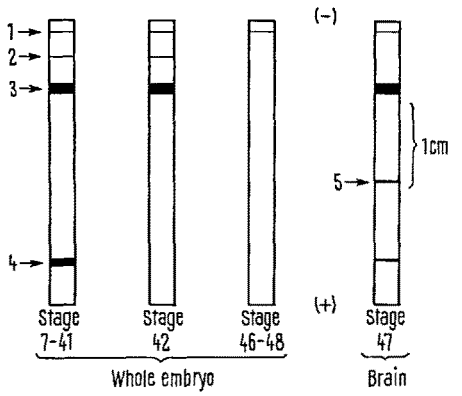
(an MAO inhibitor) was added to the anticonvection medium to a concentration of 0.25 mg/ml[13]. 100 µl of these samples were used for each run. A running buffer of *Tris* glycine at pH 8.3 was employed and the constant current supply was set to deliver 0.5 mA at 400 volts. A run required about 50 min for the bromphenol blue front to migrate through 3 cm of the running gel[11]. Gels were transferred to an incubation medium containing 25 mg tryptamine hydrochloride, 4 mg sodium sulfate, 5 mg nitro-blue tetrazolium, 5 ml 0.1 *M* phosphate buffer at pH 7.6, and 15 ml water. The gels were incubated at 37 °C for 40 min, washed in tap water, and fixed in 10% buffered formalin[13].

Results. It is possible to demonstrate 4 separate kinds of MAO in whole *Xenopus* embryos (Figure). All 4 are demonstrable for stages between blastula (Stage 7) and pre-swimming (Stage 41). One of these disappears by swimming (Stage 42), and only one remains by Stage 46. For the brain preparations at Stage 47 there are again four separate kinds of MAO. Three of these are similar to whole embryo MAO, but one is clearly different. Control runs with MAO inhibitor indicated no MAO in either whole embryo or brain preparations.

Discussion. Various investigators have been able to demonstrate multiple forms of MAO in rat liver by cellulose acetate electrophoresis[7] and polyacrylamide electrophoresis[4,8–10]. MAO assay on whole *Xenopus* embryos has shown a constant increase in enzyme activity during development. This proceeds in phases, showing a series of accelerations after hatching (Stage 38) and swimming (Stage 42), and a slowing down after Stage 46[14]. Measurements of 5-hydroxytryptamine, a major MAO substrate, show significant shifts in level at these same stages[15]. MAO bands 3 and 4 are identical to bands from similar preparations stained with the general protein stain amido black[16]. There is reason to believe that these 2 fractions are in part yolk products being consumed during differentiation[16]. This is in part supported by yolk utilization studies indicating that the terminal phase of yolk breakdown occurs around Stage 46[17]. In this same context, there is evidence for yolk formation in association with mitochondria[18], and MAO is a mitochondrial enzyme[19]. The possibility of a yolk association for MAO during development deserves serious consideration, and has been suggested previously[15]. The pattern of MAO for Stage 47 brain indicates that there are specific organ variations from the whole embryo pattern. At least one MAO band in brain is not demonstrable in whole embryo. Specific brain patterns have been demonstrated in both rat and human tissues[4,7–9,20].



Multiple forms of monoamine oxidase in developing *Xenopus laevis*.

Zusammenfassung. Das Isoenzymmuster der Monoaminoxidase verändert sich während der frühen Entwicklung der Larven von *Xenopus laevis*. Die Anzahl elektrophoretischer Banden verkleinert sich während der Entwicklung von vier auf eine.

