T R A N S P L A N T A T I O N

I N

T A D P O L E S

O F

R A N A C A T E S B E I A N A

Harold D. Ames

1941 - 1942

C O N T E N T S

INTRODUCTION

Biology is the study of living organisms. The study of the vital functions of these organisms is physiology. The common method of study is by observation. Observations may be made under natural or under experimental conditions. Experimental conditions may mean a controlled environment of an otherwise normal vital process, or that the nature of the vital process is altered in a controlled manner. Transplantation is one of the methods of experiment employed in the latter field.

An understanding of the process of development is of utmost importance if the nature of living organisms is to be comprehended. Perhaps no function of the organism is so characteristic of life as is development. It may safely be said that where there is life there is development, and where there is development there is life. Apart from psychical phenomena, nothing expresses so clearly the contrast between a living organism and a lifeless mechanism as the intricate factor-complexes conerened in development.

The study of development may be divided into two problems: (1) fertilization and the stimulus to development, (2) the process of development. The latter involves the various interrelations concerned in development, their functions, and the agencies upon which they depend. There is probably no other branch of Biology which leads one so directly to

fundamental questions concerning the living organism.

In experiments on an organism by physical and chemical methods, no results can be secured except those which can be measured in terms of Physics and Chemistry. If a really biological method is used, specifically biological results may be obtained. Some Biologists believe that such results must be explained with regard to the "wholeness" of the organism. This type of explanation is referred to as "organismic". The experimental analysis of development provides an extension of the biological method to a limited degree. By this analysis a just comprehension of the nature of the organism can be attempted. Pure observation reveals only the externals of the process, while experimentation can disclose what forces are at work, and their modes of action.

The first aim in experimentation is to analyse and reduce complicated processes to simple terms. The ultimate aim is to reassemble and synthesize the results obtained to form a coherent pattern of development. This can only be done by attacking the many individual questions involved.

The process of development may be considered under two headings, (1) growth, (2) differentiation. Growth takes place by cell proliferation, and by increase in the size of individual cells. Differentiation is much more complex. In order to discuss this topic, it is necessary to use special terms and concepts, the explanations of which are

given below.

There are two divergent views of the nature of development. According to the preformistic theory, the development of the individual from the egg is a process of unfolding of rudiments, as in the opening of a flower bud. The mosaic theory of Weismann is a slight modification of this view. He believed that the egg is a mosaic of parts, each of which will develop into a predetermined part of the adult animal. A pure epigenetic view differs fundamentally from the above. Interpreting this theory, development is not a simple unfolding but a real origination; all the parts develop from beginning to end in continual correlation. Potency is the power possessed by any part of the germ to develop under abnormal conditions into more or different morphological parts than it would normally. The conception of potency is especially important in the experiments to be described. If the part of the germ does not possess this power, and will not develop under abnormal conditions into anything more than it normally does, it is said to be determined. If a part of the organism is labile (possesses potency) changes may be induced in that part by processes occuring in other parts. This induction may be illustrated in Embryology by the method of formation of the lens of the eye by the inducing action of the optic cup on the external ectoderm. If a part which is labile becomes adapted to new conditions and takes on characteristics

which depend upon position rather than on origin, it is said to have been regulated. When a part is capable of developing to its predetermined destiny independent of any outside influence, it exhibits self-differentiation.

Transplantation

In the analytical investigation of development is is often necessary to isolate individual parts of the germ, or individual tissues and organs, by removing them from their normal position, and thus from the action of factors to which they are exposed in the complete organism. Further, part of one germ may be brought in the zone of influence of another germ, and thus the interaction of two living complexes may be studied. In making such experiments the procedure is that of transplantation. In certain cases explantation, tissue culture, may be more effectively employed. Of course, in the process of fertilization, two living complexes interact. The science of Genetics is a statistical study of this interaction. However, it is difficult to perform experiments in the field of Genetics; the best that can be done is to control the conditions for normal development.

Transplantation, or grafting, means the artificial fusion of two organisms, or the transference of some tissue or organ to a different position in the same individual, or to any position in another individual. Apart from the theoretical importance of transplantation, it has great

from one region to another should retain its pecularities. If the transplanted tissue should acquire the characters of the region into which it has been moved, the result would indicate that it had become regulated and that its local differentiation depends upon position and other factors external to the epidermis itself.

MATERIAL AND METHODS

The experimental animals were second year bull-frog tadpoles (Rana catesbeiana) obtained from Roland Brown's "Aquarium Supply House". The equipment consisted of aquaria, fingerbowls, watch glasses, crystallizing dishes, wax, anaesthetic, isotonic solution, operating instruments, and a binocular dissecting microscope with a spotlight attachment; also the usual glassware and reagents for histological preparations.

It was necessary to have an anaesthetic which would keep the tadpoles quiet during the operation, but would have no prolonged adverse effect on the animal. Chloretone is the anaesthetic commonly used in such cases. A series of comparisons was made, using various concentrations of chloretone and of M.S. 222. M.S. 222 is the trade name of a product manufactured by "Chemical Works", (formerly Sandoz), Basle, Switzerland. It was found that 2 parts in 10,000 of M.S. 222 was much the most satisfactory.

The tadpole was taken from the aquarium and placed in a solution of 2 parts M.S. 222 in 10,000 parts of tap water. It was left here until it became quiet - usually 3 to 5 minutes - and then transferred to a solution of 1 part M.S. 222 in 10,000 parts of Ringer's solution for cold-blooded vertebrates. The tadpole was transferred in this solution to an operating dish prepared from a syracuse watch glass, by partially filling it with wax and making a mould to fit the tadpole. This served to keep the animal in position during the operation.

In the cases of skin transplantation, a piece of skin (about 4 by 6 millimeters) was removed from the back of one tadpole and replaced by a similar sized piece from the ventral side of another. In one case - tadpole # 13 - a reciprocal transplantation was made. Some of the difficulties involved were (1) the slippery and tough nature of the tadpole skin, (2) the problem of cutting the graft the exact size of the place prepared for it in the host, (3) the fact that the transplant had to be cut larger than the area it was going to cover because of shrinkage during the transfer. Careful manipulation and sharp implements overcame the first difficulty. It was found that sharp scalpel, needles, and forceps were much better for most purposes than scissors. The use of one tadpole as the donor for several of the experimental animals overcame the last two problems.

After the operation, the tadpole was left in the operating dish, under anaesthetic, for a period of about two hours in order to allow the graft to become firmly attached. During this time it was kept covered with a crystallizing dish to prevent any desiccation. After this period, the tadpoles were transferred to individual fingerbowls of fresh water. Most of them regained consciousness within five minutes. In the cases of a few which did not, it was thought advisable to apply artificial respiration. This was done by forcing water through the gills with a pipette until breathing movements started. Resuscitation was immediately accomplished in all but one case. It was found necessary to cover the fingerbowls with glass plates in order to prevent the tadpoles from jumping out.

Following the operations, the tadpoles were examined daily; later at weekly intervals. Notes and sketches were made of the size and shape of the grafts, and particularly of their pigmentation. It was hoped that by watching the nature of any pigment change, it would be possible to gain some information regarding the specificity of the grafted tissue, and of the nature of the interaction between the host and graft.

The tadpoles were fixed in Bouin's fluid, and preserved in 70 % alcohol. Histological preparations were made of

twelve representative types. Where it was possible, the graft was cut in two and a whole mount prepared from the posterior portion, and serial sections cut from the anterior portion. The whole mounts were mounted in 'clarite' unstained except for a small residue of picric acid from the fixing solution. The sections were stained in Mann's Methyl-Blue Eosin mixture, and destained in Dobell's differentiating solution.

EXPERIMENTS

All of the transplants are homoioplastic. A total of 39 tadpoles were operated on, of which 10 were used as donors, or were otherwise unsuitable for observation. Of the 29 experimental animals, 15 were used for transplantation of skin, 7 were the object of limb transplantation, 4 of limb excision, and 2 of eye transplantation. The latter groups were done in order to make a qualitative investigation of the relation between homoiotransplants and host in Amphibian larvae, and in the hope that they might give some clue as to the reaction taking place in the case of the epidermal grafts.

The observations of the individual cases follow. The time elapsed from operation to observation is given in 'number of days'. All the sketches, unless otherwise stated are twice natural size.

Skin Transplantation

Case # 1.

This animal was found desiccated on the table 2 days after the operation, and therefore served no purpose except to instigate precautions (glass plates over fingerbowls) against a similar fate for the others.

Case # 2.

This was used as a donor for # 1.

Case # 3.

A square of skin was cut from the right middle region of the back and replaced with skin from the ventral side of tadpole # 4.

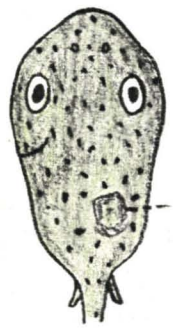
1 day - graft established, tadpole recovered.

7 days - graft pulling up in the center, slight pigmentation around the margin.

16 days - graft bunched in the center, and wound area filled in by scar tissue.

20 days - graft area very little pigmented. Sketched.

27 days - dead, preserved, not sectioned.

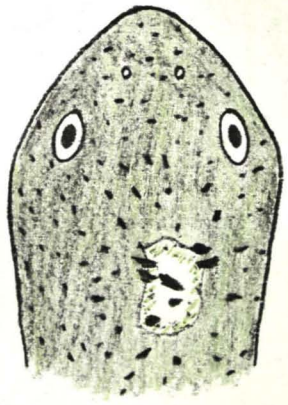


20 days.

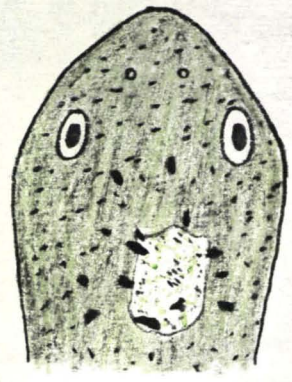
30 days - shape of graft is becoming more "shield-like"; green pigment (lipochrome) is appearing just within the margin of the graft. The pigment aggregations have changed shape, and two of them are continuous from the host to graft area. The pigment in the host about the graft area is not as dense as elsewhere, and there is some indication of radial arrangement of pigment spots in the host area about the graft. Sketched.

39 days - general conditions are unchanged, band of greenish pigment about the border slightly wider, small specks of green and of black pigment throughout the graft. Sketched.

46 days - little change, some rearrangement of pigment aggregations, one large black spot is continuous from graft to host; there are more definite black specks throughout the graft area. Sketched.

