutes) into the antecubital vein of selected patients. Terminal patients with life expectancy of from 2 to 10 days were selected for this study. These patients were aged and had clinical evidence of arteriosclerosis. Postmortem examination revealed that all patients had well defined atheromatous plaques. Some of these plaques, in addition to being calcified, had adjacent ulcerations. Samples of the aorta of each individual were carefully obtained by use of a metal punch. Disks were obtained at intervals throughout the length of each aorta, representing an even

* All radioactive materials were obtained on allocation from U. S. Atomic Energy Comm.

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distribution from upper to lower aorta (2 disks from the arch, 4 from thoracic region and 4 from abdominal region). Each disk was then dissected and the intima, media and adventitia separated. The separated intima and media sections of each disk were homogenized, treated with metaphosphoric acid and extracted continuously with ether in an automatic continuous extractor under nitrogen. The ether extracts were taken to dryness and the oil obtained dissolved in ethanol and treated with 1% solution of digitonin. The cholesterol digitonide obtained was repeatedly washed with the aid of centrifugation with acetone, ether and petroleum ether. The purified cholesterol digitonide was dissolved in methanol and assayed for radioactivity in a Packard "Tri-Carb" spectrometer with the aid of carbo-cell and a suitable phosphor (Popp). Blanks for radiometric assays were performed by homogenization of both intima and media disks of non-radioactive aortas similarly obtained. The results have also been corrected for background. Counting was done for a sufficiently long period to ensure a ± 0.3% counting error per determination. This probable per cent error has been determined by counting each sample to 30.000 total counts when the background was 25 counts/min(6).

Results. In the media portion of the aorta of the patients studied, the recovered cholesterol showed a radiometric value approximately equal for each sample disk tested throughout length of aorta. The recovered cholesterol from the intima of the aorta gave a total radioactive value (counts/min.) for the region of the arch of the aorta that was slightly higher than for the disks obtained from other regions of the same aorta. In the areas where the punch included a large atheromatous plaque, values obtained showed a lower radioactivity value, thus indicating a lower exchange rate between cholesterol circulating and in the plaque. Values for patient A are presented in Table I. Results for radioactive assays of the disks of the other 4 patients were in all respects comparable to those obtained for patient A. In Table II are depicted average results obtained for the 5 patients studied. Our results are in some respects similar to those reported by Hollander(7). Cholesterol absorption in dog aorta has been reported to show a sharp reducing gradient going from thoracic to abdominal region. This gradient has also been observed in this study with human aortas. This gradient, however, was very gentle as seen by data in Table I. These results may be a consequence of a slower equilibration rate for the human aorta than the one observed in dog aortas(8).

TABLE I. Radioactivity Recovered* in Aorta of Patient "A" (Received 3-C^{14}-cholesterol 2 Days Prior to Expiration).

One inch diameter disks from aorta				Recovered radioactivity in cholesterol;† total cpm in disk	
Sequence No.		Aorta region	Atheromatous condition	Intima	Media
1	↑	arch	mild	875	380
2		"	severe	973	420
3		thoracic	"	812	430
4		"	medium	735	400
5	16"	"	plaque	239	530
6		"	medium	653	480
7		abdominal	"	685	435
8		"	"	227	385
9		"	"	295	430
10	↓	"	medium	842	480

* From 20 repeat determinations on aliquots of the same sample a spread of ± 12% was found for the experimental technics used.

† Cholesterol-digitonide precipitable material.

The results conclusively show that circulating cholesterol can exchange with the available cholesterol pool of the aorta. Exchange or deposition on calcified structures (plaques) was slower than that found in non-calcified fatty deposits of the human aorta. The data were found to be significant for the assay used when submitted to standard statistical

TABLE II. Average Radioactivity Recovered in Aortas of 5 Patients (Received 3-C^{14}-cholesterol 2-10 Days Prior to Expiration).

One inch diameter disks		Recovered radioactivity in cholesterol; total cpm in disk	
No. of disks	Aorta region	Intima ± S.E.M.*	Media ± S.E.M.*
10	arch	895 ± 25	430 ± 35
15	thoracic area	780 ± 37	400 ± 50
15	abdominal "	730 ± 50	380 ± 55
15	with plaques	240 ± 80	390 ± 66

* Stand. error of mean.

analysis (calculation of standard error of mean and Chi square analysis.)

Summary. The results suggest that circulating cholesterol exchanges with available cholesterol pools of intima of human aorta. This exchange is slow. Cholesterol in the atheromatous plaques appears to exchange or accept circulatory cholesterol with the greatest difficulty. The highest exchange or depositions were observed for area of the arch of human aorta; abdominal part of aorta showed a smaller exchange. The media showed about the same cholesterol rate of exchange through length of aorta. A disk technic suitable for various types of quantitative assays of intima, media, etc. of the aorta was described.

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Uptake and Excretion of Radioactive Rose Bengal Dye in Normal Dogs.* (26069)

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Delprat demonstrated that rose bengal dye (tetra-iodo-tetra-chlorfluorescein) is rapidly concentrated by liver cells and subsequently excreted into the biliary tract(1). Since the initial studies on radioiodine^{131}-tagged rose bengal dye by Taplin and his associates(2), various investigators have utilized this substance for liver scintiscanning as a diagnostic procedure for detection of tumors, cysts and abscesses within the liver(3,4). Although initial studies on nonradioactive rose bengal dye suggested that little or no dye is concentrated by spleen, kidney or pancreas(1,5), the possibility remained that minute amounts might be taken up by these organs. Our studies were stimulated by a recent liver scan performed at this hospital in which a faint area of radioactivity was noted over the left upper quadrant of the patient's abdomen. Employing the radioactive form of the dye on rats, Glaser and co-workers recently concluded that "no large portion of the injected dye could be observed outside of the liver, blood stream, or intestinal tract(6)." To assess the role of various abdominal organs in concentration and excretion of even trace amounts of rose bengal dye, studies in normal dogs were undertaken. Our experimental findings indicate that in normal dogs uptake and excretion of the dye occur mainly through the hepatobiliary system and that pathways through other organ systems are negligible.

Method. Mongrel dogs, weighing from 5 to 10 kg, were anesthetized with 30 mg/kg of sodium pentothal. Using aseptic technic, the abdominal cavity was entered through a midline incision. During period of anesthesia, the dogs were maintained on a constant intravenous infusion of 5% dextrose in water. The animals were given 10% dextrose in water intravenously in experiments in which the urine was collected in order to assure an

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