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[13] G. C. GLENNER, H. J. BURTNER and G. W. BROWN, J. Histochem. Cytochem. *5*, 591 (1957).
[14] P. C. BAKER, Devl Biol. *14*, 267 (1966).
[15] P. C. BAKER, Acta Embryol. Morph. exp. *8*, 197 (1965).
[16] P. C. BAKER, Acta Embryol. Morph. exp. in press.
[17] G. G. SELMAN and G. J. PAWSEY, J. Embryol. Morph. exp. *14*, 191 (1965).
[18] J. WILLIAMS, in *The Biochemistry of Animal Development* (Ed. R. WEBER; Academic Press, New York 1965), vol. I, p. 14.
[19] B. JARROTT and L. L. IVERSON, Biochem. Pharmac. *17*, 1619 (1968).
[20] The author was supported by a grant from the Cleveland State University Faculty Research Committee. The author would like to express his thanks to JOHN Y. PUN the president of Bionix (El Cerrito, California) for the use of the apparatus.

Combined Surgical and Radiation Injury V. The Effect of Bone Marrow Transplantation

Previous studies in this laboratory have demonstrated a delay in wound contraction in irradiated rodents which is most pronounced when the surgical wound follows irradiation by 4 days[1]. This retardation of wound contraction is corrected, in part, by the prior administration of radioprotective compounds[2] or partial bone marrow shielding during radiation[3], but not by the administration of antimicrobials[4]. The purpose of the present study was to evaluate and compare the wound contraction process in non-irradiated rodents, irradiated rodents and rodents subjected to whole body X-irradiation and subsequently transplanted with synginaic bone marrow.

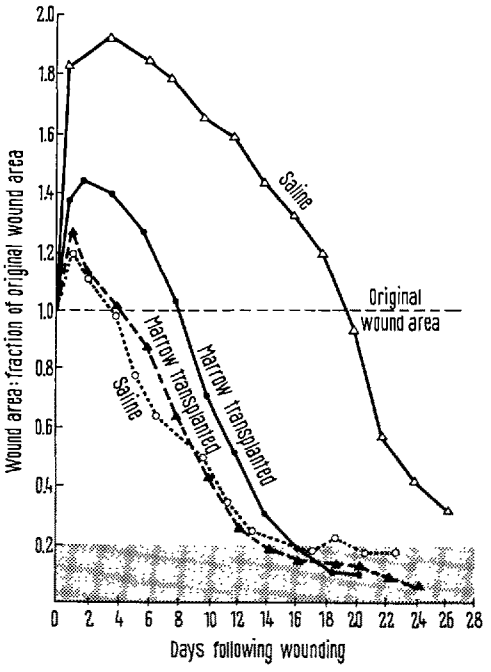
Inbred female Lewis rats, 6–9 weeks of age, housed in a controlled environment and allowed water and food ad libitum, were used in all experiments. In preliminary studies, the LD 50/30 was determined to be 675 R X-ray. The rats were divided into 4 groups of 32 rats each. One group was not irradiated and marrow transplanted, a 2nd group was irradiated and given saline, a 3rd group was irradiated and marrow transplanted and the 4th group was not irradiated and given saline.

Harvesting of bone marrow was accomplished following sacrifice of donor rats by injecting Nembutal i.p. Femurs, tibias and humeri were removed and placed in Hanks' solution. The end of each bone was removed and aspirated in Hanks' solution at 37 °C using a 20 G needle. In order to obtain a homogenous suspension, a series of decreasing sized needles down to 25 G were used. The bone marrow suspension was then filtered through sterile gauze and washed twice in Hanks' solution at 37 °C. The average of 4 hemocytometer counts of nucleated cells were used to measure the cell concentration.

Animals were exposed 16 at a time in a perforated rounded flat plexiglass cage, on a rotating turn-table to 675 R X-ray produced by a 250 KVP X-ray unit operated at 15 MA, with a HVL of $^1/_4$ mm copper and 1 mm aluminium. The dose rate was 30.4 R/min. Controls were treated in a similar manner without X-ray exposure. One hour following exposure, a bone marrow suspension containing 2×10^8 cells was injected i.v. into one-half of the irradiated animals, physiological saline solution (0.9%) was injected i.v. into the remaining animals. One-half of animals which were sham irradiated were also transfused with bone marrow cells as above.

