IRRADIATION LESIONS OF THE GROWTH PLATE IN RABBITS

F. ARGÜELLES, F. GOMAR, JUN., A. GARCIA and J. ESQUERDO, VALENCIA, SPAIN

The effects of gamma irradiation on the growth plate have been studied in nineteen rabbits with a 1,000 rads/skin dose. The rabbits were killed after one to ninety days. The growth plates were studied by microscopic examination, thymidine- H^3 autoradiography, and fluorescence with radiographic measurement. Changes were already detected after twenty-four hours at the cell mitosis level, which showed the sensitiveness of the chondrocyte itself. The lesions were clearly seen with the optical microscope after seven days, and they were most advanced between the fourteenth and twenty-first day after irradiation. Regeneration of the cartilage began in the fourth week and the histological appearance became normal after seventy days. Fluorescence with tetracycline showed a temporary retardation of growth, with consequent shortening of the affected limb.

The effects of irradiation on growth have been known since the reports of Perthes (1903), Tribondeau and Récamier (1905), Försterling (1906), Latarjet (1912), Segale (1920) and Phemister (1926), among others. Between 1930 and 1940 the published papers on this subject became more numerous, all of them emphasising that irradiation brings a series of histological changes in the growth plate and consequently an alteration of bone growth; this has been demonstrated in man as well as in the experimental animal (Baserga, Lisco and Cater 1961).

Nevertheless, some aspects of the effect on the growth plate have not been clearly shown, doubt remaining about whether the apparent histological lesions in the growth plate and the consequent retardation of growth were caused by direct action on the chondrocytes or were secondary to damage to the neighbouring structures such as blood vessels, osteoblasts and cells of the bone marrow (Hinkel 1943; Kember 1967; Moss and Brand 1973; Vaughan 1973).

For this reason we have not confined our studies to the histological alterations already noted by other authors, but have also examined by thymidine- H^3 and by autoradiography, the action of the gamma rays upon the cartilage cells so far as their reproductive capacity is concerned, and we have studied the formation of new bone with the aid of the fluorescence technique after administration of tetracycline.

It is well known that the growth plate is very sensitive to irradiation compared with other tissues even from the same origin such as fibrocartilage and hyaline cartilage (Engel 1938).

From the results obtained by various authors (Brooks and Hillstrom 1933; Dahl 1934; Bisgard and Hunt 1936; Hinkel 1942, 1943; Kember 1967) it seems that a number of factors influence the histological changes found in the growth plate after irradiation, and that the changes are proportional to the radiation delivered and inversely proportional to the animal's age

at the time of irradiation. Other factors that influence the changes include the species of animal, sex, weight, metabolic level, tissue growth, presence or absence of dehydration and, experimentally, the interval between irradiation and death of the animal (Barr, Lingley and Gall 1943).

The dose of rays is undoubtedly the most important factor and following Kember's (1967) work, we found that up to 800 rads produced a retardation of bone growth which was temporary and reversible, characteristically with precocious histological changes after sixty hours and evidence of regeneration beginning after the second week (Dahl 1934; Regen and Wilkins 1936; Gall, Lingley and Hilcken 1940; Hinkel 1943; Sissons 1956). With 800 to 1,800 rads cellular survival was less, bone growth was held back for a longer period, and there might be premature cessation of growth if irreparable damage were produced in the germinal cells (Sissons 1956; Kember 1967). With a dose of more than 1,800 rads complete arrest of growth was achieved, though evidence of later regeneration was found on occasions (Regen and Wilkins 1936; Lacassagne and Gricouroff 1941; Woodard 1954; Barnhard and Geyer 1962; Bensted and Courtenay 1965).

MATERIAL AND METHODS

In this study we used young rabbits, animals with which we were already familiar from previous experiments and whose chondrogenesis, mitotic capacity, metabolism and reaction of the cartilaginous tissue from various situations were known.

We used a total of nineteen immature rabbits, divided into three series. The rabbits were three weeks old at the beginning of the study and had an average weight of 350 grams.

The left knee was used for irradiation, the right as a control. Feeding and living conditions were identical for all animals.

