for polishing cutting facets are developed.

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Absorption and Distribution of Vitamin A in X-Irradiated Rats.* (17932)

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(Introduced by H. A. Blair)

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The gastro-intestinal mucosa is one of the tissues most sensitive to ionizing irradiation. Various studies have indicated physiologic alterations in gastro-intestinal function following irradiation with impaired absorption of certain foods. In the comprehensive review of Warren and associates(1), several experiments are cited which indicate impaired absorption of sugars and fat. Studies on absorption in radiated rats indicated a diminished absorption of glucose at 24 and 40 hours following irradiation(2). This has been recently confirmed by Barron(3) in rats at 2 and 4 hours post-radiation. Impaired and altered fat absorption has also been described in human leukemia following irradiation(4). Studies on dogs receiving a moderately large dose of x-ray to a single loop of the bowel showed no fat in the lymphatics of the radiated loop after a test meal(5). Certain indirect evidence, however, suggested that the absorption defect following whole body irradiation in the mid-lethal range cannot be as large as those earlier experiments suggested. Dogs show little weight loss following irradiation with mid-lethal doses; and such weight losses as are observed can be nearly accounted for by anorexia which is frequently seen(6). It would seem reasonable that if absorption were more than minimally impaired, the difference in weight between the irradiated animals and pair fed controls would be much larger.

The findings in the present studies indicated that, after the first day, post-radiation absorption of vitamin A alcohol is essentially normal or increased; however, there is an abnormality in the tissue distribution.

Methods. In these studies young female rats of the Wistar strain were used. The rats were fed a standard Purina Fox Chow mixture ground into a fine granular form. The irradiated rats received 625 r whole body radiation which is an LD_{50} in our laboratory. Rats were studied at intervals from one hour post-radiation up to 7 days post-radiation. The test dose of vitamin A alcohol was administered to irradiated and control rats by stomach tube from a tuberculin syringe. During the test period, the animals were allowed water and food *ad libitum*. The animals were anesthetized with chloroform and exsanguinated from the vena cava at either 6 or 10 hours. At autopsy, the gastro-intestinal

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TABLE I.
Percentage Dose of Vitamin A Recovered 6 Hours After Administration.

Time post-radiation		Intestine	Liver	Carcass	Total recovery
3 days	R*	51.4	10.1	24.5	86.0
	C	60.7	13.6	18.6	92.9
3 "	R	29.0	8.6	16.2	53.8
	C	58.0	11.4	5.5	74.9
3 "	R	23.5	4.1	34.0	61.6
	C	62.5	7.8	4.6	75.1
3 "	R	24.8	3.3	12.0	40.1
	C	55.5	8.0	2.4	65.9
3 "	R	78.3	3.8	2.7	84.3
	C	80.2	5.4	1.8	87.4
3 "	R	32.0			
	C	65.0			

* R = Radiated. C = Controls.

tract was removed in 2 segments. Ties were placed at the pylorus in order to avoid loss of either gastric or intestinal contents. Recoveries of vitamin A were carried out on the stomach, small and large intestine, liver, and remaining carcass of each rat. The adrenal glands were pooled for each group of control and radiated animals. Both the esophagus and trachea were removed and analyzed with the stomach. The tissues were frozen and stored until used. The tissues were homogenized in a Waring Blender in alcohol in the presence of hydroquinone, and then were analyzed for vitamin A by the Clausen and McCoord modification of the Carr-Price reaction(7).

Results. Effect of x-rays on the G-I tract. Following whole body irradiation in the mid-lethal range, rats reduce their food intake by 75% during the first 24 hours. In this same period the stomach becomes greatly distended, and the small bowel loses tone and fills with fluid. During the second 24 hours, anorexia becomes more marked and food intake is reduced by 80-100% of pre-radiation. The obstruction at the pylorus disappears. The food passes into the small intestine, which now becomes distended with fluid and gas. Watery diarrhea usually appears at this time. By the fifth day, the diarrhea subsides, and by the 8th to 10th day the bowel appears grossly normal in surviving animals.

Effect of x-ray on intestinal absorption. Studies were made using groups of 4-6 animals. Control and irradiated groups were handled in an identical manner except for the radiation, and were studied simultaneously in each experiment. Since the absolute values of the dose administered in terms of International Units varied from 20,000-25,000 I.U. in different experiments, the data have been tabulated as percentage of dose. Absorption has been measured in two ways. First, by determining the amount of vitamin A remaining in the gastro-intestinal tract; and secondly, by measuring the amount present in the remainder of the animal. Experiments were made on the 1st, 2nd, 3rd, 4th, 6th and 7th days post-irradiation. After the 6 hour test period, there was less recovered in the intestine of the radiated animals than in the controls, except for one experiment which began 1 hour after irradiation. Vitamin A recovered in the remainder of the animal, except for 2 experiments, also showed greater absorption in the radiated animals. The results of experiments performed on the 3rd day post-irradiation are given in Table I. In the 10 hour test period experiments (Table II) the intestinal recovery values show little difference between the control and irradiated animals. In these 10 hour test period experiments, it has been necessary to base absorption entirely on the amount retained in the intestine since an analysis of the carcass and liver values indicated a mean recovery at 10 hours which was considerably below the carcass-liver recovery at 6 hours in the radiated animals. Differences between the absorption at 6 and 10 hours can probably

