# BLOOD HISTAMINE LEVELS IN SWINE FOLLOWING TOTAL BODY X-RADIATION AND A FLASH BURN*

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IN 1927 SIR THOMAS LEWIS[19] reported the triple response of blood vessels of the skin to various stimuli such as heat, cold, ultra-violet and roentgen rays. It was postulated that these reactions were due to the production or liberation of H- or histamine-like substances. He pointed out the parallelism between the effects of histamine and irradiation on the skin. Among the theories endeavouring to explain the cause of acute radiation sickness has been suggested the possibility of abnormal or increased production of certain enzymes or tissue hormones. Ellinger[14] has attributed some of the symptoms of acute radiation sickness to histamine action. He emphasized that animals, such as the guinea pig and dog, which are sensitive to histamine, also succumb readily to much lower doses of total body radiation than is necessary to kill rats and mice, species which show high resistance to histamine.

It is known that certain organs such as the skin, gastro-intestinal tract, lungs, liver and spleen contain high concentrations of histamine. In these tissues, histamine is held almost entirely in a loosely combined form within the cells. In this state it is thought to be physiologically inactive. A small quantity circulates in the blood plasma in the free state. When histamine passes from the intra to the extracellular spaces it exerts its principle pharmacological actions. These are contracture of smooth muscle, stimulation of glandular secretion, dilatation of capillaries and venules, and increased capillary permeability. Free histamine is destroyed by the enzyme histaminase, which was first isolated by Best.[6] It is formed by the intestine[22] and kidney.[1] Histamine may also be inactivated or bound by tissues which do not contain histaminase, for example the kidney and liver of the rat.[28] It is possible that histamine may be liberated from its "bound" state in cells due to the ionizing effects of radiation. Furthermore, there may be radiochemical formation of new histamine. Radiation of the amino acid histidine with cathode rays will, for example, form a substance which has a histamine-like action on the small intestine and uterus of the guinea pig and this has finally been identified chemically as histamine by Holtz.[17] Since it has been considered that histamine may play an important role in the acute radiation syndrome as well as in shock[12, 34] and thermal burns,[2, 5, 29] it was decided to investigate the histamine content of blood in swine following total body radiation and after the combined injury of total body radiation plus a thermal burn.

## METHOD

Twenty young Yorkshire swine weighing from 30 to 40 pounds were used. They were

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fed a standard hog diet consisting of commercial hog food and water, which provided an adequate diet for growing hogs. Both female and castrated male animals were used. It has been reported that no marked variation in results due to sex has been observed following total body radiation.[7] The animals were immobilized in a plywood box (previously described[4]) and exposed to X-radiation at 220KVP, TSD 80 cm., with filters of 0.5 mm. Cu., 1.0 mm. Al, with an HVL equal to 1.1 mm. Cu. The dosage rate was 10r per minute as measured in air with a Victoreen counter. One half the total dosage of 400r was delivered to *each side* of the hog, the exposure being interrupted for a few minutes to reverse the position of the animal for irradiation of the opposite side. The thermal burn was administered under light ether anesthesia shortly after exposure to radiation, and covered an area amounting to 10 to 15 per cent of the total body surface on the lateral aspect of the trunk. The extent of burn was calculated according to the method of Dubois.[13] The apparatus for administering a flash burn has been described previously.[4] Prior to each experiment the animals were weighed and a preliminary hemogram performed. Subsequent to irradiation and thermal trauma careful clinical observations were made daily, as well as hemograms and a record of body weight at periodic intervals. A complete autopsy was carried out on all animals. Determinations of whole blood histamine levels were made prior to the thermal and radiation trauma and at intervals throughout the subsequent 30-day period of observation. The blood histamine was determined according to Code's[7] modification of the method of Barsoum and Gaddum.[3] The results are expressed as micrograms of histamine per milliliter of whole blood.

## RESULTS

The ten swine which received 400r only, suffered a mortality of 20 per cent in 30 days, while the mortality of the ten which received a combined dose of 400r plus a 10 to 15 per cent flash burn was 90 per cent. It should be noted that the mortality in a third group of ten swine receiving only a 10 to 15 per cent flash burn was zero. It is apparent, therefore, that combination of the two forms of trauma markedly increased the mortality. This supports the observations of those who studied the causes of death at Hiroshima and noted that the incidence of casualties dying from only moderately severe thermal burns was unusually high and required explanation. Autopsies of the swine succumbing in the above two groups revealed signs common to acute radiation syndrome which included multiple petechial hemorrhages of skin and viscera. A number of animals showed massive hemorrhage into the gut and partial or complete perforations of the bowel associated with deep ulcers in which were embedded plugs of feces. In all hogs which died there was rapid loss of weight. In general, there was considerable similarity between the hemograms of those swine receiving only irradiation and the group receiving irradiation plus a flash burn.