Histological study—Nine rabbits were used. The left knee was irradiated after the animal had been anaesthetised by ether inhalation, the rest of the animal being protected by lead sheets. Conventional radiotherapy apparatus was used at 180 kilowatts, using a filter of 0.5 millimetre of copper and 1 millimetre of aluminium. The distance was 30 centimetres

Dr F. Argüelles, Menedez Pelayo, 33, Valencia, Spain.

Dr F. Gomar, Jun., Primado Reig, 116, Valencia, Spain.

Dr A. Garcia, Orthopaedic Department, Faculty of Medicine, University of Valencia, Valencia, Spain.

Dr J. Esquerdo, Matemático Marzal, 24, Valencia, Spain.

FIG. 1

FIG. 2

Figure 1—The irradiated growth plate. Normal histology, but virtually no uptake of thymidine- H^3 . Figure 2—Control growth plate. (Methyl green—pyronin, $\times 400$.)

and the area was 2×4 centimetres. The dose administered to all the animals was 1,000 rads (skin) in a single session on the same day.

The rabbits were killed at successive intervals of twenty-four hours, and seven, fifteen, twenty-one, twenty-eight, forty-five, seventy-five and ninety days after irradiation.

Histological sections were made from both knees, using two techniques of staining: haematoxylin and eosin, and toluidine blue, the last one to study the metachromasia of cartilage.

Autoradiographic study with thymidine- H^3 —Five rabbits were used. Irradiation was as described above and the rabbits were killed at the same intervals thereafter as in the previous series. The study was not prolonged further because, once the rabbit is two months old the mitotic capacity of cartilaginous tissue diminishes considerably, and this would make the study very difficult.

Thymidine- H^3 was injected intracardially two hours before the animal was killed, in a dose of 1 microcurie per gram weight. This time was sufficient for the thymidine to be incorporated in chondrocytes at the moment of mitotic division.

The autoradiographic study was done with AR-10 Kodak stripping film. After development, the cells stained by thymidine- H^3 were examined under an optical microscope, the specimens having previously been stained with methyl green-pyronin.

Fluorescence studies of bone growth after administration of tetracycline—Four animals were used. They too received the 1,000 rads to the left knee. One animal was killed one and a half months after irradiation, which allowed enough time for regeneration of the growth plate to be evident, and the others

were killed after two months, two and a half months and three months. A fifth animal of the same litter served as a control and was killed with the last of the irradiated animals.

A dose of 50 milligrams of oxytetracycline per kilogram of weight was given intramuscularly to the animals twenty-four hours before they were killed.

After death of the animals, both femora and tibiae were removed, radiographed and measured. Next, the lower end of the femur and the upper end of the tibia were removed, sectioned in the coronal plane and photographed under ultra-violet light; the areas of newly-grown bone are coloured greenish-yellow.

RESULTS

After twenty-four hours—The histology and metachromasia of both growth-plates of the irradiated knee appeared normal, with no histological difference from the non-irradiated knee (Figs. 1 and 2). On autoradiographic examination the premitotic index of the irradiated knee was practically zero, compared with the finding in the control knee of many marked cells in the proliferative and germinative strata.

After seven days—There was marked alteration of the whole growth-plate of the irradiated knee. The nuclei in the germinative and proliferative layers showed obvious pyknosis, as well as degeneration of the matrix and marked disorganisation of the columnar arrangement, with isolated chondrocytes and signs of necrosis. The

degenerated zone showed an obvious reduction of thickness (Fig. 3). On the other hand, the metachromasia of the ground substance was unchanged.

The number of cells marked with thymidine- H^3 was the same as that in the animals killed at twenty-four hours.

After fourteen days—The growth-plate of the irradiated knee showed normal structure and vascularity. However its thickness was reduced, because the germinative and proliferative strata had disappeared. Dying chondrocytes with cytoplasmic acidophilia were evident (Fig. 4). A slight metachromatic loss was seen in this cartilage.

The results of autoradiographic study were similar to those obtained after seven days.

After twenty-one days—The growth-plates were normal, with noticeable vascularisation in the germinative strata. Cells with an apparently lively aspect began to appear, and there was abundant proliferation of piling cells. However, the thickness of the plate remained less than that of the protected knee. The metachromatic properties were restored to normal.