7. McCoord, A. B., personal communication.

TABLE II.
Percentage Dose of Vitamin A Recovered 10 Hours After Administration.

Time post-radiation		Intestine	Liver	Carcass	Total recovery
3 days	R*	22.0	6.5		
	C	38.0	17.0		
3 "	R	46.7		11.2	70.6
	C	50.7		5.8	66.4
3 "	R	77.3	8.5	3.2	
	C	59.2	16.2	3.0	
4 "	R	49.7	9.8	4.0	64.2
	C	58.6	13.7	5.0	67.3

* R = Radiated. C = Controls.

TABLE III.
Recovery of Vitamin A in Segments of the Gastrointestinal Tract.

		6 hr vitamin A experiments			10 hr vitamin A experiments		
Days post-radiation		Stomach	Intestine	Total GI tract	Stomach	Intestine	Total GI tract
3 days	R*	52.1	26.2	78.3	28.6	18.1	46.7
	C	56.7	23.5	80.2	30.7	20.0	50.7
3 "	R	37.6	13.8	51.4	47.1	30.2	77.3
	C	44.2	15.5	60.7	46.2	13.0	59.2
3 "	R	29.5	2.5	32.0			
	C	55.8	9.2	65.0			
3 "	R	15.7	13.3	29.0			
	C	40.5	17.5	58.0			
3 "	R	21.5	2.0	23.5			
	C	59.8	2.7	62.5			
4 "	R				38.0	11.7	49.7
	C				41.3	17.3	58.6

* R = Radiated. C = Controls.

be best explained by differences in motility of the gastro-intestinal tract, the controls reaching the same degree of total absorption but at a slower rate. This is shown in Table III where the stomach and intestinal values are separated for the 3 day experiments. In 6 hour experiments, controls showed slower gastric emptying and less absorption. At 10 hours the control stomach values were slightly lower than the radiated.

Effect of diarrhea on absorption. It would seem reasonable to expect reduced absorption during the period of diarrhea. However, both the intestinal recovery values and the carcass values showed an increased absorption in the 3rd and 4th days' experiments when diarrhea was most severe, and the small bowel was flaccid and distended with gas.

Effect of x-ray on the distribution of alcohol vitamin A. The vitamin A content of the liver was lower in the radiated than in the control groups. This was observed even when the recovery from the intestinal tract and liver-carcass showed increased absorption in the radiated animals. In column II of Tables I and II are given the liver values. At the same time the carcass values are markedly increased after the 6 hour test (column III, Table I). The site of deposit of vitamin A in the radiated rats has not been determined. A limited number of studies in which individual organs were studied showed no clear cut shifts. The redistribution, therefore, seems to be widespread and probably indicates a storage of absorbed vitamin A in the peripheral fat deposits. The peripheral vitamin A appears to be rapidly metabolized since the large amounts present at 6 hours in the carcass of the irradiated animals has largely disappeared by 10 hours. Recoveries in the carcasses of both irradiated and control rats are essentially the same at 10 hours.

Effect of x-ray on adrenal vitamin A. A redistribution of vitamin A also occurs in the

TABLE IV.
Percentage Dose Recovered in the Adrenals.

Time post-radiation		6 hr	10 hr
3 days	Radiated	.018	.045
	Control	.023	.013

case of the adrenal gland. At 6 hours the radiated adrenals contain less vitamin A than the controls in the 3 days post-radiation experiments. While at 10 hours the irradiated adrenals contain more vitamin A than the controls (Table IV).

Effect of vitamin A ester. The alcohol form of vitamin A was used in all but one experiment. By using the alcohol the step requiring the hydrolysis of the ester by pancreatic enzymes was eliminated. This simplified the interpretation of the data on absorption. In the one 6 hour experiment where the ester was given to 3 days post-radiation rats, absorption by the radiated animals was slightly lower than that of the controls. The distribution was essentially the same as with the alcohol.

