the values were too low (<1 γ/ml) to be reliable and therefore are omitted. Analysis of sucrose-prepared mitochondria for isoleucine was not complete due to a variation in the properties of the organism which developed at that point.

It is clear that the concentrations of amino acids should be lower than the true values *in situ* because of the losses due to the washing procedures employed in the isolation. The sucrose preparations have a one third less nitrogen content and, in general, show a lower concentration of the free amino acids, although there is no 3:2 proportionality, as would be expected from the nitrogen analysis. Table I shows that for certain of the amino acids the concentration is the same prior to incubation for the buffer or sucrose-prepared mitochondria. In other instances the buffer-prepared mitochondria and the supernatant fluid have closely agreeing concentrations. The free amino acid concentrations in the mitochondrial preparations were calculated to range between 25 to 75% of that determined in our laboratory for whole liver homogenate.

Those amino acids which are relatively stable metabolically and are also essential amino acids (Fig. 1), or are derived from essential amino acids, increase in both mitochondrial fractions upon incubation, but remain approximately unchanged in the microsome-containing supernatant. This may indicate an extensive hydrolysis of protein within the mitochondria and but little in the supernatant and microsomal fractions. In the group of essential amino acids, a significantly greater increase occurs in the buffer-prepared than in the sucrose-prepared mitochondria with the exception of phenylalanine.

The data show that there are appreciable concentrations in the mitochondria of the 6 essential amino acids determined, as well as of the non-essential amino acids and that, with two apparent exceptions, the concentrations of the free amino acids increase upon incubation. Therefore, all the required amino acids appear to be available as substrates for the biosynthesis of proteins.

Summary. The concentrations of 13 free amino acids were determined in mitochondrial preparations of liver and in the supernatant fluid. Appreciable concentrations of the amino acids were found and, in general, these increased during a 2-hour period of incubation.

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Effects of Total-Body X-Irradiation on the Tissue Mast Cell. (20068)

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Hemorrhage is a very striking characteristic of the syndrome which follows total-body irradiation. Strong indications that hyperheparinemia is the primary cause of post-irradiation bleeding have been found in the X-irradiated dog where 1) clotting time is greatly prolonged, 2) toluidine blue or protamine administration effects normal clotting time and controls hemorrhage, 3) material having the properties of heparin appears in the blood, and 4) toluidine blue or protamine is effective in controlling hemorrhage in the presence of marked thrombocytopenia(1,2). The importance of hyperheparinemia has been questioned, however, by workers who have failed to establish prolonged clotting time as an essential component of the radiation syndrome(3-8), and the recent finding that

TABLE I.

Exp. No.	Species	No. of animals	X-ray dosage, r	Sacrifice time (days post-irrad.)
1	Hamster	16	1200	1, 2, 3, 4
2	"	37	775	3, 5, 7, 8, 9
3	"	43	600	3, 5, 7, 10, 12, 14, 17, 19, 20, 24, 26, 29, 31, 33, 35
4	Mouse	9	850	2, 5, 7

thrombocyte transfusions control hemorrhage in irradiated dogs has been interpreted as formidable evidence that thrombocytopenia is the fundamental cause of post-irradiation bleeding(9).

Reasoning that changes in circulating heparin should be reflected in the tissue mast cells, generally considered as a source of heparin(10,11), we have undertaken to determine the behavior of the mast cells in the X-irradiated animal. The present paper is concerned with the results of these studies and their possible relationship to post-irradiation hemorrhage.

Methods. The cheek pouch of the young adult male Syrian hamster and the mesentery of the hamster and of the young adult female CF#1 mouse were selected for study, since they contain mast cells in large numbers and can be observed *in toto* rather than as tissue sections. The several experiments performed are outlined in Table I. On each sacrifice day cheek pouches and/or mesenteries were removed from 3 to 5 animals under Nembutal® anesthesia. Whole mounts were prepared by placing the tissues between concentric rings (stainless steel). These were fixed for 24 hours in absolute alcohol and then treated with 0.1% toluidine blue 0 in 50% alcohol (3 hours) or with 50% alcohol saturated with thionin (10 minutes), both of which react metachromatically with heparin. On each sacrifice day tissues were also prepared from 2 or 3 nonirradiated but otherwise similarly treated controls. The *radiation factors* were 250 kv, 15 ma, 0.5-mm Cu and 3.0-mm Bakelite filters, 26.7 cm target distance, 1.5 mm Cu half-value layer, and 215-225 r per minute.

