β -Glucuronidase Activity in Lymphatic Tissues of the Rat after X-Ray Irradiation of the Whole Body

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As communicated in a preceding paper (Pellegrino & Villani, 1956) increase in β -glucuronidase activity has been observed in lymphatic tissue during fasting, which causes a marked atrophy of lymphatic organs. Subsequent feeding, which brings about the reconstitution of the tissue, is accompanied by decrease in the level of enzyme activity. From these results a relation between atrophy of the tissue and increase in β -glucuronidase activity seemed apparent.

It was therefore thought advisable to follow the behaviour of the β -glucuronidase in lymphatic tissue during atrophy induced by other experimental procedures. One of these is irradiation of the whole body, which causes in this tissue very marked regressive phenomena (De Bruyn, 1948). It was established that the enzyme increased to a maximum in the first few days after irradiation. The effect of the dose on the variation of the enzyme activity was examined as well as the effect on the period during which the activity was increased; relations between the alterations of the β -glucuronidase levels and the weight and histological structure of the tissues were also examined.

MATERIAL AND METHODS

Male albino rats weighing from 69 to 130 g. were used throughout. They were kept on a standard balanced diet (Randoin & Causseret, 1947) supplemented by green vegetables.

Conditions of irradiation were as follows: all doses of X-rays were given in single total-body exposures (140 kv, 2 ma, 3 mm. Al filter, 30 cm. target distance, 25 r./min.). Total doses administered were measured with a Victoreen 70 r. meter (Picker X-ray Corporation). Groups of four rats were irradiated simultaneously. Each animal was enclosed in an individual compartment of a wooden container, each compartment being equidistant from the centre of the field. Various doses were administered by varying the time of exposure.

From 4 hr. to 15 days after the irradiation the rats were killed by stunning and bleeding; the lymphatic organs (six superficial lymph nodes in the neck, the thymus and the spleen) were dissected out and dispersed in water (adjusted to pH 7.4) with a glass homogenizer (Potter & Elvehjem, 1936) kept in cracked ice. The final dilution of the dispersions was 1:200 (w/v). Determination of β -glucuronidase activity (with phenolphthalein glucuronide as sub-

strate, at pH 5.2) was carried out as previously described (Pellegrino & Villani, 1956).

In some cases the dry weight of the tissue was determined by drying to constant weight in an oven at 105°.

In order to determine whether sublethal doses of whole-body X-ray irradiation altered the activity of β -glucuronidase in lymph nodes, thymus and spleen of rats, various doses of X-rays (200, 400 and 600 r.) were given to groups of animals and the enzyme activity was determined 4, 8, 12 and 18 hr. after the irradiation and later after 1, 2, 3, 5, 7, 10 and 15 days.

In order to attempt to correlate the variations observed in enzyme activity with the histological changes of the tissues after irradiation, the organs or portions of them were fixed in Bouin's fluid and embedded in paraffin wax, cut in sections 10 μ thick and stained with Carazzi's haematoxylin and eosin. Samples from at least three rats for each experimental point were examined.

RESULTS

Weight and enzyme variations in lymphatic tissues after irradiation

Lymph nodes. It is seen from Fig. 1 that after any of the three doses of X-rays the lymph nodes markedly and rapidly decreased in weight, reaching a minimum corresponding to about 50% of the initial weight between the first and the fifth day after irradiation. Eventually an increase in weight took place at a rate and to an extent which did not bear any clear relationship to the dose administered.

The activity levels of β -glucuronidase showed in every case an increase corresponding with the decrease in weight of the lymph nodes. They reached a maximum between the second and the seventh day, according to the dose of X-rays, and then returned to normal values between the fifth and the fifteenth day, according to the dose.

Thymus. The values for the thymus gland are shown in Fig. 2. The loss in weight due to irradiation was more pronounced: the minimum was reached 2 days after irradiation with 200 r. and 3 days after irradiation with 400 and 600 r. In the last two cases the organ weight was reduced to about one-fifth of the normal. A rapid increase followed with a maximum at 7–10 days for the two smaller doses, at 15 days for the larger one. The enzyme activity showed an increase, already evident 1 day after

irradiation, with a maximum 2 days after the dose of 200 r. and 3 days after the two larger doses. After 7 days the β -glucuronidase values were back to normal or even subnormal values, and no other change occurred within the experimental period.