The changes in the blood histamine followed a significant course. After the second day, there was a fall in blood histamine in both groups. The level then decreased steadily until the animals died. The swine exposed to 400r plus a flash burn, with the exception of one survivor, died before the 19th day. Those animals given only 400r radiation survived, with the exception of two hogs. When these died on the 18th and 25th days respectively, the blood histamine was at a low level. As the condition of the survivors in both groups improved the histamine rose steadily and approximated pre-radiation levels.

In the group of ten swine receiving irradiation only, the blood histamine levels fell from an average of 1 microgram per ml. to a low level of 0.3 to 0.2 about the

tenth to the 15th day post-radiation. Subsequently, there was a gradual rise, although the initial level had not been regained by the eight surviving animals on the 30th postradiation day. In the group of swine receiving radiation plus a flash burn, the average curve showed several variations.

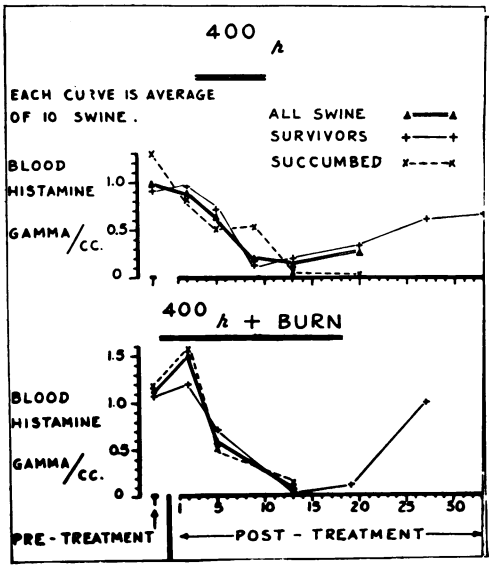


FIG. 1

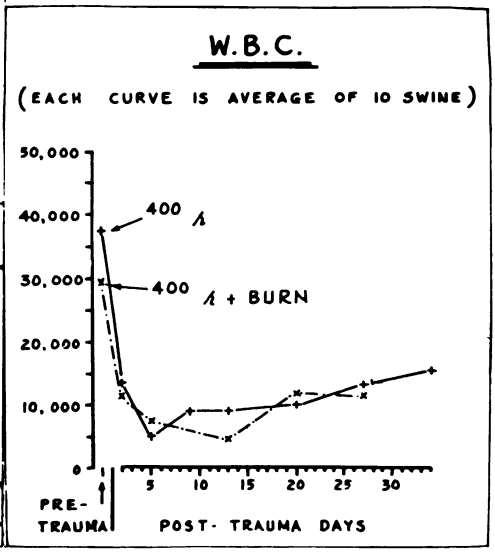


FIG. 2

FIG. 1. The blood histamine was determined at intervals both before and after exposure to 400r and in a separate group of animals, to 400r plus a 10 to 15 per cent flash burn. It is interesting to note that with the exception of an initial rise in the group exposed to radiation and a thermal burn, the blood histamine curves followed a rather similar pattern.

FIG. 2. The white blood cells of swine exposed to 400r as well as a group exposed to 400r plus a 10 to 15 per cent flash burn fell rapidly to approximately one third of the initial level two days after irradiation. After a further decline, which was reached on the fifth day, the number of white blood cells rose very slowly.

From a pre-radiation average level of 1.1 micrograms per milliliter of blood, the histamine rose to 1.5 by the second postradiation day, then fell rapidly to 0.5 by the fifth day, and in those still alive on the 13th day again fell to an extreme low of 0.1 micrograms per ml. of blood. In this experiment most of the swine succumbed between the 11th and 18th postradiation day. The blood histamine of the sole survivor rose gradually until at the termination of the experiment it had almost reached its preradiation level (Fig. 1). A fourth group of ten swine was given a larger dose of radiation, but the blood histamine and hemograms followed the same trend as described above.*

## DISCUSSION

*Radiation.* During the past few years a considerable amount of experimental and clinical investigation has been carried out on the assumption that increased or irregular production of histamine or histamine-like substances might be partly responsible for the general toxemia of radiation sickness. Loftstrom and Nurnberger[20] reported good results with Benadryl administered to patients suffering from radiation sickness. Montag[21] has treated a large number of patients with a proprietary antihistaminic preparation and various vitamins. He stated that patients benefited, although data were not given. On the other hand, Brown[8] reported that his patients obtained no relief from their symptoms after treatment with

* We have found no significant or consistent change in histamine blood levels within a few hours after exposure to total body radiation in a subsequent group of swine.