Results. The tissue mast cells of the irradiated animals presented the same appearance as those of the nonirradiated controls until 3 to 5 days after exposure when numerous atypical mast cells became apparent in the tissues of all irradiated animals regardless of the radiation dosage employed. Occurring singly or in combination, the mast cell changes consisted of 1) conglomerations of cytoplasmic granules of varying size, 2) colorless or metachromatic cytoplasmic vacuoles of various sizes, some being so large as greatly to distend the cell, 3) abnormally shaped cells, 4) discontinuities in the cell boundaries, the cell contents remaining intact, and 5) ruptured cells, the granules and granule conglomerates being scattered throughout the surrounding tissue (Fig. 1-6). The number of atypical cells increased with time, becoming greatest at 7 to 10 days post-irradiation. At this time metachromatic remnants of mast cells were present throughout the tissues, and few normal mast cells were encountered. By the 12th to 14th day after exposure the number of atypical mast cells and the amount of metachromatic material dispersed in the tissues started to decrease and continued to diminish thereafter. Simultaneously the numbers of normal tissue mast cells increased until by the 30th to 35th day, the tissues from the irradiated animals were similar to those of the controls. This picture of recovery was seen only in the 600 r group, for the higher radiation dosages were lethal, and animals were available for sacrifice only to the limits noted in experimental outline. Atypical mast cells similar to those described above were found in nonirradiated animals, but only occasionally. Attempts to determine the influence of irradiation on the number of mast cells met with little success, for distribution of the cells was not uniform, many cells being found in some areas and few in others. Observations at low magnifications gave the impression that the total number of mast cells was decreased