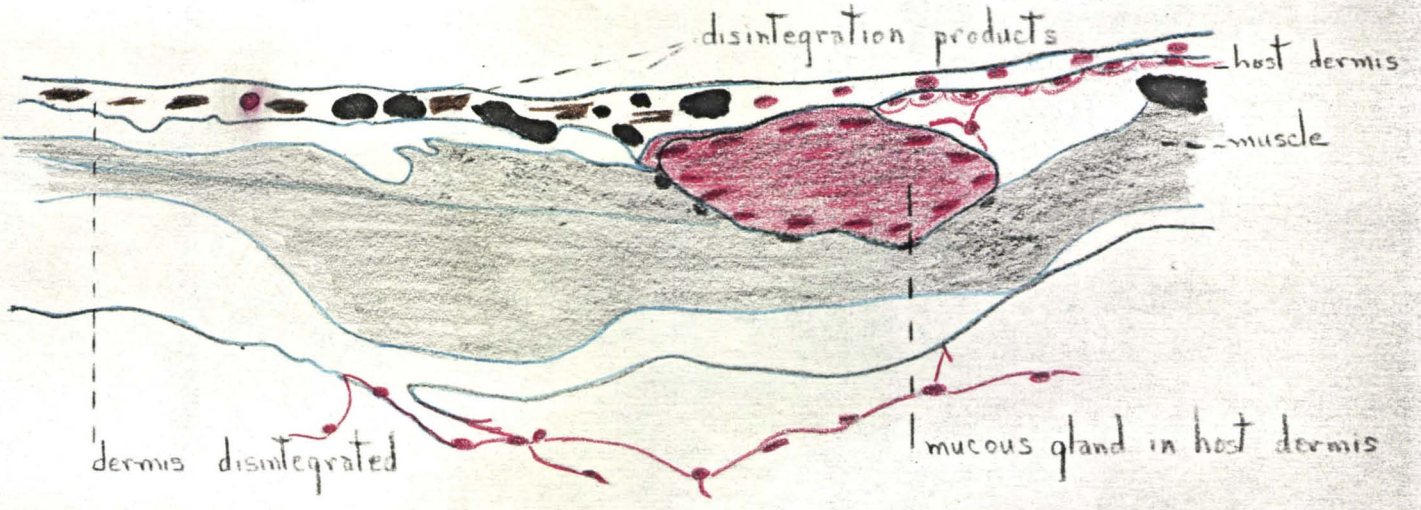


39 days



46 days

82 days - dead, fixed, whole mount and sections prepared. The whole mount shows less pigment in the graft area than in the host area. In the graft area there is a considerable amount of irregular dark brown material which is not pigment.



graft area host area

A typical section, X 280.

Case # 6.

A piece of skin was cut from the median line of the back near the tail, and replaced by a strip from the ventral side of tadpole # 4.

1 day - graft established, a roll of host tissue bunched at the anterior edge of the graft.

7 days - some scattered pigment spots.

16 days - dark pigment areas at the anterior and the posterior.

20 days - regularly scattered greenish pigment spots, no really black spots except at the very anterior and posterior. Sketched.

29 days - dead, preserved, not sectioned.

20 days

Case # 7.

This animal was used as a donor for tadpoles # 8, 9, 10, 11, and 12.

Case # 8.

A strip of skin was cut from the left side of the tail near the base, and replaced with a strip of ventral skin from tadpole # 7. A blood vessel was cut accidentally.

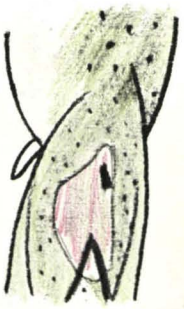
1 day - graft established, host well.

7 days - graft pinkish, some black pigment at the posterior.

12 days - graft slightly red, little pigment.

16 days - three large black pigment aggregations, two of them continuous into the host skin; still a pinkish tinge in some areas. Sketched.

26 days - shape of graft has become much more irregular; pink colour has all gone; one very black spot, some greenish streaks, some radial arrangement of pigment in the host about the graft. Sketched.

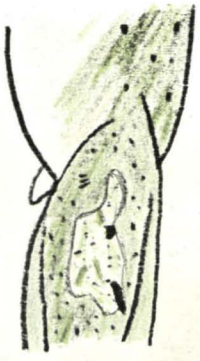


16 days

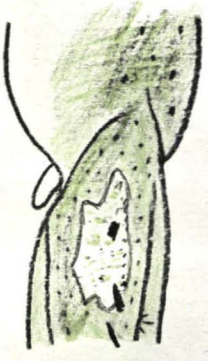
26 days

35 days - only three greenish streaks left; two large black pigment aggregations, one of which is continued into host tissue; numerous specks of pigment, mostly black. Sketched.

42 days - shape of graft has become still more irregular; all the grren streaks are gone; one large black pigment aggregation, another smaller one continuous with the host epidermis, numerous small black pigment specks, especially along the dorsal margin. Sketched.

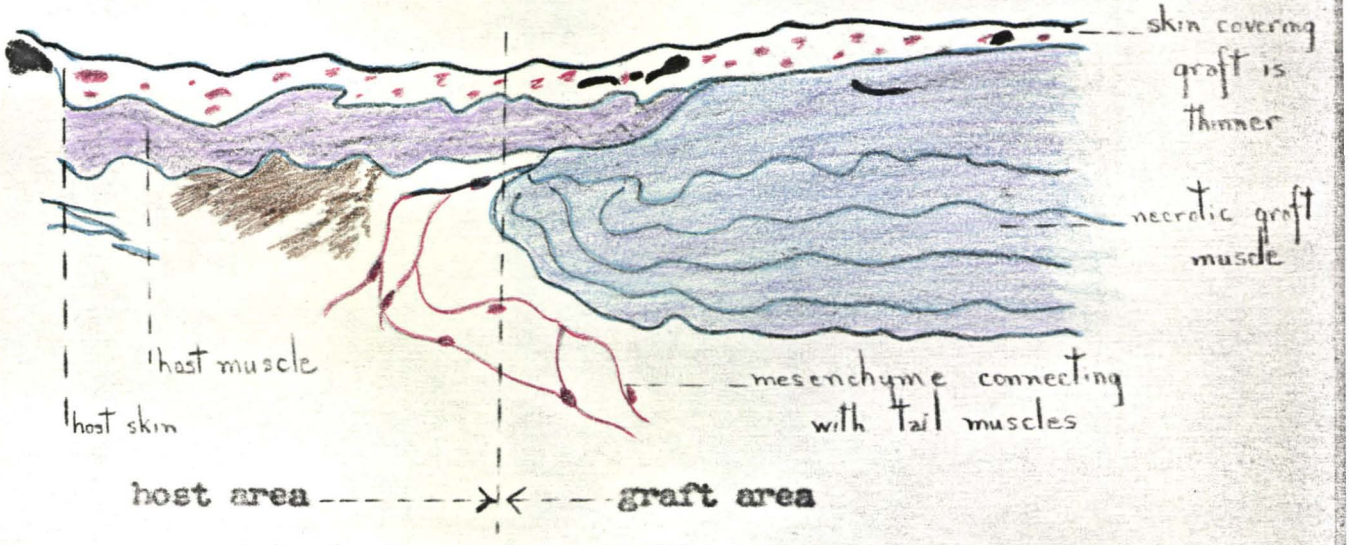


35 days



42 days

58 days - tadpole fixed while under anaesthetic, whole mount and sections prepared.



A typical section X 280.

Case # 8.

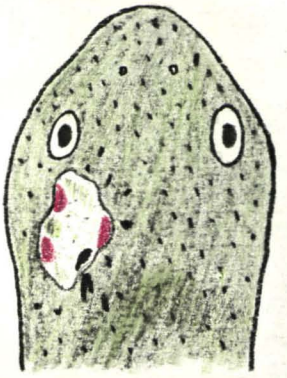
A piece of skin was taken from the back just behind the left eye, and replaced with skin from the ventral side of tadpole # 7.

1 day - graft only fairly established, some unevenness.

12 days - graft somewhat wrinkled at the edges, parts of it quite red.

16 days - three red areas, one black pigment spot, and a few specks. Sketched, (on next page).

23 days - dead, preserved, not sectioned.



16 days.

Case # 10.

An opening was accidentally cut into the body cavity during the operation. The animal died within a day, discarded, not preserved.

Case # 11.

A square of skin was cut from the left side of the tail, and replaced with ventral skin of # 7. A blood vessel in the tail was accidentally cut.

1 day - graft well established.

12 days - pigmentation scarcely noticable, some pinkish area.

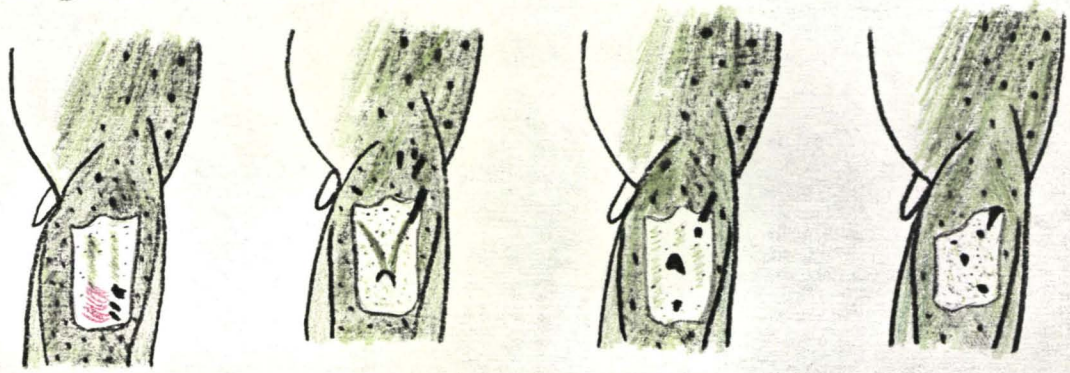
16 days - some areas of greenish pigment, and three small black spots; all of these are well away from the edge of the graft; still some pink. Sketched.

26 days - all pink gone, green and black areas changed considerably; one black pigment aggregation is continuous

from host into graft. Sketched.

35 days - graft very light coloured, four spots and a few specks of black, and a few specks of green. Sketched.

42 days - all green pigment gone, four small aggregations, and numerous small specks of black; the longitudinal axis of the graft has become shorter. Sketched.



16 days 26 days 35 days 42 days

65 days - dead, preserved, not sectioned.

Case # 12.

A piece of skin was removed from the right side of the tail, and replaced with skin from the throat of # 7.

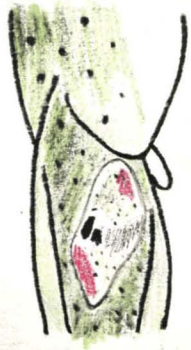
1 day - graft well established, a good transplantation.

7 days - a small amount of green pigment.

12 days - some pinkish areas in the graft; it appears somewhat like the neighbouring tail skin.

16 days - pink areas still present, two black pigment aggregations, and scattered areas of green and black pigment

specks; it blends fairly well with the surrounding host skin.



16 days

In considering this it must be rembered, however, that the graft tissue was from the throat, and hence possessed more pigment than ordinary ventral skin, before it was transplanted.

25 days - dead, preserved, but not sectioned.

Case # 13.

This is the reciprocal operation to all the other skin transplantations. A piece of ventral skin was successfully removed just posterior-ventral to the branchial opening, and was replaced with dorsal skin from tadpole # 14. The results in this case are more important than those of any other single case.

1 day - graft appears like a scab since it is thicker and darker than the surrounding skin.

5 days - graft has lost some of its green colour, and hence is more brown than normal dorsal skin; the amount of black pigment seems to be normal. The graft is in a difficult position to observe, since it is almost impossible to keep the tadpole on its back without anaesthetizing it.