Antistine.* Weber and Steggerda[33] found that histamine in the plasma fraction of blood rose two hours after an LD50 dose of radiation in the rat. Between the second and fifth days there was a second rise. Both these coincided with a fall in blood pressure. If the rats survived, the histamine disappeared from the plasma about the ninth or tenth days postradiation. Haley[16] observed the release of a vasodepressor substance in the blood during the first week following total body radiation in rats and dogs. Venters and Painter[32] reported that rabbits and dogs irradiated to LD/50/30 days showed a marked increase in sensitivity to injected histamine about two hours after radiation. On the other hand, although Prosser and co-workers[23] observed a rise in blood plasma histamine concentration in some dogs receiving a lethal dose of total body roentgen radiation, this rise was not consistent enough to be significant in causing terminal symptoms. In some dogs there was no change in plasma histamine levels. Field and Rekers[15] found in experiments on rats and dogs that treatment with Benadryl and Pyribenzamine did not alter the course of acute radiation syndrome.

*Thermal Burns.* Since one group of swine received a 10 to 15 per cent flash burn in addition to 400r total body radiation, a brief review of the effects of thermal burns on blood and tissue histamine levels is required. Barsoum and Gaddum[2] reported a marked rise in whole blood histamine of seven burned patients about the fifth or sixth day after the burn. While they considered that a close relationship existed between the magnitude and duration of increase of blood histamine and the area of the burn, no such correlation was observed between the blood histamine and clinical condition of their patients. Code and MacDonald[10] noted a steady rise in blood histamine in one patient until the 12th day post burn, followed by a very gradual fall to normal. Kisima[18] reported a rise in whole blood histamine after a few hours in four burned patients, which reached a maximum in two to four days and returned to normal levels in ten days. Kisima and also Behrmann[5] both reported a rise in blood histamine of dogs, following extensive thermal burns, which gradually returned to normal. The former noted that the histamine content of various organs was increased. Immediate resection of the burned skin prevented the increase in blood and tissue histamine.[18] The results of the present experiments are strikingly similar to those found in certain other shock-like states. Rose and Browne,[29] reporting results on seven patients, noted marked elevation in whole blood histamine within one hour after the burn. In contradistinction to previous workers, they found in every patient a rapid fall to low levels by the third to fifth days, as edema and shock developed. As the edema subsided, the blood values rose to normal. Moreover, there was a marked decrease 12 to 36 hours before death in moribund patients. Similarly, surgical and traumatic shock in man are associated with very low blood histamine values.[30] There is some resemblance as well to observations on the blood histamine in certain skin lesions such as atopic dermatitis, where the blood histamine is elevated, and in urticaria and angio-edema, where it is low.[24] Rabbit anaphylaxis, for example, is also associated with a profound and rapid disappearance of histamine from the blood.[25] It appears, therefore, that edematous states are more commonly associated with a low blood histamine than with an elevated histamine content of the blood. The significance of this general pattern is not clear, but it would seem that the results of the present experiments follow a similar course. It is considered that the initial, brief rise in blood histamine on the second day of the group of swine exposed to total body radiation plus a thermal burn, was

* Ciba Company Limited.

due to acute inflammation of the skin of the burned area (Fig. 1). A few days later the blood histamine decreased markedly.

*Distribution of Histamine in Blood.* It is known that the amount of histamine contained in plasma and red cells is very small, and that from 70 to 100 per cent of the blood histamine is contained in the "buffy coat" of centrifuged blood which contains the leukocytes and platelets. While the platelets of the rabbit contain most of the blood histamine, those of the dog, horse and man contain very little.[11] Furthermore, in lymphatic leukemia in man there is little rise in whole blood histamine, while individuals with myelogenous leukemia show greatly increased amounts of blood histamine. It has been shown also that the eosinophils and monocytes contain little histamine.[27] In most animals, probably including swine, the transport of histamine in the blood is chiefly the concern of the myeloid series of cells. These originate in the cells of the red bone marrow which contain a higher content of histamine than the blood.[11] When they leave the bone marrow and enter the blood stream they probably carry histamine with them. It is well established that histamine may be present in large quantities in the blood without exerting any demonstrable activity, providing it remains within the cells. It is only when it is released into the plasma that it produces its characteristic effects. It was not possible to obtain sufficient blood for estimation of both plasma and whole blood determinations in this series. Comparison of Figures 1 and 2 reveals interesting time relationships in the changes of both the blood histamine and the white blood cells. It may be observed that a precipitous fall in white blood cells had occurred by the second postradiation day. In the group treated with radiation plus a thermal burn, the blood histamine had reached its highest level at this time, but had only declined slightly in the swine treated with radiation alone. Furthermore, the blood histamine in both groups of swine did not reach its lowest point until the 13th postradiation day, at which time the white blood cells were beginning to rise. Therefore, it would appear that the early and dramatic effects of radiation on white blood cells and bone marrow do not entirely account for the decrease in blood histamine. When one considers that large quantities of histamine are present in the skin, gastro-intestinal tract, lung, liver and spleen, it is possible that these tissues may contribute in some way to the changes in the blood. Further investigation is under way to determine what changes occur in histamine content of these organs following total body radiation.