Spleen. In the spleen also (Fig. 3) the irradiation caused a marked decrease in weight with minima at the first and second day followed by an irregular recovery. The enzyme activity, after an initial sharp increase with a maximum at the second day, showed a decrease, followed in some cases by a second increase between the seventh and the fifteenth day.

It was thought advisable to determine when the increase of the enzyme activity first became apparent. Fig. 4 shows that as early as 4 hr. after irradiation (400 r.) the organs examined undergo a loss of weight (more marked in the spleen) and in the same time show an increase in the β -glucuronidase activity (as much as 60% in the spleen).

Fig. 1. Effect of total-body X-ray irradiation (200, 400 and 600 r.) on the weight and β -glucuronidase activity of neck lymph nodes of rats. Upper curves: wet weights (mg./100 g. body wt. at time of irradiation). Lower curves: β -glucuronidase activities (glucuronidase unit/g. wet wt.). For definition of glucuronidase unit (g.u.) see Pellegrino & Villani, 1956. Each experimental point corresponds to the mean of from four to eleven individual results.

In case variations in the water content of the tissues were concerned in the fluctuations of β -glucuronidase activity found in the first 24 hr. after irradiation, dry weights were determined in this period. No important variations in dry weight were found.

Early histological findings

The main changes in the tissues taking place within 24 hr. of total-body irradiation with 400 r. were as follows:

Lymph nodes. At 4 hr. after irradiation numerous pycnotic lymphocytes were present in the corona which surrounds the germinal centres of the lymphatic nodules and also outside in the cortex and medulla of the organs. The germinal centres showed many large phagocytes with abundant debris inside their cytoplasm. At 8 hr., pycnoses were still very evident, and phagocytosis was even more pronounced. Later the pycnotic cells were less numerous and the corona of small lymphocytes around the nodules had almost completely disappeared; at 24 hr. almost all the debris had cleared from the cells in the germinative centres. The latter were much enlarged at this stage and were composed of packed reticulo-endothelial cells with vesicular nuclei. During all this period very few mitoses could be seen.

Fig. 2. Effect of total-body X-ray irradiation (200, 400 and 600 r.) on the weight and β -glucuronidase activity of thymus glands of rats. Upper curves: wet weights (mg./100 g. body wt. at irradiation). Lower curves: β -glucuronidase activities (g.u./g. wet wt.). Each experimental point corresponds to the mean of from four to eleven individual results.

Thymus. In this gland the changes were even more striking than in the lymph nodes. Pycnoses in the periphery of the thymic lobules were already so numerous at 4 hr. as to affect most of the small lymphocytes present. Pycnotic nuclei were still visible in smaller numbers 24 hr. after irradiation. In this interval debris was visible everywhere in the gland, inside and outside the phagocytes.

Spleen. Here the early visible damage was mainly localized in the lymphatic nodules, which represent, in the rat, a relatively small part of the organ. Extensive pycnosis was evident in them, as well as debris, mainly phagocytosed. In the red pulp, the haematopoietic centres underwent pycnosis and were markedly reduced at the end of the first day after irradiation.

Later histological findings

The subsequent changes from 24 hr. to 15 days in the lymphatic tissues after 400 r. can be summarized as follows.

Lymph nodes. These remained almost completely devoid of lymphocytes until the eighth day after irradiation; later, increasing numbers of these cells appeared in the cortex and in the medulla; the normal structure of the nodules had, however, not been recovered even 15 days after irradiation.

Fig. 3. Effect of total-body X-ray irradiation (200, 400 and 600 r.) on the weight and β -glucuronidase activity of the spleen of rats. Upper curves: wet weights (mg./100 g. body wt. at irradiation). Lower curves: β -glucuronidase activities (G.U./g. wet wt.). Each experimental point corresponds to the mean of from four to eleven individual results.