9 days - the tadpole was anaesthetized in order to make more careful observations. The graft is a shade lighter

near the margin; the black pigment has diminished; the graft as a whole is mainly brown with a greenish tinge. Sketched.

19 days - black and green pigment almost all gone; the brown colour is not so abundant at the anterior and posterior ends of the graft. Sketched.

28 days - the brown colour is disappearing from larger areas, especially at the posterior, and even in the center the brown portion is broken up to some extent by interspersed colourless areas; there is a slight black elevation near the anterior of the graft. Sketched.

9 days

19 days

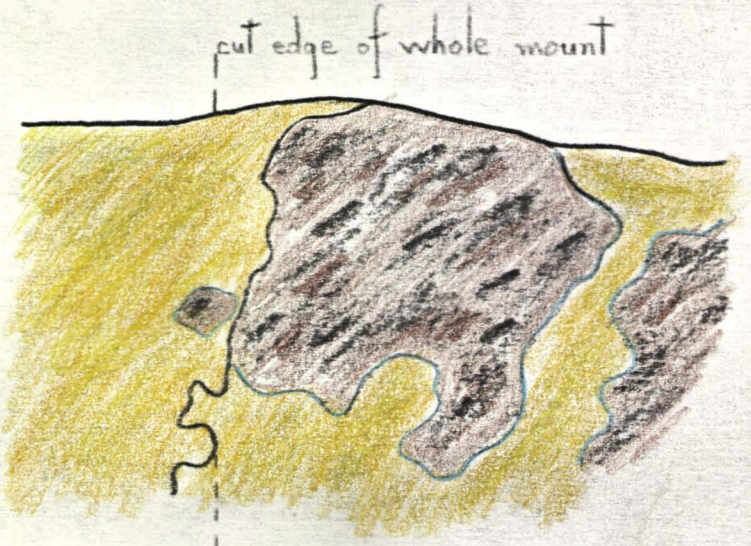
28 days

35 days - the ventral side of the graft is folded under to a slight extent; the brown colour is disappearing everywhere; the graft is still very distinct from the neighbouring ventral skin due to greater pigmentation and greater thickness.

56 days - dead, fixed, whole mount and sections prepared. The sections are rather unsatisfactory, but a representative

one is illustrated. The whole mount shows the demarcation between the host and graft; The difference between host tissue and graft tissue is very obvious when the whole mount is studies under the microscope; the graft contains considerable black pigment, while the surrounding host tissue contains none; in the graft area there are large, diffuse, irregular patches of a brownish black appearance; most of the colour of the graft area is due to this substance which will be discussed later.

35 days



Whole Mount X 280 - 56 days.

typical section, X 280.

Case # 14.

This animal was used as donor for tadpoles # 13, 15, 17, 18, and 19.

Case # 15.

A square of skin was cut from the right side of the tail, and replaced with skin from the ventral side of # 14.

1 day - graft slightly wrinkled at three places along the border.

5 days - the graft is not smooth, greenish pigment is appearing along the dorsal side, there is a kink in the tail.

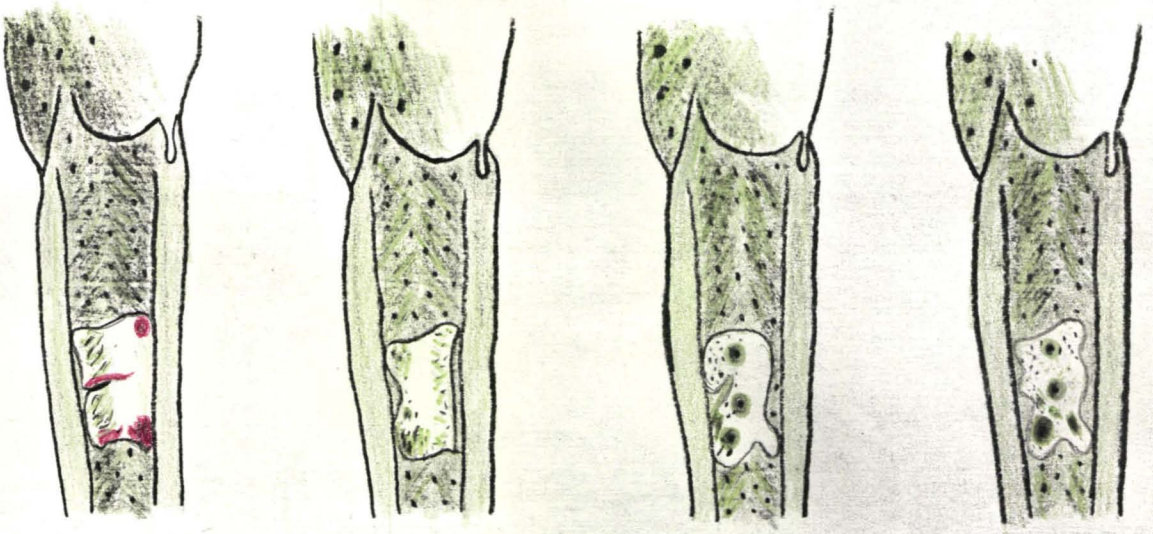
9 days - There are three protrusions along the border which have become red; green and black pigment specks are appearing along the dorsal side. Sketched.

19 days - graft is slightly more irregular in shape; the red areas are gone, black and green pigment along the dorsal border is breaking up into patches. Sketched.

28 days - graft has become still more irregular in shape, and the edges more rounded; three small black pigment spots surrounded by green now appear in the center; many black pigment specks near the dorsal edge. Sketched.

35 days - shape of graft still more irregular; more black pigment spots and specks scattered throughout the graft.

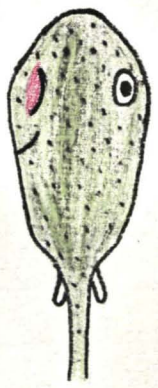
93 days - dead, preserved, not sectioned.



9 days 19 days 28 days 35 days

Case # 16.

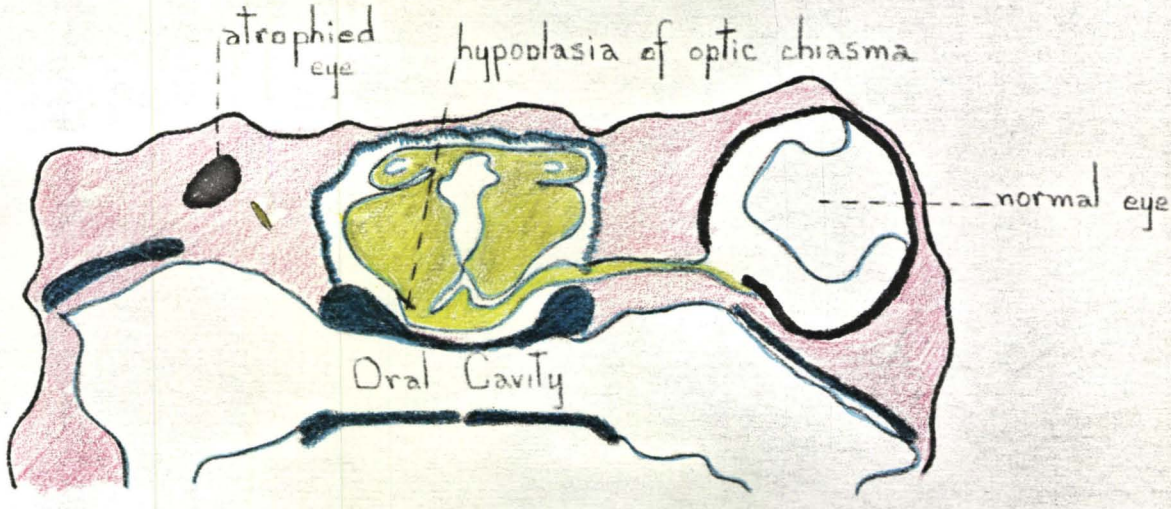
This animal was not operated on. It was noticed that the left eye was apparently missing, and the animal was kept in order to investigate. In the place where the left eye should have been, there was only a transparent circular area in the skin through which the optic cavity could be seen, but no eye was visible. The cavity was pinkish. Sketched.



natural size.

The tadpole was later fixed and sectioned. The sections

showed that the left eye was not missing, but rather had atrophied. The sections ellustrate very well the effect of undeveloped organs on the central nervous system. This is similar to the effect of grafted organs that are out of scale. These results are included because they illustrate in an interesting way the relations between organs and nervous system. In the section illustrated, the optic chiasma is unequally developed. A similar section through the midbrain shows hypoplasia of the right side.



Section through the optic chiasma.

Case # 17.

A piece of skin was removed from the right side of the tail, and replaced with a piece from the ventral side of tadpole # 14.

1 day - graft well established, very smooth and even.

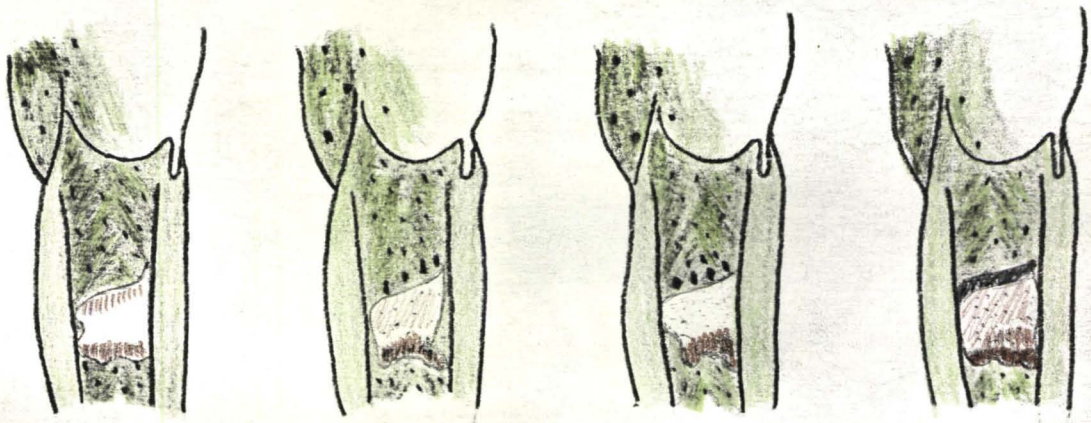
5 days - a small band of pigment is appearing along the anterior and posterior edges.

12 days - some bunching of the graft has appeared at the posterior, and the fold thus formed contains considerable black pigment. Sketched.

19 days - the posterior portion of the graft has become quite brown; pigment at the anterior has dispersed. Sketched.

28 days - there seems to be less black pigment in the brown portion at the posterior of the graft, several large black pigment aggregations have appeared in the host tissue just anterior to the graft. Sketched.

35 days - very little changed, but more pigment specks throughout the graft area; the pigment in the host tissue just anterior to the graft has become spread out along the anterior margin of the graft. Sketched.



12 days 19 days 28 days 35 days

51 days - dead, preserved, not sectioned.

Case # 18.

A piece of skin was removed from the left side of the tail and replaced with skin from the ventral side of tadpole

14. The area from which the graft was taken included the posterior throat region.

1 day - graft well established, no new pigment.