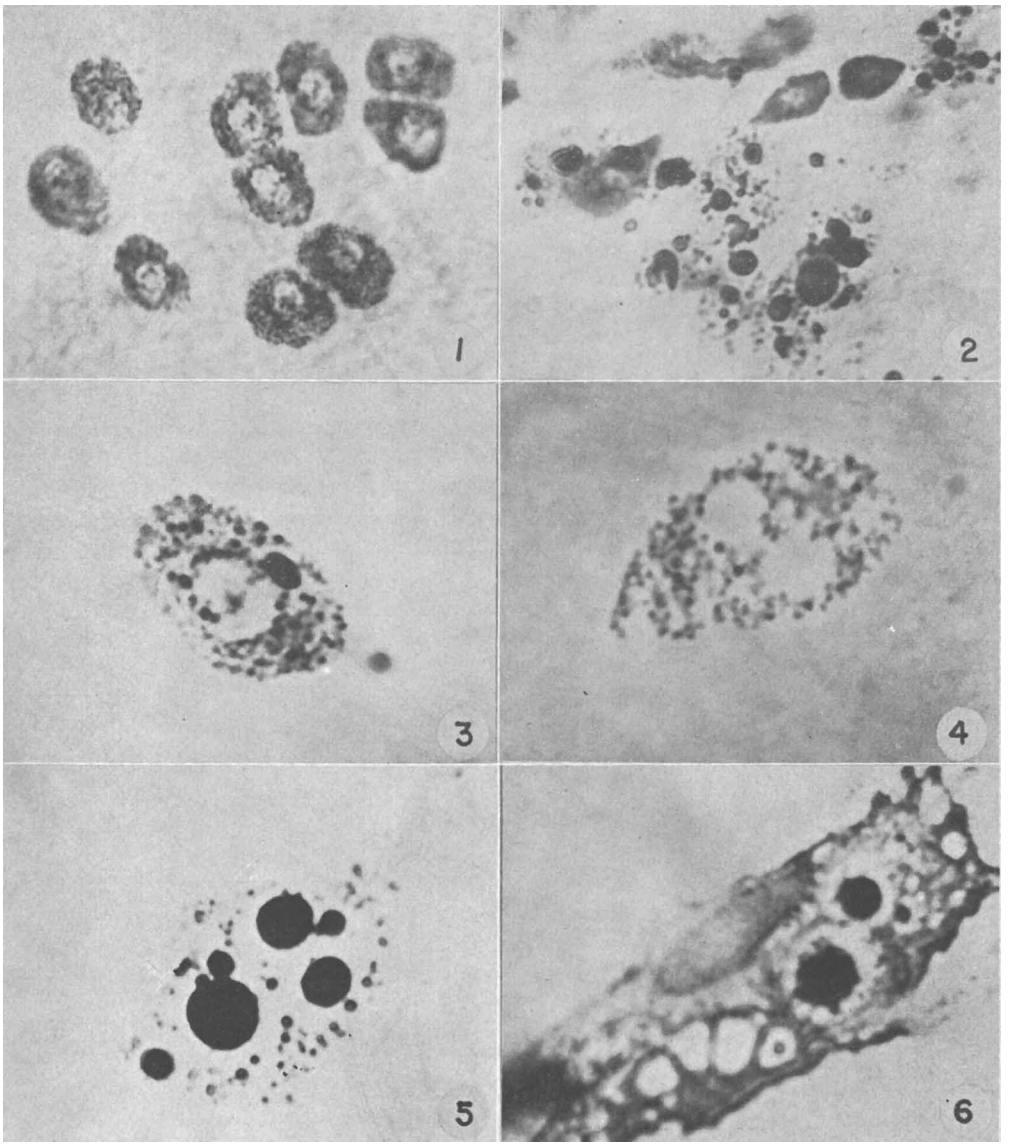

FIG. 1. Control. Hamster cheek pouch; toluidine blue; × 750.
FIG. 2. Irradiated animal. Hamster cheek pouch; 7 days post 775 r; toluidine blue; × 750.
FIG. 3-6. Irradiated animal. Hamster cheek pouch; 7 days post 775 r, toluidine blue; × 1800. Clumping of metachromatic granules, vacuolation, cell deformation, cell disruption.

when large numbers of atypical cells were apparent, but unchanged at all other times.

Discussion. The present study clearly demonstrates that total-body X-irradiation is followed by striking changes in the morphology of the tissue mast cells, the end point of which is the complete disruption of the cells with dispersion of their contents into the surrounding tissue. The probable sequence of events is 1) clumping of granules and vacuole formation, 2) increase in size of granule clumps and vacuoles, and 3) distension and rupture of the cell, allowing the granule clumps and vacuoles to be dispersed into the nearby tissues. Similar changes have been found in the tissue mast cells after a variety of treatments: allowing tissue cultures of dog mast cell tumors to age(12); administration

of cortisone(13,14), and toluidine blue(15); and stimulation of urticaria pigmentosa lesions(16). Since the several types of atypical mast cell were occasionally seen in control tissues, the results of these treatments have been interpreted as demonstrations of secretion by the mast cell. The same interpretation can be given to the present results for the same reason.

Fulton *et al.*(17) have observed that the whole blood clotting time of hamsters exposed to 1200 r gradually increases from a pre-irradiation control level of approximately 2 minutes to a value of about 5 minutes on the sixth day after irradiation. The correlation between these results and the time of occurrence of widespread mast cell changes indicates that upon release from the cell the heparin-containing material is more available to the circulation and, in part at least, can be responsible for the post-irradiation clotting defect. Previous reports on the influence of irradiation upon the tissue mast cells have been primarily concerned with changes in the cell population. The results conflict. Thus, increases(18) or lack of change(19,20), or decreases(20) in the mast cells of the irradiated skin of the rat have been noted. Increased numbers of mast cells have been reported for practically every human tissue (21,22) and for the thymus glands of hamsters(23) exposed to total-body irradiation. It is possible that species and organ differences may account for the discrepancies between these results and those of the present study. The conclusions reached in the thymus gland experiments, however, are open to question. In these studies cortisone, adrenal cortical extract, and starvation, all of which bring about thymic involution, were employed to determine whether the mast cell count would increase as the organ diminished in size. Finding no change in the cell counts under these conditions, it was concluded that the increased number of mast cells seen after irradiation was real. This conclusion seems valid until one takes note of the fact that cortisone *per se* causes depletion and disruption of the mast cells(13,14).

