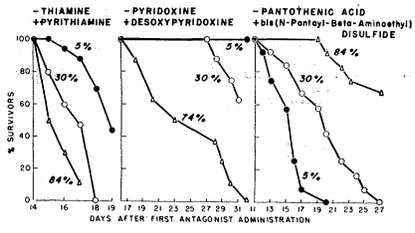

Fig. 1. Survival rate of B-factor-deficient rats on purified diets with varying casein levels and supplied with the appropriate antivitamin.

doxine; observations were made of growth, food intake, development of the "acute deficiency" state (*8*), and survival time. In all these aspects, the animals on 74-percent casein showed the most severe changes; those receiving 5 percent showed scarcely any and the ones fed 30 percent were in between. Death, in particular, occurred earliest with 74-percent casein and not at all during the time of observation among those on 5-percent casein (Fig. 1). Thus, the use of an antagonist does not alter, but strongly intensifies, the differences in pyridoxine requirements, which go parallel to the casein content of the diet. The rapid occurrence of a severe deficiency state at high dietary protein levels is indicative of the great importance of pyridoxine in the protein-metabolizing enzyme systems. As in the case of riboflavin and thiamine, pyridoxine and protein are mutually limiting factors.

It has been found that the signs of pantothenic acid deficiency become less pronounced if the carbohydrate in the diet is partly replaced by protein (*9*); no antagonists were used in those experiments. In our experiments, groups of 12 rats received 5,- 30-, and 84-percent casein in pantothenic acid-deficient diets and daily injections of 10 milligrams of the pantothenic acid antagonist, bis(*N*-pantoyl-beta-aminoethyl) disulfide (*10*). In Fig. 1 it is demonstrated that the survival rate of these animals increased very significantly when carbohydrate in the diet was replaced by protein. Although the casein had been specially purified, these experiments (just as previous ones) did not entirely rule out the possibility that the milder form of the deficiency state was the result of the presence of traces of pantothenic acid in the casein. This argument could be entirely refuted by an experiment in which eight animals that received 30-percent casein were restricted in their food intake so that they just maintained their body weights. This group survived the freely eating, slowly growing, deficient animals on the same diet. Therefore, the longer life span of animals on the high casein level is not the result of traces of pantothenic acid in the casein because the animals on the restricted food intake that survived their controls received less casein. The experiment further demonstrates that the signs of the deficiency state are less pronounced if the food intake is restricted.

Despite the rigid exclusion of pantothenic acid from the diet and the use of a potent antagonist, increased dietary protein levels decrease the pantothenate requirements; thus, pantothenic acid and protein are probably not mutually limiting factors. Inasmuch as, in these experiments, high dietary protein levels mean low carbohydrate levels and vice versa, these experiments indicate that such mutual limitation exists between carbohydrate and pantothenate.

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References and Notes

1. H. M. Evans and S. Lepkovsky, *Science* 68, 298 (1928); *J. Biol. Chem.* 83, 269 (1929).
2. H. Kaunitz *et al., J. Nutrition* 52, 467 (1954).
3. Leo A. Pirk of Hoffman-La Roche, Inc., Nutley, N.J., generously supplied us with most of the vitamins used.
4. W. J. Dann, *Federation Proc.* 4, 153 (1945).
5. B. C. P. Jansen, in *The Vitamins*, W. H. Sebrell, Jr., and R. S. Harris, Eds. (Academic Press, New York, 1954), vol. 3, p. 472.
6. D. W. Woolley, *A Study of Antimetabolites* (Wiley, New York, 1952).
7. L. R. Cerecedo and J. R. Foy, *Arch. Biochem.* 5, 207 (1944).
8. H. C. Stoerk, *Ann. N.Y. Acad. Sci.* 52, 1302 (1950).
9. M. M. Nelson and H. M. Evans, *Proc. Soc. Exptl. Biol. Med.* 60, 319 (1945); 66, 299 (1947).
10. We are very grateful to Merck and Company, Rahway, N.J., for supplying us with galactoflavin, desoxypyridoxine, and the pantothenic acid antagonist. The properties of the latter have been discussed by G. E. Boxer, C. E. Shonk, H. C. Stoerk, *Federation Proc.* 14, 185 (1955).

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Regeneration of X-rayed Salamander Limbs Provided with Normal Epidermis

It has been suggested that a large part of the regenerate of a salamander limb arises from epidermal cells (*1, 2*). The evidence for this is that, as the blastema forms, approximately the same number of mesenchymal regeneration cells appear as there are epidermal cells disappearing. It is believed that the epidermal cells change into mesenchymal cells *in situ* in the epidermal cap. This transformation had been described earlier (*3*) but had not been interpreted as evidence for a permanent change. Other investigators, while confirming the loss of many epidermal cells during blastema formation (*4, 5*), believe that these cells die, but this has not been studied quantitatively. Still others, for various indirect reasons, believe that a transformation from differeniated epidermis to a variety of internal limb tissues is unlikely (*6–10*). Direct evidence that epidermal cells can transform to mesenchymal cells was obtained by tracing polyploid epidermis on a diploid limb stump and later recovering it as mesenchymal tissue (*11*). There is still a question whether the marked mesenchymal cells that had been epidermal could have completed the transformation to differentiated mesodermal tissue.