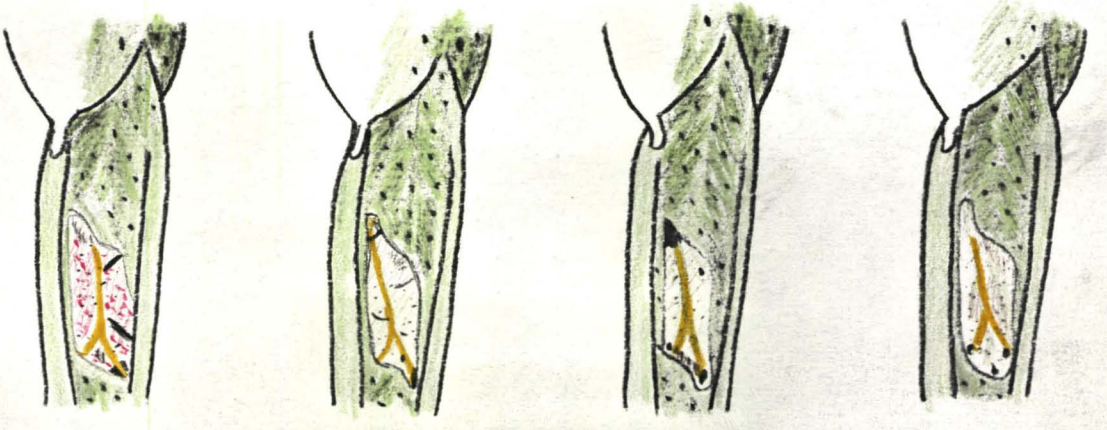
5 days - slight pinkish tinge, no pigment.

12 days - miscellaneous specks of pigment; a large yellow "Y" down the middle; two black streaks, one of which is continuous from host to graft; some pinkish area. Sketched.

19 days - pink colour gone; some pigment appearing near the anterior border; the yellow "Y" is even more evident. Sketched.

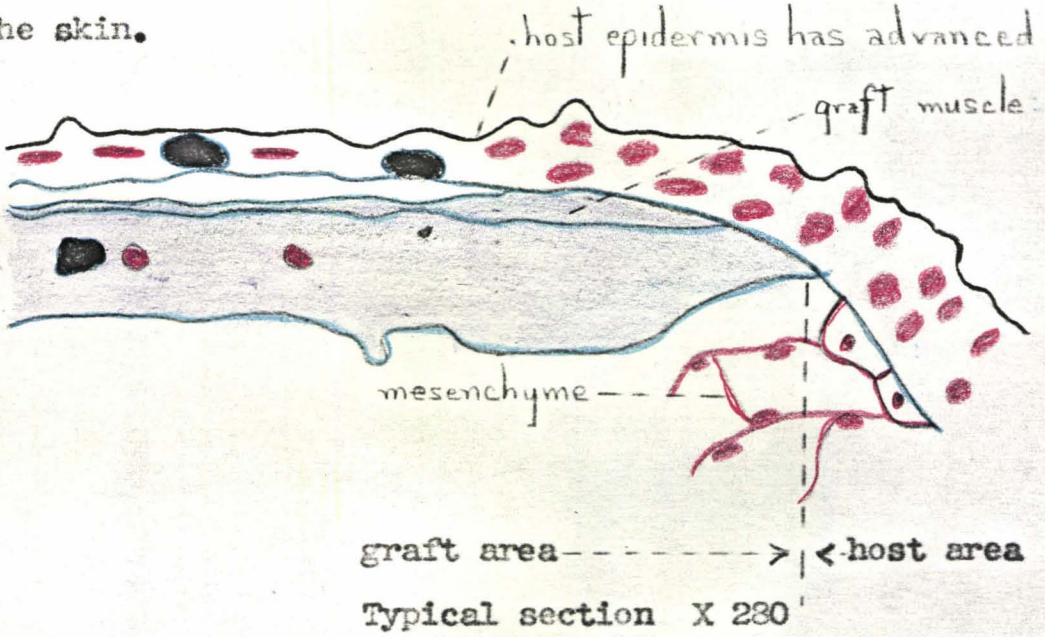
28 days - the yellow "Y" has become faint and greenish; some bunching at the anterior ventral corner, the fold thus formed containing much black pigment; a greater number of pigment specks throughout the graft area, and a narrow band of pigment at the posterior. Sketched.

35 days - a fine speckling of pigment all over, with a few minor aggregations at the anterior and posterior; the fold at the anterior has disappeared; the greenish-yellow "Y" is more evident again. Sketched.



12 days 19 days 28 days 35 days

86 days - dead, fixed, sections and whole mount prepared. The sections show that a layer of muscle was transplanted with the skin.



Typical section X 280

Case # 19.

A piece of skin from the left side of the tail was removed and replaced with skin from the throat of # 14.

1 day - graft is well established, a good transplantation.

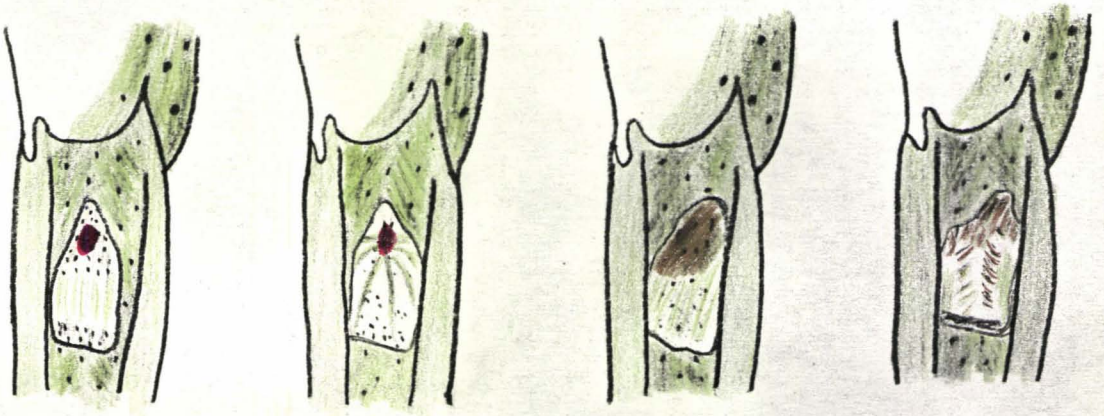
5 days - graft has become uneven at the anterior end; no new pigment.

12 days - graft somewhat curled under at the deges; some new pigment in the anterior third; a large brown spot near the anterior apex. Sketched.

19 days - the brown spot is not so dark, streaks of greenish pigment radiate posteriorly from it; a speckling of black pigment throughout the graft. Sketched.

28 days - considerable brown and green pigment in the anterior half of graft area; black specks throughout. Sketched.

35 days - considerable black and brown pigment in the area where the spot was, but no regular arrangement; an infolding of the graft at the posterior edge, and considerable black pigment in the resulting fold; the longitudinal axis of the graft has shortened. Sketched.



12 days 19 days 28 days 35 days

48 days - dead, preserved, not sectioned.

Case # 20.

This animal was used as a donor for # 21, 22, 23, 24, 25, 26, and 27.

Case # 21.

This tadpole never recovered from the anaesthetic, artificial respiration did not revive; discarded, did not preserve.

Case # 22.

A piece of skin was removed from well down the left side of the tail and replaced with ventral skin of # 20.

1 day - graft well established.

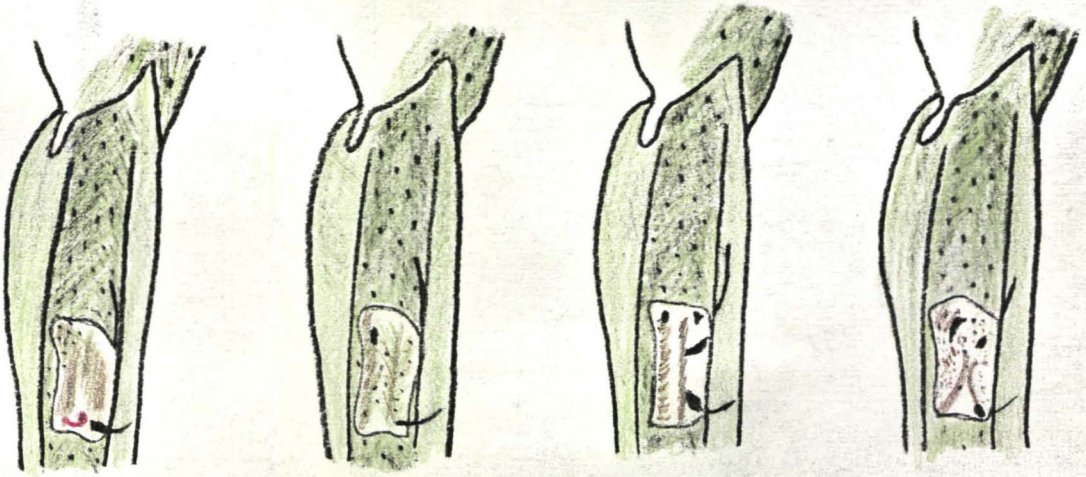
3 days - two cuts which were made in the skin outside of the graft area show up black.

10 days - graft pinkish, a few small spots of melanin, and scattered specks of green. Sketched.

17 days - pinkishness gone; black cuts in the host skin much less distinct; a green streak the length of the graft; considerable melanin along the ventral margin, and some scattered throughout the graft. Sketched.

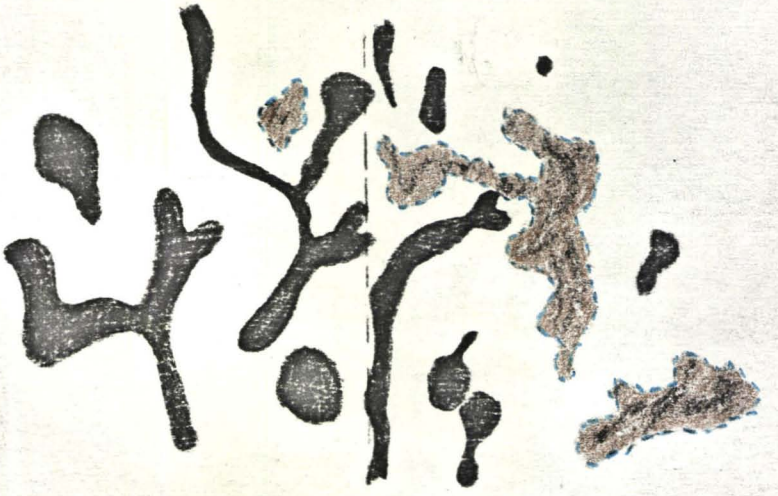
26 days - considerable green and brown pigment; one large aggregation of black at the posterior dorsal corner; no obvious reason for the arrangement of the pigment. Sketched.

33 days - black specks all over, especially along the ventral margin; some green and brown scattered through the center. Sketched.