Discussion. Intestinal absorption like many other physiological processes responds to irradiation in a complex manner. From our own studies, and those in the literature, it becomes obvious that a number of variables, such as dosage time and species must be considered in making any statement about the effect of radiation on intestinal absorption. Since the results obtained in this experiment are quite different from those which might be predicted from earlier work, certain fundamental differences between the studies should be pointed out. Most of the earlier work was done with large doses of irradiation limited to the abdomen or a single loop of the bowel. The exact dosages used were not known, although they were in the range which causes an erythema. This resulted in direct irradiation to the intestine with larger doses than we obtained with the whole body radiation doses we have used. The experiments of Buchwald(2), Martin and Rogers (5), and Mottram, Cramer and Drew(9) come in this group. That absorption may be impaired with such large doses seems quite reasonable since very large doses of x-ray will cause irreversible damage to the intestine.

The distribution of vitamin A in tissues indicates that certain major changes in vitamin A metabolism may occur as a result of irradiation, but this is probably not specific since McCoord(8) has described a similar change in rats infected with mouse paratyphoid bacilli, and in rats severely depleted of vitamin A, or subjected to hyperthermia. Liver content is consistently low in the radiated animals and does not support the claim that liver content is an accurate measure of absorption(12). Our studies indicate a rapid destruction of absorbed vitamin A. The radiated carcass values are high at the 6 hour period, and low at 10 hours, and since there are no other sites for storage, the vitamin A had to be either destroyed or transferred back to the intestine. The latter does not seem likely in view of present-day concepts of intestinal function.

The adrenals are known to contain relatively large amounts of vitamin A which presumably participates in the formation of adrenal steroids. In these studies we found evidence of definite disturbances in adrenal vitamin A metabolism. Such findings are not surprising in view of the marked changes in the adrenal reported by Patt(10) after whole body irradiation of rats. However, these changes probably are not specifically related to radiation, since similar changes in vitamin A content have been observed following experimental infection, hyperthermia, chloroform poisoning(8) and after cold stress(11).

Summary. The absorption of vitamin A alcohol by young female rats at varying intervals during the first week following 625 r, whole body irradiation has been studied 6 and 10 hours after a test dose of vitamin A.

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Absorption after a 6 hour test is increased from the 2nd to 6th days in irradiated animals. After a 10 hour test there was little difference between irradiated and control animals. Liver content of vitamin A is decreased in irradiated animals, and there is an increase in carcass vitamin A. Adrenal vitamin A is less in irradiated than control rats at 6 hours and is increased above control rats at 10 hours.

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Presence of Desoxyribonuclease Activity in Human Serum.* (17933)

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Greenstein and Jenrette(1) studied the depolymerization of thymonucleic acid by extracts from various tissues and body fluids. The enzyme responsible for this depolymerization was found to be present in the serum of the guinea pig, mouse, rabbit and dog, but not in that of man. McCarty(2) isolated the enzyme from beef pancreas, termed it "desoxyribonuclease", showed that it was activated by magnesium, and found that the optimal concentration for such activation was 0.003 M. In view of McCarty's findings, it was of interest to determine whether the addition of magnesium ion to human serum would reveal any desoxyribonuclease activity and, if so, to study this activity in normal individuals and in patients with cancer and noncancerous disease.

Desoxyribonucleic acid was prepared from calf thymus, essentially in accordance with Hammarsten's method(3), and dried in vacuum to constant weight. Analyses gave a value of 8.11% for the phosphorus content. The desoxyribonucleic acid was dissolved in 0.028 M veronal buffer of pH 7.5 so as to yield a concentration of 0.8 mg desoxyribonucleic acid per cc. Batches of this solution were filtered just before each series of determinations. McCarty's method for determination of the desoxyribonuclease activity was employed. A volume of 4.0 cc of the 0.08% solution of desoxyribonucleic acid was placed in an Ostwald viscosimeter immersed in a water bath at $37.0 \pm 0.1°$. The relative viscosity, η, of this solution was usually between 2.80 and 3.00. When this solution had attained the temperature of the bath, any substance such as Mg^{++} ion, the effect of which was being investigated, was added. The contents were mixed thoroughly by raising and lowering the solution several times, and several viscosity determinations were made in order to obtain an initial value. At a stated time, 1 cc of serum, brought to 37.0°, was added; the contents were mixed again and the change of viscosity with time was determined. In accordance with McCarty's procedure, the slope of the straight line portion of this curve was taken as the reaction velocity. In this paper, the velocity is expressed as the change in the relative viscosity, η, per 10^3 seconds.

Effect of Mg^{++} concentration. One-tenth cc of distilled water, or of various concentrations of Mg^{++}, was added to 4.0 cc of the desoxyribonucleic acid solution, and 1.0 cc of a pooled specimen of serum was then added. Table I shows that in agreement with the observations of Greenstein and Jenrette, only very slight activity was demonstrable in human serum in the absence of added magnesium over a period of several hours. If the reaction was allowed to proceed overnight (20 hours) measurable activity was manifest. The addi-

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