In the previous reports(18-23) no mention was made of atypical cells of the kind observed in the present experiments. Failure to find such abnormalities may be accounted for by the fact that the previous work was done on tissue sections, which in our experience rarely afford opportunity for visualizing the alterations readily apparent in whole mounts. The morphological changes noted in the present work have since been confirmed in the cheek pouch of the irradiated hamster by Maynard and Fulton(24).

Summary. 1. The effect of total-body X-irradiation upon the state of the tissue mast cells in the cheek pouch and/or mesentery in the hamster and the mouse was studied. 2. Following exposure there occurred in the tissue mast cells a series of striking changes the end result of which appeared to be disruption of the cell with dispersion of its contents into the surrounding tissues. 3. The possible relationships of these findings to the hemorrhage and clotting defect of the radiation syndrome were discussed.

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Ultra-Short-Acting Thiobarbituric Acids. (20069)

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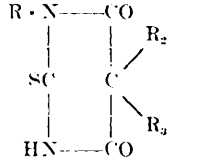
N-alkyl barbiturates were first prepared in 1904(1). The brief action of evipal, or N-methyl-cyclohexenylmethyl barbituric acid, was demonstrated in 1932(2). Subsequent workers(3,4) synthesized newer N-alkyl and N-aryl substituted barbituric acids. One of us(5) studied the series prepared by Shonle and Doran(4) and stressed the short duration of their action. American investigators(6,7) further succeeded in preparing short-acting thio-analogs of barbituric acid. Their work led to the introduction of thiopental(8,9) and other sulfur-containing barbiturates(10-13) to anesthesiology. Later N-substituted thiobarbiturates were synthesized and found to have a short induction and short duration of anesthesia. Similar work was undertaken by the Organic Chemical Division of our laboratories.



	R	R_2	R_3
[I]	CH_3	C_2H_5	$CH(CH_3) \cdot CH_2 \cdot CH_2CH_3$
[II]	CH_3	C_2H_5	$CH(C_2H_5) \cdot CH_2 \cdot CH_3$
[III]	CH_3	$CH_2 \cdot CH : CH_2$	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$
[IV]	CH_3	$CH_2 \cdot CH : CH_2$	$CH(C_2H_5) \cdot CH_2 \cdot CH_3$
[V]	H	$CH_2 \cdot CH : CH_2$	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$
[VI]	H	C_2H_5	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$

Forty-five new N-substituted thiobarbituric acids were made available to us for pharmacological evaluation. It was hoped that both replacement of sulfur for oxygen and alkylation of nitrogen might result in products having an even shorter action than thiopental.

Preliminary studies showed that the introduction of an ethyl or allyl radical to nitrogen gives rise to many compounds that hemolyze the mammalian blood and, following injection, cause phlebitis. A few members have convulsant action. The best hypnotic and anesthetic compounds by intravenous injection are N-methyl thiobarbiturates. It was very difficult to predict the desired activity of a product on the basis of its structure. However, four closely allied derivatives, [I], [II], [III], and [IV], appeared more outstanding than the remaining 41. More extensive experiments were therefore undertaken in order to demonstrate with greater certainty the relative activity of these compounds. In all experiments comparisons were made with sodium 5-allyl-5-(1-methylbutyl)-thiobarbiturate [V] and thiopental [VI]. The formulas of the 6 acids are as follows:

To ascertain the median anesthetic dose (AD_{50}) and median lethal dose (LD_{50}) of each compound, the solution was injected intravenously into rats, rabbits, cats, and dogs. The figures were read off from the logarithmic-probit graph paper as devised by Miller and Tainter(14). The total number of animals used was as follows: 510 rats with an