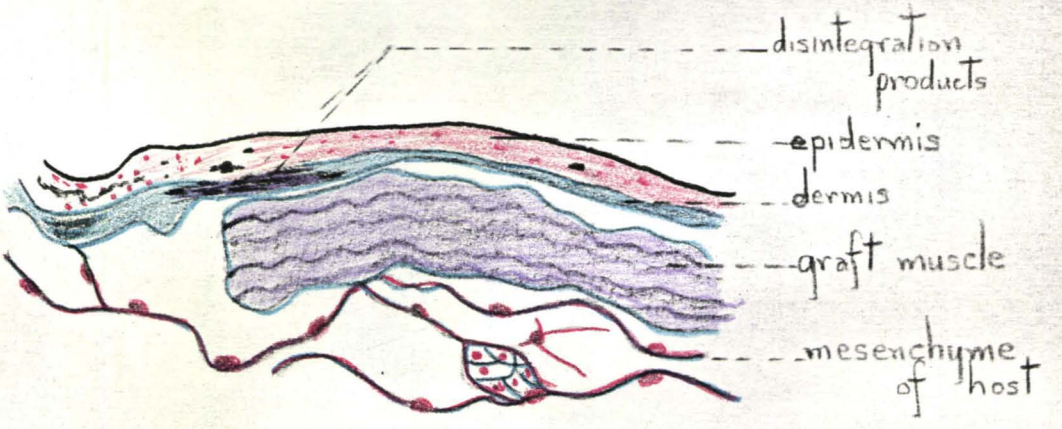


10 days 17 days 26 days 33 days

80 days - killed by fixing, prepared whole mount and sections. The sections show that muscle was transplanted with the epidermis and dermis. The illustration of the whole mount, prepared with a camera lucida, shows clearly the difference between host tissue and graft tissue. The nondescript brownish masses are also very evident, small portions of them extending a short distance into the host tissue.



Whole mount X 280.



host area graft area

Typical section X 280.

Case # 23.

A patch of skin was removed from the left side of the tail near the base, and replaced with skin from the ventral side of tadpole # 20.

1 day - graft established.

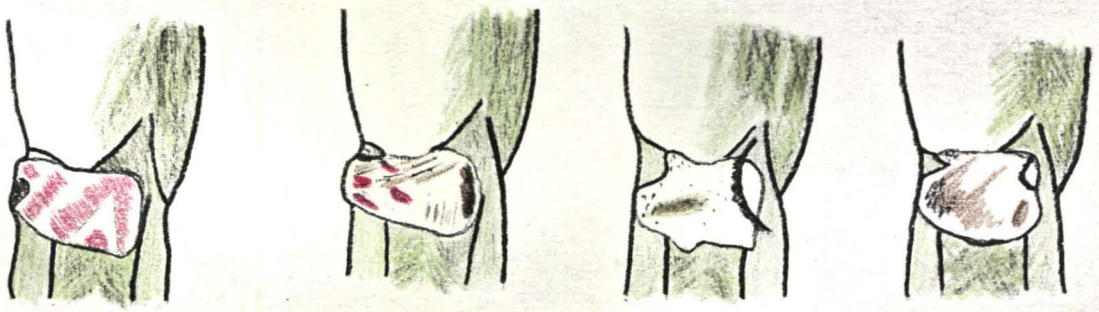
3 days - graft red around the edges.

10 days - the whole graft pinkish, with some red areas; a little dark pigment has appeared in a small fold on the ventral margin. Sketched.

17 days - still three red spots; some brown and black pigment at the dorsal and ventral margins.

26 days - all pink gone; irregular brown and black pigment arranged in streaks and specks; one black streak is continuous from host to graft and back to host. Sketched.

33 days - two black areas near the ventral edge; black specks throughout; some brown colour. Sketched.



10 days 17 days 26 days 33 days

95 days - dead, preserved, not sectioned.

Case # 24.

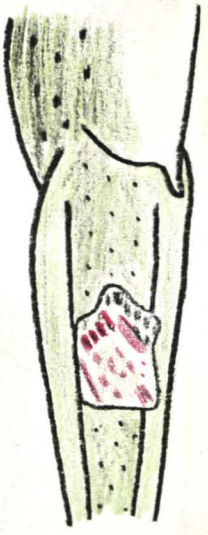
A patch of skin removed from the left side of the tail, and replaced with a piece from the ventral side of tadpole # 20.

1 day - graft established.

3 days - a small amount of loose host skin at the anterior edge.

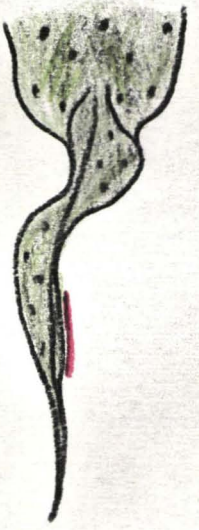
10 days - loose skin gone; many pink spots; a kink in tail; pigment appearing along the anterior border. Sketched.

17 days - most of pink colour gone; the kink in the tail has become very pronouned ; a large reddish-brown spot has appeared in the center of the anterior border with green streaks radiating from it; most of the black pigment is along the anterior border. Sketched. Also sketched a dorsal view to show kink in the tail.



10 days

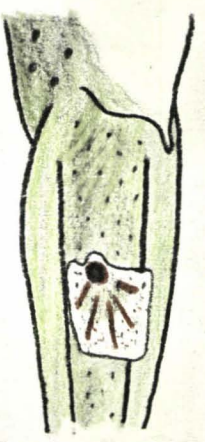
17 days



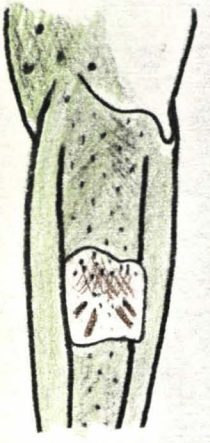
17 days
dorsal view

26 days - considerable black, brown and green pigment at the anterior; the reddish-brown spot has become black, and the green streaks have become brown; scattered black specks in the posterior region also. Sketched.

33 days - the dense pigment area has disappeared, and the pigment has become more dispersed; less green pigment. Sketched.



26 days



33 days

49 days - dead, preserved, not sectioned.

Other Transplantations

Case # 25.

A slit was cut in the right rump and the left hind leg of tadpole # 20 was inserted.

1 day - graft is well established.

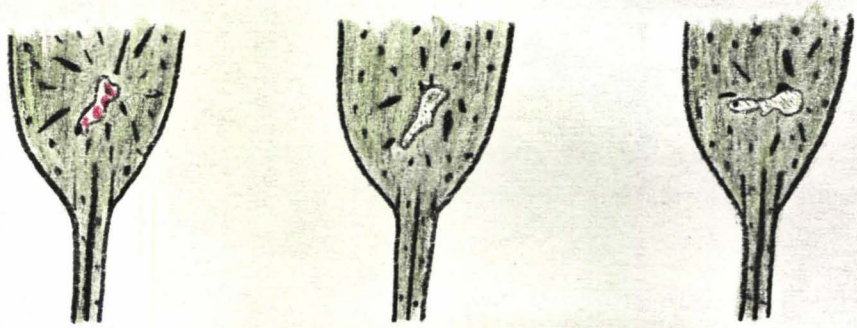
3 days - graft and host appear normal.

10 days - the graft has acquired some pink spots and appears to be losing its specific shape; there is an indication

of radial arrangement of pigment in the area about the graft. Sketched.

24 days - pink colour all gone; graft has become specked with pigment; shape of graft has become still more generalized; the pigment aggregations of the host about the graft have taken on a very definite radial arrangement toward the graft, and seem to be less numerous about the graft than elsewhere. Sketched.

31 days - graft has become rotated 90° from its original position, otherwise much the same shape; pigment aggregations in the host tissue sround the graft are much reduced in numbers, but increased in size and length, the long axes being directed toward the graft. Sketched.



10 days 24 days 31 days

49 days - dead, preserved, not sectioned.

Case # 26.

A slit was cut in the right rump and the right hind leg of tadpole # 20 inserted.

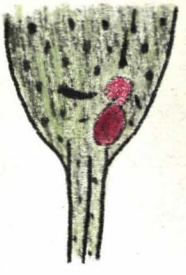
1 day - graft normal; host rests on its right side.

3 days - graft had turned red, especially at the base.

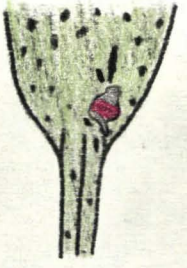
10 days - graft has lost all its specific shape; the anterior portion is pink, and the posterior bright red; the attachment to the host has shifted to the center; the host has regained its equilibrium and seems to be well; the area of the host about the graft exhibits the radial arrangement and paucity of pigment aggregations mentioned in other cases. Sketched.

24 days - the graft is much reduced in size; only a small area of red remains. Sketched.

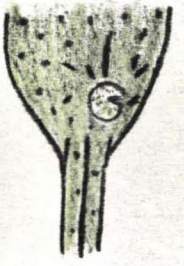
31 days - all the red is gone, and there is considerable black pigment in the graft region; the graft is reduced in size to a mere mound in the right rump of the host. Sketched.



10 days



24 days



31 days

37 days - dead, preserved, not sectioned.

Case # 27.

The right hind leg was removed and the wound covered with a piece of skin from the throat of # 20.

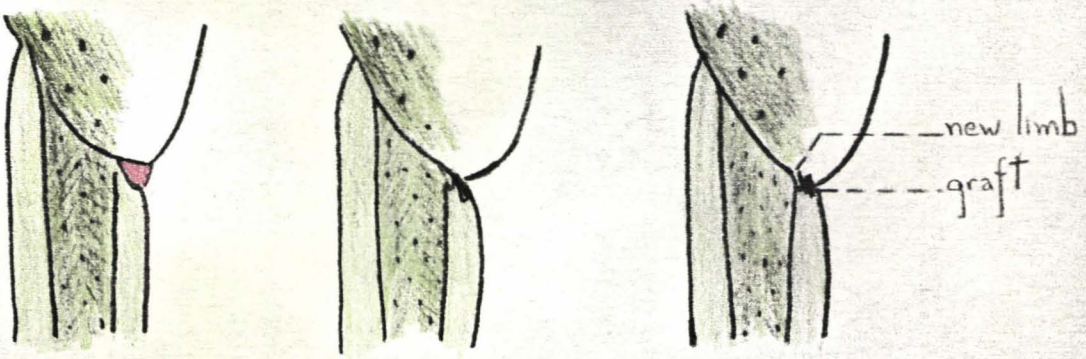
1 day - graft in place, host well.

3 days - the graft has become red; no other change.

10 days - graft pink and becoming smaller. Sketched.

24 days - all that can be seen is a small lump with a black tip; possibly a new limb bud. Sketched.

31 days - there appears to be a new limb bud above the old scar which is black. The structure that was thought to be a limb bud after 24 days was probably the grafted skin rolled into a gold. Sketched.



10 days 24 days 31 days

33 days - dead, fixed, and sectioned. The sections show a small but definite new limb bud.

Case # 28.

A slit was cut in the right rump and the right leg of tadpole # 27 was inserted.