In the autoradiographic study with thymidine- H^3 , the number of marked cells was only slightly less than in the protected knee.

After twenty-eight days—The structure of both growth plates in the irradiated knee appeared normal, although the proliferative strata were not very well developed. The metachromasia of the intercellular substance was normal.

The premitotic indices were practically the same in both knees.

After forty-five days—The growth plates of the irradiated knee showed signs of regeneration. There were still vascular remnants of necrosed growth plate, but there was no metachromatic alteration.

Autoradiographic studies were not carried out because the rabbits were more than two months old, and the proliferative capacity of the growth plate diminishes considerably. Also, the recovery of the premitotic index was checked three or four months after irradiation.

FIG. 3

Seven days after irradiation. The germinative layer shows a shrinking of the matrix with pyknosis of the nuclei. The proliferative layer shows mucous degeneration of the matrix and disorganisation of the columnar arrangement. (Haematoxylin and eosin, $\times 190$.)

FIG. 4

Fifteen days after irradiation. The growth plate has become thinner. The germinative and proliferative layers have disappeared. Dying chondrocytes with cytoplasmic acidophilia are predominant. (Haematoxylin and eosin, $\times 250$.)

FIG. 5

Eight weeks after irradiation. A clear regeneration and reorganisation of the growth plate is seen. There are remnants of the necrosed plate. (Haematoxylin and eosin, $\times 100$.)

A clear reduction of tetracycline uptake was observed in the growth plates, up to two and a half months after irradiation. At three months the uptake was practically the same in both knees. Radiographic measurement of femur and tibia showed that the irradiated limb was 4

millimetres shorter than the control limb three months after irradiation, tibia and femur contributing equally to the disparity.

DISCUSSION

Gamma radiation produces anatomical and pathological alterations in both growth plates of the rabbit's knee. There is a relatively brief, latent period with a temporary closure of the growth plate which is, however, totally reversible, with regenerative changes beginning about three weeks after irradiation.

Our histological results conform with those of Dahl (1934), Hinkel (1943) and Sissons (1956) in their studies of albino mice subjected to irradiation of 600 rads. Nevertheless, the lesions were not so marked as those obtained by Sissons (1956) and Kember (1967) who used a dose of 1,500 to 2,000 rads to achieve a total halt in growth.

The absence of lesions in the control knee clearly indicates that alterations were provoked by local and not by general action of the irradiation. We think that the lesion in the growth plate is mainly produced by direct action on the chondrocyte, though a secondary action, caused by vascular alteration, may occur.

In support of this theory, there was no thymidine H^3

uptake twenty-four hours after irradiation, which indicates suppression of the proliferative capacity of the chondrocytes, long before the histological changes and thrombosis of small vessels appear.

The inhibition of mitotic capacity of the growth plate was maintained during the period of maximum histological alteration, between seven and fourteen days. Its recovery ran parallel to the process of regeneration: great premitotic activity was detected in the newly formed germinative and proliferative zone after about twenty-one days.

The histological and premitotic changes in the chondrocytes suggest an effect on the growth of the bone (Sinclair and Morton 1965). Because we did not obtain very important differences of length as compared with those found by Hinkel (1943) and Kember (1967), we studied osteogenesis by means of tetracycline and by detailed radiographic measurement. An important difference of length was found, although it was not so great as that found by other workers using similar doses.

Through the study of bone formation by means of fluorescence with tetracycline we showed re-formation of the growth plate of the irradiated knee. It began to recover after two to two and a half months and, after three months was similar to that of the control knee (Fig. 5).