Because of general interest in the extent of cellular transformation during regeneration and because there have been different interpretations of data already presented, a more rigorous test of the possibility of epidermal transformation has been made.

The best method for testing the potency of a tissue during limb regeneration is the method of transplanting it to an x-rayed limb that is incapable of growth or regeneration. Sufficient x-irradiation completely prevents growth and regeneration. The change appears to be permanent and irreversible (*12*). If normal tissue is transplanted to an x-rayed limb and the limb is subsequently amputated at the level of the graft, whatever regenerates must arise from the grafted tissue. The method has been used previously to demonstrate that bones, muscles, and whole skin can each produce whole limbs (*13, 14*). In the present work (*15*), an attempt has been made to learn whether the epidermis alone can furnish the cells for limb regeneration.

Both forelimbs of 55 adult *Triturus viridescens* were x-rayed below the elbow with single dosages of 500 to 10,000 roentgens. The conditions of irradiation were 150 kv and 8 ma with a delivery rate of 745 r per minute at a distance of 11.5 cm. The upper arms and the rest of the animal were protected by lead shields. Left forelimbs served as controls. These were irradiated and either 7 or 28 days later were amputated midway between wrist and elbow. Right forelimbs were treated in the same way except that, in addition, whole skin was stripped off the stump up to the unirradiated tissue 1 mm above the elbow at the time of amputation. It was known from work with polyploid skin transplants that only the epidermis migrates and piles up at the tip of the stump (*11*). Hence, in the right limbs, the epidermis that migrates to cover the stumps was unirradiated.

On all 55 animals the limbs that became covered with unirradiated epidermis regenerated. No complete hands regenerated from the control stumps. After the 3 lowest x-ray dosages, 500, 1000, and 2000 r, there was some outgrowth

from the irradiated control stumps, but these outgrowths in all cases were simple cartilaginous spikes covered by skin. After a dosage of 3500 r or more, there was complete absence of regeneration on the control side, but all x-rayed limbs that were provided with unirradiated epidermis showed some hand regeneration. This included the experimental limbs in the groups that received 3500, 5000, 7500, or 10,000 r. There were at least 5 useful cases in each radiation group except in the group that received 10,000 r. All but one animal in the 10,000-r group died before regeneration had been completed. The type and amount of regeneration is shown for 4 representative cases in Fig. 1.

Appreciable growth potential remained in the 500-r series when all tissues were irradiated. When unirradiated epidermis has been added, the form of the regenerate is better and the amount of regeneration is greater. With dosages from 3500 to 10,000 r there was no outgrowth after both internal tissues and epidermis had been irradiated. However, there was some regeneration even from atrophied stumps whose internal tissues had received higher dosages, provided that they had been covered with unirradiated epidermis. Diminishing size of regenerate with increasing dosage may be accounted for in large part by the decreasing growth potential of the internal tissues. At 3500 r and above there was no outgrowth from controls and the stumps became smaller. From this dosage upward, any regeneration that occurred in the right limb must have resulted from the unirradiated epidermis.

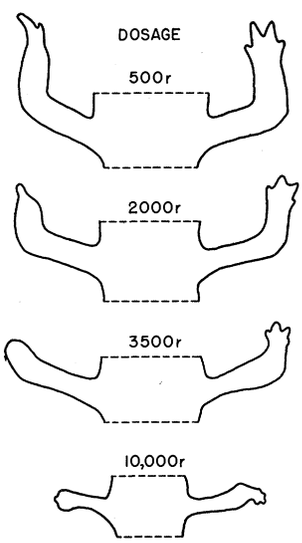

Fig. 1. Representative regenerates showing extent and type of regeneration after various x-ray dosages. The limbs were irradiated below the elbow. All limbs were amputated half way between the wrist and elbow. Left, regeneration after simple amputation through forearm; right, regeneration after amputation and removal of skin to a point above the elbow. The limbs on the right received unirradiated epidermis. The diagrams are drawings from photographs of the same magnification.

Some of the decrease in size of regenerates may result from increasingly deleterious effects of the x-rayed stumps upon the regenerates. This was quite apparent in the 10,000-r series, where failure of the epidermis to maintain a complete cover around and over the stump was observed. In spite of this there was regeneration of a recognizable hand on the one 10,000-r animal that lived.