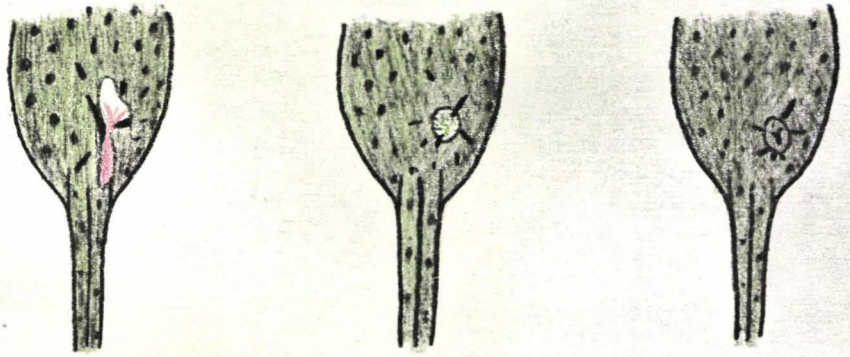
1 day - graft and host are normal.

3 days - graft has become quite pink; there is no black pigment in the host skin immediately surrounding the graft.

10 days - graft still pinkish; no black pigment aggregations very near to graft; no pigment in the graft. Sketched.

24 days - graft almost completely resorbed; the few pigment aggregations that are present in the surrounding host skin are radially arranged toward the graft area; the host skin appears to have overgrown the graft. Sketched.

31 days - conditions very little changed from the preceeding. Sketched.



10 days 24 days 31 days

56 days - tadpole found dead on table in morning; desiccated, therefore discarded.

Case # 29.

This tadpole was used as donor for # 30, 31, 33, 35, 36, 37, 38, 39.

Case # 30.

A slit was cut in the right rump and the left hind leg of tadpole # 29 was inserted.

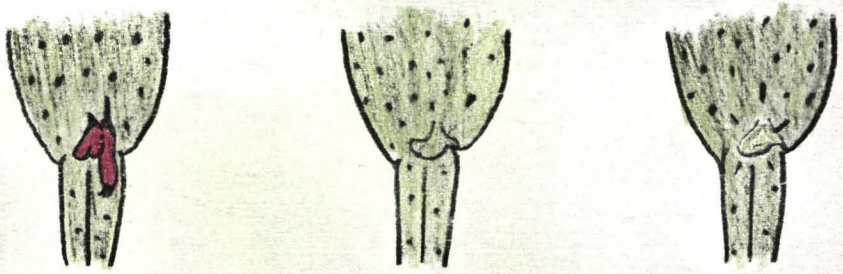
1 day - graft and host normal.

3 days - graft has become reddish.

10 days - point of attachment has shifted to the middle of the graft; the graft is red and has one black pigment streak near the point of attachment; the specific shape of the graft it only slightly modified. Sketched.

24 days - graft is much reduced in size; only one band of red left; graft much the same colour as the surrounding host skin, except that there is little black pigment. Sketched.

31 days - the graft is reduced to a lump, much the same colour as the surrounding host skin, except that it is outlined in black. Sketched.



10 days 24 days 31 days

54 days - dead, preserved, not sectioned.

Case # 31.

A slit was cut between the eyes and the right hind leg of # 29 inserted.

1 day - graft and host normal.

3 days - graft well established.

10 days - there is a large round pink swelling at the

point of insertion; the rest of the graft is also pinkish; otherwise normal. Sketched.

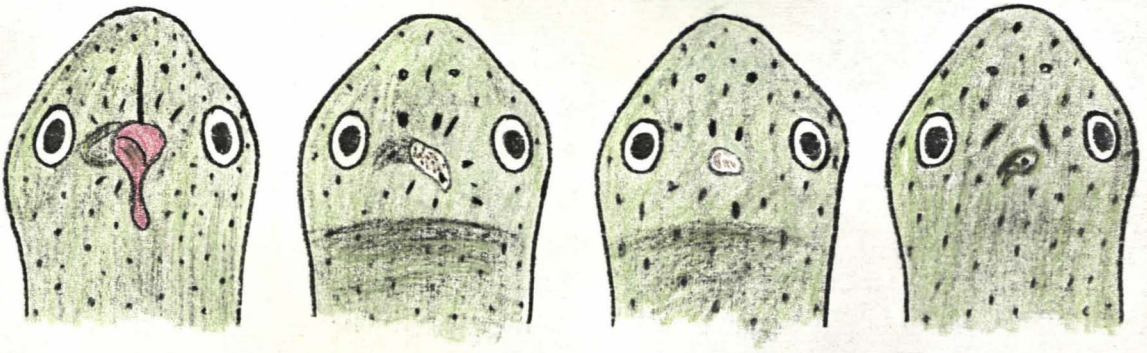
18 days - tadpole accidentally sucked into cleaning host. This caused a circular bruise about the middle of the body, just behind the spiracle; no adverse effect was observed, on the tadpole.

24 days - graft very much resorbed; all reddishness gone; considerable green and black pigment has appeared. Sketched.

31 days - host skin growing over the graft from all sides, and at the same time the lump beneath is becoming even smaller. Sketched.

50 days - all that can be seen is a small lump with a black pigment spot; all suggestion of radial arrangement of pigment aggregations is now gone - it never was very obvious in this case.

173 days - there is still a very slight lump with a terminal pigment spot. Sketched.



10 days 24 days 31 days 173 days

Case # 32.

A slit was cut in the left rump and the left hind leg of tadpole # 33 was inserted.

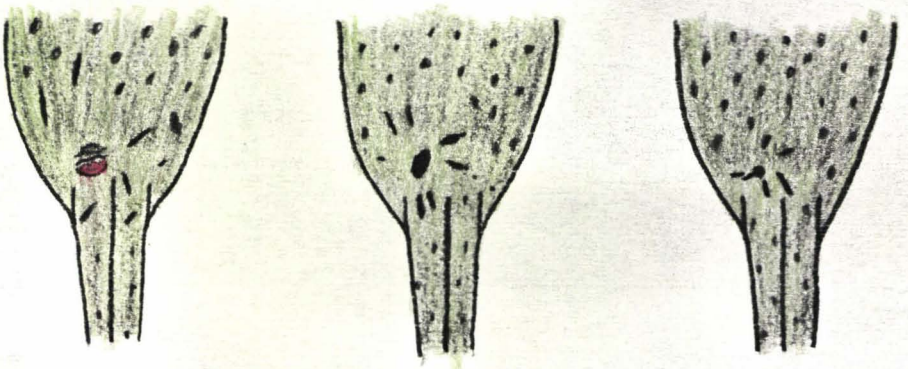
1 day - graft has become red.

3 days - graft has reduced in size to just a stump.

10 days - just a small round red bud left protruding, and the surrounding host skin is growing over this. Sketched.

24 days - graft is just a slight lump under the host skin; the host skin is very black above the lump; the pigment about the lump exhibits a distinct radial arrangement. Sketched.

31 days - the lump has become even smaller, and much of the dense blackness has gone, but the radial arrangement of the surrounding pigment aggregations persists. Sketched.



10 days 24 days 31 days

119 days, dead preserved, not sectioned.

Case # 33.

The left hind leg was removed and the wound area covered with ventral skin from tadpole # 29.

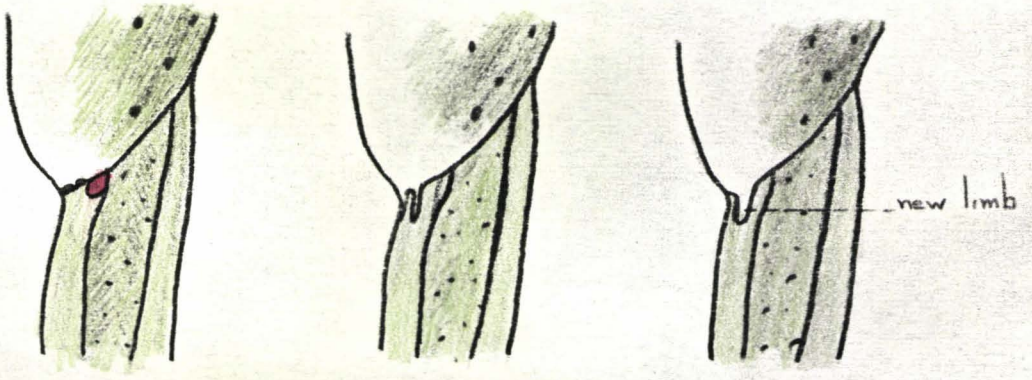
1 day - graft and host appear normal.

3 days - graft has been lost, but the wound and host seem to be fine.

10 days - wound well healed, even a suggestion of a new bud forming. Sketched.

24 days - the new limbbud can definitely be identified. Sketched.

31 days - conditions similar; limb bud larger. Sketched.



10 days 24 days 31 days

32 days - dead, preserved, not sectioned.

Case # 34.

A slit was cut in the dorsal skin between the eyes, and the left hind leg of tadpole # 35 was inserted.

1 day - graft and host normal.

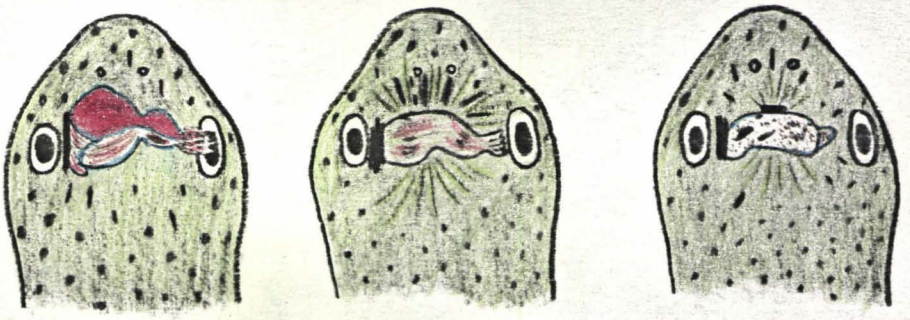
3 days - graft has become quite red, otherwise unchanged.

10 days - graft very much swollen and red in a few regions; it still retains its specific shape. Sketched.

24 days - the swelling has disappeared, as has most of

the red colour; the graft is slightly decreased in size; there is a ridge of very darkly pigmented skin at the point of insertion; the surrounding skin of the host exhibits the radial arrangement of melanin aggregations. Sketched.