Thymus. After an extreme atrophy between the first and the fifth day after irradiation, with an almost complete disappearance of lymphocytes, these reappeared after 5 days, and their proliferation was so fast that after 10 days the thymus had almost recovered its normal appearance.

Spleen. In this organ, although atrophy of the lymphatic nodules was still evident up to 15 days, when only a small number of lymphocytes was again visible in them, in the red pulp haematopoietic tissue was again visible at 5 days and after 15 days was developed to an extent at least as great as in the normal animals.

Irradiation with 200 and with 600 r. As a consequence of the smaller dose of X-rays little damage was evident in the lymphatic organs, which

Fig. 4. Short-term effects of total-body X-ray irradiation (400 r.) on the weight and β -glucuronidase activity of lymphatic tissues of rats. Upper curves: wet weights (mg./100 g. body wt. at irradiation). Lower curves: β -glucuronidase activities (G.U./g. wet wt.). Each experimental point corresponds to the mean of from four to eleven individual results.

substantially maintained the appearance of the normal ones. On the other hand, 600 r. caused very marked lesions, with patterns similar to, but quantitatively more evident than, those after 400 r.

DISCUSSION

The results here presented demonstrate that as early as 4 hr. after X-ray irradiation an increase in the activity of β -glucuronidase is evident in lymphatic tissues (lymph nodes, thymus and spleen). The enzyme-activity levels reach eventually a maximum around the second and the third day and afterwards decline. A second less-pronounced increase in the enzyme activity can be observed later (from the seventh day onwards) in some of the organs and after some doses of X-rays.

In the first period after the irradiation the clear increase in the β -glucuronidase activity was related to the abrupt decrease in weight of the tissues (as much as 80% for the thymus). Histology showed that this period was characterized by regressive processes, which consisted mainly in extensive pycnoses of the lymphocytes, subsequent fragmentation of these cells and formation of a mass of debris, which was taken up by large phagocytes. In the thymus, where regressive processes were more evident, because of the greater proportion of lymphocytes present, this was the only period in which the activity of β -glucuronidase was clearly increased.

In the second period, in which reconstitution of the tissues took place and which extended in general from the third day to the end of the experiment, the behaviour of β -glucuronidase activity was variable. In the thymus, as soon as the regeneration of lymphocytes began, the enzyme activity fell to normal values and stayed at these levels until the end of the experiment. In the lymph nodes (Fig. 1) and in the spleen (Fig. 3) a second increase in the activity of the enzyme was evident with the doses of 200 and 400 r. but not with that of 600 r. Although these increases took place in periods of increase in weight of the organs and of regeneration of lymphocytes, no clear correlation could be found between these facts. For instance, after 600 r. the weight of the lymph nodes increased notably in the period from 7 to 10 days, coincident with the re-appearance of many lymphocytes, but the β -glucuronidase activity fell in comparison with the values reached previously.

The histological examination of the tissues did not give any indication of the reason why, at 4 hr. after irradiation with 400 r., β -glucuronidase activity showed a large increase in the spleen whereas only a small increase could be observed in the lymph nodes and thymus. The fact that in the spleen the areas which showed histological damage

(i.e. the lymphatic nodules) were relatively small in comparison with the red pulp, in which nothing significant could be seen at such a short interval, might indicate that at this stage the increase in enzyme activity is related to modifications which do not have an immediate histological counterpart.

Also the high levels of glucuronidase in organs almost devoid of lymphocytes as a result of the irradiation show that the main contribution to the total activity comes in these cases from other cells.

A comparison, from the data of the present and a previous paper (Pellegrino & Villani, 1956), between irradiation and fasting, both conditions in which an increase in β -glucuronidase activity could be demonstrated in lymphatic tissues, seems interesting. In both cases a rapid loss in weight of the tissues can be observed. This is more rapid after irradiation than during the fast. However, the extent of the maximum weight reduction is similar in the two conditions, being 50–55% for lymph nodes, 78–79% for the thymus and 59–70% for the spleen. The increases in the β -glucuronidase activity were similar for the lymph nodes (about 150% in both) but clearly less after irradiation for the thymus (170% after 400 r. as against 278% after 4 days of fasting) and for the spleen (228% after irradiation as against 282% after 4 days of fasting).