The possibility that the unirradiated epidermis reactivated the internal tissues has been considered seriously (*16*), but all evidence is against it. As shown by Brunst (*12*), x-rayed limbs remain for years incapable of regeneration. There is additional counterevidence in the present work. The stumps of all x-rayed limbs lost some volume. The presence of an unirradiated epidermis does not reactivate to protect against this loss. In all cases the shrinkage of the stump was the same whether irradiated or unirradiated epidermis was present. This is evidence against reactivation.

Direct evidence that only the unirradiated tissue participates in regeneration was obtained by Umanski (*47*). After a dosage of 5000 r to hind limbs of black axolotls whose internal tissues are dark, followed by transplantation of forelimb skin from white axolotls and subsequent amputation, completely white forelimbs regenerated on the stumps of the hind limbs. Umanski suspected that the dermis was the source of the regeneration cells, but the hypothesis was not tested.

It is concluded that epidermis *can* serve as the only source of cells for a regenerating limb. It now appears likely that any limb tissue can serve as a source of regeneration cells. Whether one is the predominant source both in larvae and adults is not clear. Better regenerates were obtained from x-rayed stumps when normal whole skin was transplanted than when either muscle or bone was used (*18*).

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References and Notes

1. E. Godlewski, *Wilhelm Roux' Arch. Entwicklungs-mech. Organ.* 114, 108 (1928).
2. S. M. Rose, *J. Exptl. Zool.* 108, 337 (1948).
3. A. Ide-Rozas, *Wilhelm Roux' Arch. Entwicklungs-mech. Organ.* 135, 552 (1936).
4. D. T. Chalkley, *J. Morphol.* 94, 21 (1954).
5. H. W. Manner, *J. Exptl. Zool.* 122, 229 (1953).
6. E. G. Butler and O. E. Schotté, *ibid.* 112, 361 (1949).
7. P. Weiss, *Quart. Rev. Biol.* 25, 177 (1950).
8. H. D. Heath, *J. Exptl. Zool.* 122, 339 (1953).
9. A. G. Karczmar and G. G. Berg, *ibid.* 117, 139 (1951).
10. J. S. Nicholas, in *Analysis of Development* (Saunders, Philadelphia and London, 1955) sect. 13, chapt. 2.
11. E. D. Hay, *Am. J. Anat.* 91, 447 (1952).
12. V. V. Brunst and E. A. Cheremetieva, *Arch. zool. exptl. et gen.* 78, 57 (1936); V. V. Brunst, *Quart. Rev. Biol.* 25, 1 (1950).
13. E. Umanski, *Biol. Zhur.* 6, 757 (1937).
14. C. S. Thornton, *J. Exptl. Zool.* 89, 375 (1942).
15. This study was in part supported by the National Science Foundation grant G 115.
16. F. C. Rose, H. Quastler, S. M. Rose, *Anat. Record* 117, 619 (1953).
17. E. Umanski, *Bull. biol. med. Exptl. U.R.S.S.* 6, 141 (1938).
18. H. A. L. Trampusch, *Koninkl. Ned. Akad. Wetenschap. Proc.* 54C, 373 (1951).

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Action of Pectic Enzymes on Surface Cells of Living Brassica Roots

Robert's conclusion (*1*) that the root-hair wall consists of an inner layer of cellulose and an outer layer of calcium pectate continuous with corresponding layers in the hair-forming cell has been confirmed and denied (*2*). I obtained strong confirmatory evidence (*3*) from microchemical tests and by growing roots of *Brassica* seedlings in a variety of cultural solutions that were designed either to prevent or to stimulate calcification of the pectic layer. In this connection, the experiments with ammonium oxalate solutions were particularly convincing (*3*). A theory of root-hair development was formulated, that was based on the gradual hardening of the outer pectic layer to calcium pectate (*2–4*).

More recently, Ekdahl (*5*) considers the pectic and cellulosic substances to be uniformly distributed in the hair-wall and not separated into two distinct layers. In his opinion, calcification does not occur and hardening is due entirely to changes in the cellulosic substances.

Ekdahl's view still lacks direct proof and does not explain the fact that anything which prevents calcification also prevents root-hair formation. Furthermore, in denying the existence of an outer pectic layer or so-called middle lamella substance, his view fails to explain many well-known cellular phenomena such as the normal sloughing of root-cap cells, the formation of intercellular spaces, and the maceration of multicellular tissues by pathological organisms and chemical reagents.

The recent use of pectic enzymes in the maceration of plant material (*6*) suggested that they might be useful in the present problem. If an outer cementing layer of pectic material occurs in the epidermal cell walls, then it should dissolve on treatment of the root with pectic enzymes. To test the validity of this assumption, *Brassica* seedlings (*Brassica napus* var. oleifera), were grown in several different preparations of pectic enzymes