Wounding was performed 4 days following radiation under light ether anesthesia. The hair was clipped from the dorsal surface. A circle measuring approximately 3 cm in diameter was marked over the high lumbar region in the mid-line. The circular piece of skin was removed by sharp dissection down the areolar tissue to the deep subcutaneous fascia. There was no immediate mortality. The wounds were left open and not treated in any manner. The longitudinal and lateral diameters of the wounds were measured at the time of wounding and regularly thereafter. The products of the longitudinal and lateral diameters of the wounds were calculated and the decrease in the value of these products with time was used as an index of wound contraction.

The results of the wound contraction patterns of these 4 groups of animals is presented in the Figure. There are no differences between the non-irradiated marrow transplanted or saline treated wound contraction patterns. The wounds of the irradiated animals demonstrated a marked initial enlargement of the wound to a size 90% greater than the original wound area as compared with a 25% enlargement in the non-irradiated animals. Subsequently, following a lag phase of 4–6 days, the irradiated wounds established a rate of contraction similar to the other groups. This retardation of the wound contraction pattern following radiation is compatible with our prior reports[1–4] and the reports of others[5–7]. The bone marrow transplanted irradiated animals and the non-irradiated rats treated with either bone marrow transplantation or saline demonstrated similarity as to be almost identical in the curves of wound contraction.



Wound healing curves. Each point represents the average of 32 animals. The curves of the irradiated animals are indicated by solid lines. The curves of the non-irradiated animals are indicated by the interrupted lines.

In both instances, the non-irradiated and the bone marrow transplanted irradiated wound contraction patterns are markedly different from the saline treated irradiated group of animals. These results provide further evidence that bone marrow is necessary for the normal process of wound contraction. In addition, these results provide further information which strongly suggests that the radiation induced retardation of wound healing can be corrected by the institution of a functional marrow by means of transplantation. We have suggested in prior publications[2,3] that the marrow may exert an effect on wound healing either directly by providing precursor cellular elements which are necessary in tissue repair, indirectly by providing either a humoral substance which acts to initiate or retard wound healing or alternatively by providing non-specific support for the wound healing process by rendering the animal less susceptible to infection and the catabolic effects of radiation.

Studies with antimicrobial therapy[4] would suggest that infectious agents do not markedly retard wound contraction of themselves, and that the radiation induced delay in wound healing did not appear to be mediated primarily through infection. Therefore, the two hypotheses which require further exploration are the possibility of the participation of specific bone marrow elements such as the lymphocyte in wound healing or the possibility of a humoral mediated mechanism controlling wound repair. Both of these possibilities are presently being investigated in our laboratories.

Zusammenfassung. Es wird gezeigt, dass bei Nagern Röntgen-Ganztierbestrahlung mit 675 R während 4 Tagen vor Wundsetzung zu einer beträchtlichen Verzögerung der Wundkontraktion führt. Diese Verzögerung konnte durch eine Knochenmarktransplantation aufgehoben werden.

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1 Lw. R. Stromberg, K. T. Woodward, D. T. Mahin and R. M. Donati, Ann. Surg. *167*, 18 (1968).
2 Lw. R. Stromberg, M. M. McLaughlin and R. M. Donati, Proc. Soc. exp. Biol. Med. *129*, 140 (1968).
3 Lw. R. Stromberg, K. T. Woodward, D. T. Mahin and R. M. Donati, Experientia *23*, 1064 (1967).
4 R. M. Donati, M. M. McLaughlin and Lw. R. Stromberg, unpublished results.
5 H. Langendorff, O. Messerschmidt and H. J. Melching, Strahlentherapie *125*, 333 (1964).
6 D. Zelman, I. C. Song, D. D. Porteus and B. E. Bromberg, Bull. N.Y. Acad. Med. *45*, 293 (1969).
7 H. C. Grillo and M. S. Potsaid, Ann. Surg. *154*, 741 (1961).
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