The mechanism of the rapid tissue involution which takes place during a short fast (for example, of 4 days in our previous experiments) seems to consist of both lymphocytolysis and of inhibition of formation of new cells, as indicated by the reduction of the mitotic rate (Frank, Kumagai & Dougherty, 1953).

After irradiation the most striking phenomena are those of lymphocytolysis, which clearly predominate; however, in the first period after irradiation with 400 and 600 r. a complete inhibition of mitoses is observed. Like the maximum increases in enzyme activity, the histological aspects of irradiated and starved tissues show some differences: more lymphocytes are present in the fasted group than in the irradiated one; phagocytosis is still going on in the former, whereas it is much reduced in the latter. However, one must conclude that histological findings fail adequately to explain the increase in β -glucuronidase activity and its singularities in the various cases. In this connexion, in the earlier paper (Pellegrino & Villani, 1956) it was demonstrated that an actual increase in enzyme activity per lymphocyte takes place during fasting, and it was also suggested that only a small proportion of the increased activity could be explained by the suppression, in the atrophic tissue, of an inhibitor of β -glucuronidase which is normally present in lymphatic tissues.

With reference to the search for the primary effects of irradiation on the tissues, the increase in β -glucuronidase activity observed seems to be the manifestation of effects not directly related to the irradiation, since it can be produced by a very different experimental condition, namely by fasting.

SUMMARY

1. Weights and β -glucuronidase activity have been determined in lymphatic organs of rats (lymph nodes of the neck, thymus and spleen) from 4 hr. to 15 days after total-body irradiation (200, 400 and 600 r.).

2. The activity of the enzyme increased in the first period after the irradiation (from 4 hr. to 5 days) when the tissues underwent a rapid decrease in weight. Histological examination showed then extensive pycnoses of lymphocytes, fragmentation of cells and phagocytosis. In the second period, in which reconstitution of the tissues took place, the behaviour of the enzyme activity was variable, depending on the organ and on the dose of radiation administered.

3. After X-ray irradiation the positive correlation between increase of β -glucuronidase activity and atrophy of the tissues, already found in the atrophy

of lymphatic tissues caused by fasting, was confirmed.

4. The increase in β -glucuronidase activity observed should be considered as a secondary effect of the irradiation, as it can be reproduced by different experimental conditions such as fasting.

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An Improved Method of Preparing Nisin

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Nisin, produced by some strains of *Streptococcus lactis*, is one of the family of polypeptide antibiotics which has not yet been used medically (Gowans, Smith & Florey, 1952). It has, nevertheless, a practical as well as an academic interest, since it is used in the cheese-processing industry (Berridge, 1953).

The preparation of nisin has already been the subject of several papers. Mattick & Hirsch (1947) described the production of concentrates having a specific activity of two '1947 units'/ μ g., with a yield of 20%. Owing to the absence of adequate precautions it is only likely and not certain that the '1947 units' were almost equal to those in use now. Probably the concentrates contained about 4% of actual nisin. Concentrates made by similar methods were used for the preparation of crystalline nisin (Berridge, 1949). Losses occurred in this further purification, but they were diminished by reactivation which occurred at one stage. Since the

raw material for the preparation of crystals was made commercially it was impossible to ascertain the overall yield, but it was probably of the order of 10%.

A newer method was described by Falconer (1952). In this method nisin was caused to remain with the bacterial cells by maintaining the pH at about 6. After the cells had been collected the nisin was extracted from them with acidified aqueous acetone or other solvents, and further purified by methods similar to those described earlier (Berridge, 1949). Considerable increases in yield were claimed, but no actual figures were given, and it is probable that the increases were due to the higher activity produced in the brew by techniques described at the same time elsewhere by Hirsch (1951).

The methods by which a desired substance may be concentrated and purified depend not only on the properties of the substance, but also on those of the