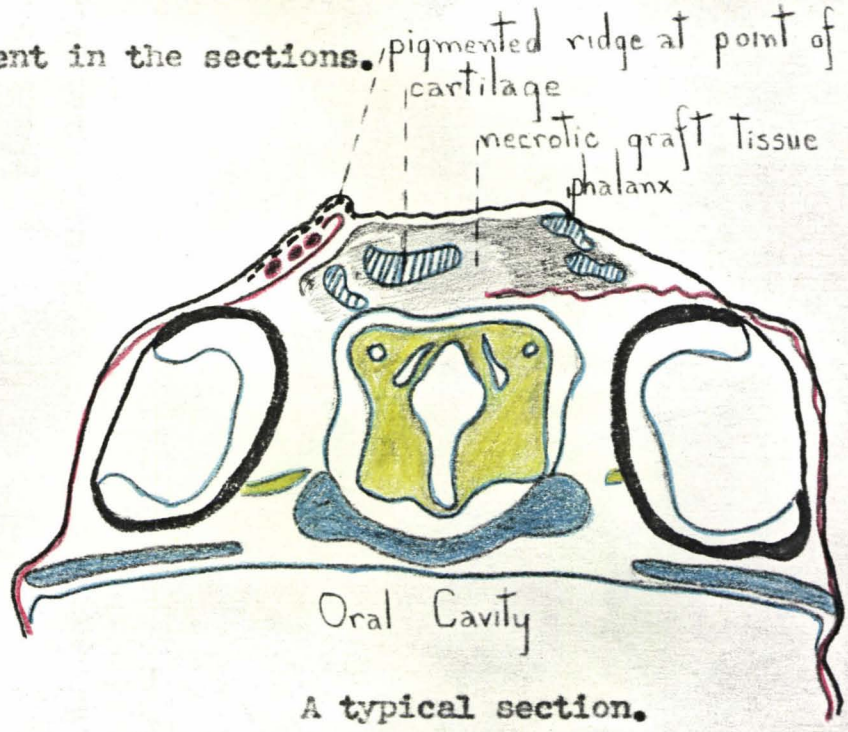
#1 days - graft considerably diminished in size, and has lost much of its specific form; it has become considerably speckled with black pigment; the host still exhibits the radial arrangement of pigment, also the black ridge at the point of insertion. Sketched.



10 days - 24 days 31 days

42 days - dead, fixed, sectioned. The sections show that the limb is completely covered by the host epidermis, but the host dermis has not yet made any progress in this regard; in fact it shows definite signs of disintegration in the region where it is overlain by the necrotic graft tissue. The cartilage elements of the grafted leg are conspicuous, and show little sign of disintegration; the rest of the graft tissue has lost most of its specific nature. The ridge of darkly pigmented skin at the point of insertion is also

evident in the sections.



A typical section.

Case # 35.

The left hind leg was excised and the wound covered with a piece of ventral skin from tadpole # 29.

1 day - dead, discarded.

Case # 36.

A slit was cut in the left side of the tail, and the left hind leg of # 37 was inserted. A little piece of ventral skin from # 29 was used to cover the wound.

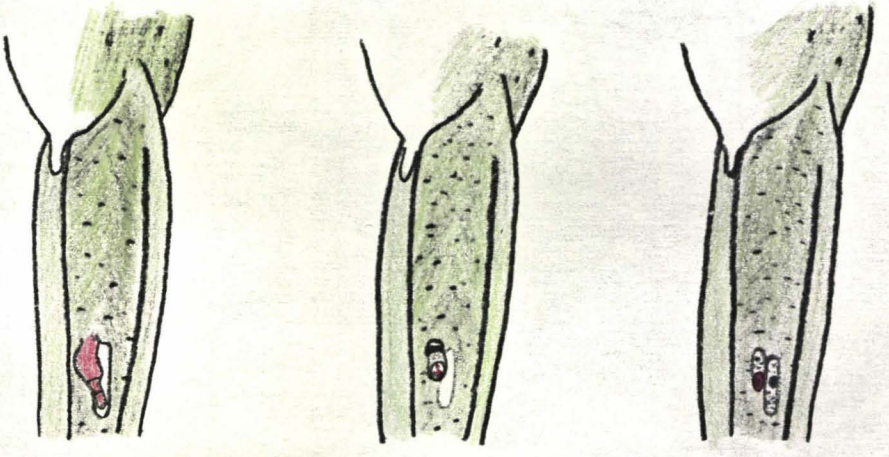
1 day - grafts and host normal.

3 days - grafted limb has become red.

10 days - the grafted leg is somewhat swollen and very red; the grafted skin is unchanged. Sketched.

24 days - the grafted limb much reduced; grafted skin somewhat reduced; three small red spots on leg; some black pigment in both leg and skin. Sketched.

31 days - a pink ring has appeared about a black pigment aggregation on the grafted limb; limb is still smaller; skin is unchanged. Sketched.



10 days 24 days 31 days

65 days - dead, preserved, not sectioned.

Case # 37.

The left hind leg was excised, and the wound covered with ventral skin from tadpole # 29.

1 day - graft and host normal.

3 days - has lost graft, but is healthy.

7 days - accidentally sucked into cleaning hose. The suction caused the gut to come out through the wound; discarded.

Case # 38.

A slit was cut in the back skin between the eyes, and the right eye of tadpole # 29 with a long optic nerve attached was inserted. During the process of resuscitation, the graft was dislodged.

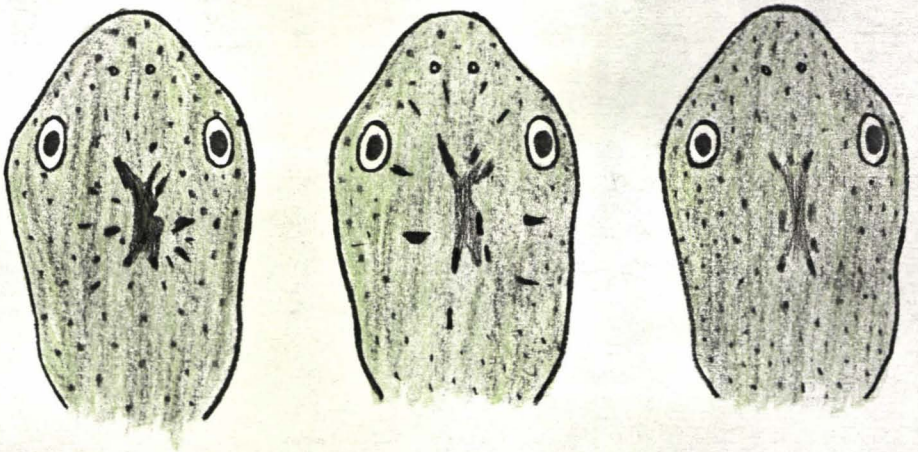
1 day - host is normal, but has a large wound in the head between the eyes.

3 days - animal is well; wound starting to heal.

12 days - the host area about the wound is beginning to show the characteristic radial arrangement and paucity of pigment aggregations. Sketched.

24 days - the wound area is covered with scar tissue, which appears black. The paucity of pigment about the wound is especially noticeable. Sketched.

31 days - the same general conditions as above; the edge of the wound area is no longer sharply definable. Sketched.



12 days 24 days 31 days

77 days - dead, fixed, and whole mount and sections prepared. It is difficult to show anything very definite from either of these.

Case # 39.

A slit was cut in the dorsal skin between the eyes, and the left eye of tadpole # 29 with a short optic nerve attached was inserted.

1 day - host and graft are well; the pupil of the grafted eye has a misty appearance.

8 days - the grafted eye has become clear black, and the skin of the host is growing over it from the sides.

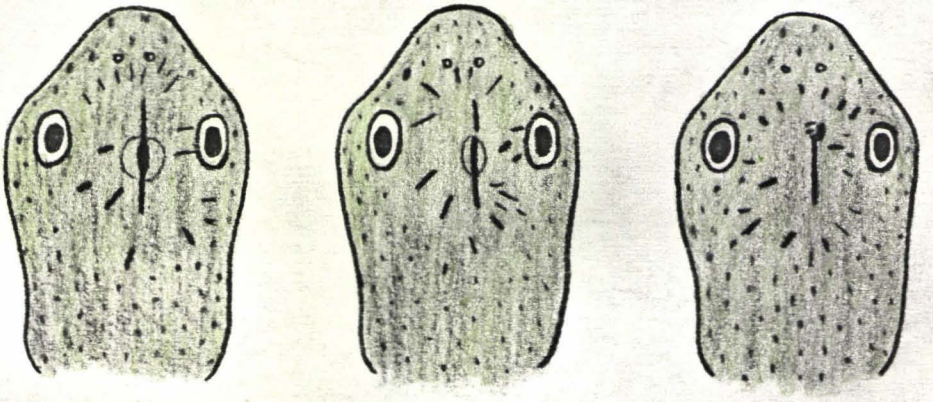
12 days - only a slit of the grafted eyes shows; it is clear black; the pigment aggregations in the surrounding skin show the characteristic phenomena of radial arrangement and paucity. Sketched.

24 days - conditions are very slightly modified from the above. Sketched.

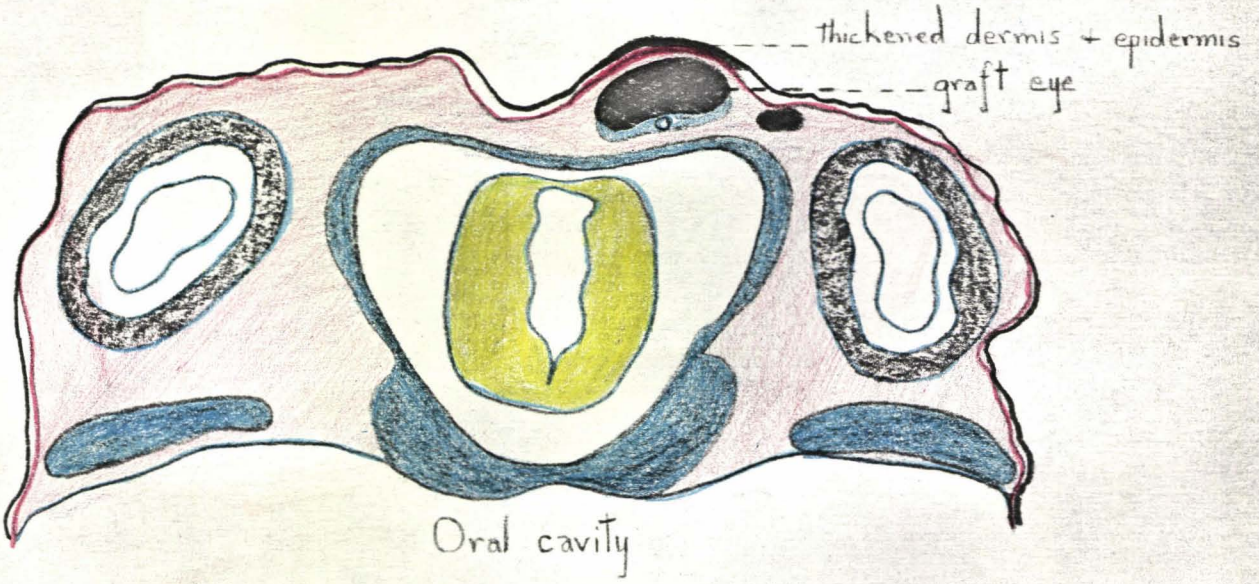
31 days - the host skin has completely healed over the graft, and at the point of meeting of the two over-growths, there is a dark black pigment streak and a slight elevation under the skin. The radial arrangement and paucity of pigment aggregations is very evident. Sketched.

37 days - dead, fixed, sectioned. The sections show that both the epidermis and the dermis of the host have

overgrown the graft, and both exhibit a greater thickness at the point where they have grown together from the two sides. The grafted eye is much reduced in size due to resorbtion, and is full of pigment from the retinal layer and other dark disintegration products.



12 days 24 days 31 days



A typical section.