REFERENCES

- Barnhard, H. J., and Geyer, R. W. (1962) Effects of X-radiation on growing bone. *Radiology*, **78**, 207-214.
- Barr, J. S., Lingley, J. R., and Gall, E. A. (1943) The effect of roentgen irradiation on epiphyseal growth. I. Experimental studies upon the albino rat. *American Journal of Roentgenology and Radium Therapy*, **49**, 104-115.
- Baserga, R., Lisco, H., and Cater, D. B. (1961) The delayed effects of external gamma irradiation on the bones of rats. *American Journal of Pathology*, **39**, 455-472.
- Bensted, J. P. M., and Courtenay, V. D. (1965) Histological changes in the rat bone after varying doses of X rays with particular reference to bone tumour production. *British Journal of Radiology*, N.S. **38**, 261-270.
- Bisgard, J. D., and Hunt, H. B. (1936) Influence of roentgen rays and radium on epiphyseal growth of long bones. *Radiology*, **26**, 56-64.
- Brooks, B., and Hillstrom, H. T. (1933) Effect of roentgen rays on bone growth and bone regeneration. *American Journal of Surgery*, N.S. **20**, 599-614.
- Dahl, B. (1934) Effects des rayons X sur les os longs en développement. *Journal de Radiologie, d'Électrologie*, **18**, 131-140.
- Engel, D. (1938) An experimental study of the action of radium on developing bones. *British Journal of Radiology*, N.S. **11**, 779-803.
- Försterling, K. (1906) Ueber allgemeine und partielle Wachstumsstörungen nach kurz dauernden Röntgenbestrahlungen von Säugethieren. *Archiv für klinische Chirurgie*, **81**, 505-530.
- Gall, E. A., Lingley, J. R., and Hilcken, J. A. (1940) Comparative experimental studies of 200 kilovolt and 1000 kilovolt roentgen rays. I. The biological effects on epiphysis of the albino rat. *American Journal of Pathology*, **16**, 605-618.
- Hinkel, C. L. (1942) The effect of roentgen rays upon the growing long bones of albino rats. I. Quantitative studies on the growth limitation following irradiation. *American Journal of Roentgenology and Radium Therapy*, **47**, 439-457.
- Hinkel, C. L. (1943) The effect of roentgen rays upon the growing long bones of albino rats. II. Histopathological changes involving endochondral growth centers. *American Journal of Roentgenology and Radium Therapy*, **49**, 321-348.
- Kember, N. F. (1967) Cell survival and radiation damage in growth cartilage. *British Journal of Radiology*, **40**, 496-505.
- Lacassagne, A., and Gricouloff, G. (1941) *Action des radiations sur les tissus*. Paris: Masson & Cie.
- Latarjet, H. (1912) Resultats expérimentaux sur l'accroissement des os en longueur. *Comptes Rendus de l'Association des Anatomistes*, **14**, 72.
- Moss, W. T., and Brand, W. N. (1973) *Radioterapia Clinica*, pp. 493-527. Barcelona: Ed. Salvat.
- Perthes, G. (1903) Ueber den Einfluss des Röntgenstrahlen auf epithelial Gewebe insbesondere auf das Carcinom. *Verhandlungen der deutschen Gesellschaft für Chirurgie*, **32**, 525.
- Plemister, D. B. (1926) Radium necrosis of bone. *American Journal of Roentgen and Radium Therapy*, **16**, 340-348.
- Regen, E. M., and Wilkins, W. E. (1936) The effect of large doses of x-rays on growth of young bone. *Journal of Bone and Joint Surgery*, **18**, 61-68.
- Segale, G. C. (1920) Sull'azione biologica dei raggi Röntgen e del radium sulle cartilagini apofisarie. *Radiologia medica*, **7**, 234-246.
- Sinclair, W. K., and Morton, R. A. (1965) *Cellular Radiation Biology*. Baltimore: Williams & Wilkins.
- Sissons, H. A. (1956) Experimental study of the effect of local irradiation on bone growth. In *Progress in Radiobiology*. Proceedings of the Fourth International Conference on Radiobiology, Cambridge 1955, pp. 436-448. Edited by J. S. Mitchell, B. E. Holmes and E. L. Smith. Edinburgh and London: Oliver and Boyd.
- Tribondeau, L., and Récamier, D. (1905) Altérations des yeux et du squelette facial d'un chat nouveau-né par roentgenisation. *Comptes Rendus de la Société de Biologie*, **58**, 1031.
- Vaughan, J. M. (1973) *The Effects of Irradiation on the Skeleton*. Oxford: Clarendon Press.
- Woodard, H. Q. (1945) Some effects of roentgen rays on mouse bone. *Radiation Research*, **1**, 567.