There are several possible reasons for the divergence of opinion both in clinical and experimental studies on the relation of histamine to acute radiation sickness. The first is that the patho-physiological disturbances which result in the syndrome of radiation sickness are multiple, and involve cardiovascular, hematopoietic, endocrine, gastrointestinal and reticuloendothelial systems. Therefore, disturbance of a single system, enzyme, or hormone cannot be expected to reflect uniformly all organic responses to this stressor agent. The second cause for variation in experimental results is the different amounts of radiation administered by various investigators, as well as the species of animal, some of which are far more resistant than others to radiation. It is well known that heavy, moderate and light doses of total body irradiation result in varying degrees of signs and symptoms which differ considerably in intensity and character. Accordingly, it may be assumed that a similar variation in production, storage, liberation, or elimination of histamine may occur following different amounts of radiation. Further studies are in progress at this time to explore the validity of this hypothesis. Finally, different investigators have employed various methods of histamine assay. It has been observed also, that unless frequent determinations are made

following a given stress, the abrupt changes which sometimes occur in the blood histamine may not be noted. We have shown, for example, in previous experiments, that following intravenous injections of curare in man, a marked rise in plasma histamine may occur within seconds, which is replaced in a few minutes by an abrupt fall and then a gradual return to pre-treatment levels. Other factors may also be concerned. The production of histaminase may be increased, or increased excretion of histamine through the kidney or bowel may occur. A lowered intake of food during the period of illness may reduce the supply of histidine, the precursor of histamine, or the bowel organisms normally responsible for breaking histidine down may be changed so that this step is not accomplished. It is possible that changes in the gut wall resulting from radiation prevent its absorption. Another concept is that changes in permeability of the capillaries and shifts in intra- and extracellular fluids may result in transfer of histamine to extravascular spaces or storage in certain visceral organs.

Rose[25] has shown that the induction of skin response in a patient with dermatographia, after a brief rise in blood histamine, in 20 minutes, may be followed by a decrease as wheal formation occurs. Intravenous injection of histamine is often followed by a fall in blood histamine in man, probably due to activation of the enzyme histaminase which destroys it. Rose[25, 30] has also noted a decrease in blood histamine during shock, or when marked edema of skin and superficial tissue was present. Schayer[31] has demonstrated that four hours after injection of $C^{14}$-histamine, none could be detected in the organs of guinea pigs. On the other hand, after injection of radioactive L-histidine, it was possible to detect $C^{14}$ histamine in the urine and internal organs for many days. Thus it is apparent that the biosynthesis, the physiological and pathological functions, as well as the excretion of histamine, pose many enigmatic questions.

## SUMMARY

1. Twenty swine were divided into two groups of ten. One group received 400r, and the other the same dose of roentgen radiation plus a 10 to 15 per cent flash burn. Mortality in the first group was 20 per cent, while in the second group it rose to 90 per cent.

2. In the group of swine treated with roentgen radiation alone, the blood histamine content fell steadily from the preradiation level until the 13th day postradiation. The histamine then rose gradually in the survivors.

3. When a flash burn was added to the same amount of radiation, a rise in blood histamine above preradiation values occurred on the second postradiation day. This was followed by a continuous decrease until the 13th day. Most of these swine died between the 11th and 18th days. In the one survivor, the histamine then rose gradually until almost the initial level had been attained.

4. There appeared to be a definite parallelism between the fall in whole blood histamine content and increasing severity of symptoms of acute radiation illness. In all survivors, the blood histamine gradually rose toward normal.

5. While the changes in blood histamine also were parallel in some respects to the decrease in the myeloid series of cells, which contain most of the blood histamine, this did not entirely account for the decrease in blood histamine.

6. Several hypotheses are tentatively advanced to account for the changes in blood histamine following whole body roentgen radiation.

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