OBSERVATIONS AND DISCUSSION

The preceding observations do not at once lead to any obvious conclusions. The results appear to be inconsistent. It is difficult to follow any definite pattern of interaction between the host and graft. All the grafts gained some additional pigmentation, yet none gained enough to look like the surrounding host epidermis. Within a few hours after the operation, the adjacent cut edges of the epidermis became joined, and there was physical continuity of the two epithelia; yet at the line of junction, there was a sharp demarcation between the two.

This sharp demarcation is due in many cases to an excess of what appears to be pigment in the region of the margin of the graft. In most cases, the pigment which occurs in the graft region appears as large dense aggregations or large spots. When these spots were situated near the margin, they were often radially arranged with regard to the center of the graft,- see especially cases #4, page 12; # 8, page 16; # 11, page 19; # 18, page 27; # 19, page 29; and # 22, page 30. In addition, the host skin for a distance of several millimeters from the edge of the graft presented a striking characteristic appearance due to the radial arrangement or alignment of the long axes of the elongated epidermal pigment aggregations. In such cases, the number of pigment spots per unit area was less in the region immediately surrounding the graft,-

normal metabolism, it could become adapted to its new environment by actively acquiring the characteristics of the surrounding host tissue. Melanophores might be induced in the graft, either by the underlying host tissue, or by diffusion of inducing substances from the host tissue situated around the graft. In the former case, the pigment would arise evenly distributed in the graft area, in the latter case it would arise first near the graft-host boundary and would appear progressively nearer to the center until the whole area was covered.

In the experiments described, nothing was observed to indicate that the graft carried on active metabolism and became adapted to its environment by actively acquiring the characteristics of either the underlying or the surrounding host tissue. Nothing approaching regulation of graft to host was observed.

The fact the the skin of the graft is not specific, and does not become regulated, does not prove that the epidermis has no regulatory capacity. When a piece of skin is transplanted, its epidermis comes into relation to the host only at its cut edge, but otherwise it remains seated upon its own dermis and often a thin layer of muscle. It may be possible that the epidermis is locally determined by its underlying dermis, or that the two are correlated into a binary system. Thus these experiments cannot determine

the reaction of the epidermis, except as it is correlated with the interaction of the skin as a whole.

Another possible explanation of thelloss of specificity would be to assume that the graft and host tissue were not compatible, and that an interaction took place that was not favourable to the graft. In this case the graft would become moribund and subject to the aggressive action of the host tissue. The host tissue would then react to overcome the disturbance of the physiological equilibrium, and re-establish normal conditions. During this process the graft might be replaced gradually by host tissue.

Many of the phenomena observed suggest that the graft tissue was not compatible with the host tissue. This is obvious in the case of the limb and eye grafts. The inflamation and swelling - see cases # 26, page 36; # 30, page 39; # 31, page 40; # 34, page 43 - combined with the rapid resorbtion and overgrowth by host epidermis, indicate conclusively that homoiotransplants of this type are incompatible. It has been stated previously that the epithelium is the adult tissue that seems to best retain the capacity for developmental activity; and it is obvious from the results obtained here, that the skin grafts were more compatible than the other types. Nevertheless, the skin grafts exhibited some signs of incompatibility; that some of them became inflamed is significant. The fact that the graft tissue stained less

well in the histological sections is a characteristic feature of necrosis. These facts indicate that the skin grafts were not only not active, but that they were to some extent necrotic. Under these conditions, how can the appearance of any pigment in the graft area be accounted for since it was not induced in the graft tissue? As has been suggested in the previous paragraph, it must have been carried there by the host epidermis during the course of some sort of invasion.

It would seem likely that this invasion would take place in either of two ways; the host epidermis might overgrow the graft, leaving the then submerged graft epidermis to disintegrate; or it might undercut the graft causing it to be sloughed off. The histological preparations showed no indication in any place of either of these actions. Instead, they showed that the two epithelia - the one of the host and the one of the graft - had in every case become joined to form a continuous layer. The host epithelium always met the corresponding layer of the graft squarely, edge to edge, and apparently pushing itself, at least for a short distance, directly into the space which had been occupied by the graft epidermis. This is readily illustrated in the prepared sections, where the host epidermis continues unchanged for a short distance into the graft region,- see cases # 5, page 14; # 8, page 17; # 18, page 28; and #22, page 31. It is also indicated by the fact that any pigment

aggregation in the host epidermis on the border of the graft usually became continuous into the graft area,- see cases # 5, pages 12 & 13; # 8, page 16; # 11, page 19; # 18, page 27; # 22, page 30; # 23, page 32; # 28, page 38, and # 30, page 39.

Another clue as to what happened is indicated by the excess of "pigment" which has been mentioned as appearing in the graft region, particularly in the marginal region and thus emphasizing the demarcation between the graft and host. In the histological preparations, both sections and whole mounts, a large part of this colouring matter appears as large, irregularly shaped bodies which are not melanophores. They are of various sizes, and appear less dense, especially in unstained material,- see cases # 5, page 14; # 13, page 22; and # 22, page 31. Their appearance, and the fact that they appear at that region where we have reason to suspect invasion by the host epidermis, displacing graft epidermis, strongly suggest that they are products of the disintegration of the graft epidermis.

It has been shown that the grafts did not become active- they did not grow, become regulated, nor even retain their specificity - but rather there is strong reason to believe that they became necrotic, and were invaded and displaced by the host epidermis; yet the host epidermis did not overgrow or undercut the graft tissue. A common way for an

animal to get rid of foreign protein matter which is incompatible, it by phagocytosis. Assuming this as a possibility, what could the phagocytizing agent be in this case? Adami (1908) states that in the regeneration of skin in warm-blooded animals, 'young' epithelial cells may be phagocytic. Dawson found in the epidermis of adult Necturus, cells which appear to be of a phagocytic nature. It thus seems possible that the host epithelial cells may be stimulated to phagocytic activity, and phagocytize the necrotic cells of the graft. This, however, has not been proved.

It is possible to get a clearer understanding of what happened by comparing the mode of interaction as illustrated by case 13, pages 20 - 22, with that illustrated by all the other skin transplantation. Case 13 may be considered the reciprocal of the others. The method of loss of pigment in case 13 may be considered the reverse of the method of gaining pigment in the other cases. There is the possibility that the loss might be influenced by the fact that the graft of dorsal skin in its new position does not receive its usual quota of light stimulus. Conversely the gain of pigment by ventral skin grafted in new dorsal positions might be influenced by the increased quota of light stimulus. It appears, however, due to the method of loss and gain of pigment respectively, that light stimulus has little if anything to do with the observed changes. Also, the graft

in case 13 was exposed to almost normal light conditions due to its lateral position and the fact that the animal was kept in a glass-bottomed bowl.

The graft on # 13 seemed to retain its specificity for about a week, although it lost much of its green colour even in this time, and became definitely brownish. From this time until its death at 8 weeks, the graft very gradually lost its pigment. It did not lose it in any regular manner, but rather the pigmented area was split up by prongs of colourless tissue. This colourless or white tissue was first observable near the boundary of the graft, particularly near the anterior and posterior extremities. Gradually the pigmented portions of the whole graft became dispersed and broken up by these prongs of tissue which seemed to spread from the anterior and posterior and to a lesser extent from the sides. At 8 weeks the pigment areas were dispersed and scattered over the whole graft to such an extent that the graft showed very little colour, and in only a few portions, except for the general brown colour which was definitely observed to be due to non-pigment matter which has been previously mentioned, and described as possible disintegration products.

This process is analogous to the phenomena observed in the other transplantations of the skin type. In the other cases the pigment which appeared in the graft would appear around the circumference, or perhaps just along one or two

edges. Then after varying periods of time, various irregular pigment aggregations could be observed throughout the graft. The above observations would seem to support the previous s suggestion that the epidermis of the host invaded the graft region. The fact that pigment aggregations in the host epidermis near the graft-host boundary often become greatly elongated into the graft area has already been mentioned, with references, in another connexion,- see top of page 54. This strongly supports the same contention.

All of the above observations indicate that the invasion was in the nature of an infiltration of host epidermis into and among the cells of the graft epidermis. Leo Loeb (1897) in his experimentation on the skin of the guinae-pig's ear, transplanted black skin into white, and white into black. He describes in both cases, an infiltration of cells from the host, including pigment, into the graft. This appears to be a process similar to that observed in skin grafts on Rana catesbieana.

Another observation that fits in with the invasion idea is the radial arrangement and paucity of pigment aggregations observed in the host epidermis for a short distance, up to 5 or 6 millimeters, around the graft. This has been described with references on pages 49 and 50. It would seem to indicate a mass migration by the epithelium of the host, and a subsequent elongation and orientation of the pigment

aggregations in the migrating epithelium. The fact that there are fewer numbers of aggregations per unit area could be accounted for by reasoning that, since some of the host epithelium had moved in to take the place of the necrotic graft tissue, the surrounding epithelium would be lessened in amount, hence thinner, and the pigment aggregations would decrease in number, at least until such time as the healing process had been completed and the cells of the epithelium had proliferated to make up their normal number.

This view is further supported by the fact that the above phenomenon was most distinct and severe in those cases in which the healing action of the host epidermis was most active. It also came to an end much sooner in these cases, and shortly after the healing action was apparently over, the characteristic arrangement of pigment aggregations gradually became less distinct and finally disappeared. Case 38 on page 46 illustrates this most clearly.

Other Transplantations

Certain aspects of the other transplantations has already been discussed in connexion with certain points regarding skin transplantation. Much of the preceding discussion will also apply to all of the grafting experiments in addition to the skin grafts. Thus the conclusions reached regarding radial arrangement and paucity of pigment aggregations

and regarding inflamation of the graft tissue, have the same significance with regard to the other transplantations.

The limb transplants exhibited very definite incompatibility, and were more or less rapidly resorbed in every case. In case 34 on page 40, the graft had been resorbed in 50 days, except for a small lump beneath the skin, and yet after 173 days, this lump was still present. In two cases, the grafts were almost completely resorbed within 3 weeks,- see cases # 28, page 38, and # 32, page 41. In case 34, page 44, histological sections were prepared, and it can be seen that the host epidermis grew over the grafted limb long before it was resorbed to any great extent. It can also be seen that the host dermis does not grow over the graft with the epidermis. Where the dermis has been covered by the graft, it shows evidence of being necrotic. The great activity which was necessary on the part of the host epidermis in order to accomplish the overgrowth, is indicated by the very definite radial allignment of pigment aggregations about the graft. This gives added assurance to the deductions made previously.

In the four cases of excised limbs, two died in a short time. The other two exhibited rapid regeneration of new limbs, quite definite buds being developed in 2 to 4 weeks,- see cases # 27, page 37; and # 33, page 42. This serves to give an idea of the regenerative powers of

the experimental animals. The power of regeneration bears a direct relationship with the ability to survive transplantation successfully. Thus tissues from these tadpoles should be able to survive transplantation more successfully than corresponding tissues in animals with lower powers of regeneration.

Only one case of eye transplantation was successfully performed,- see case 39, page 48. The observations and discussion of this case is very closely allied to that regarding limb transplantation. The grafted eye tissue became disorganized and necrotic and eventually became filled with black pigment, from the pigment layer of the retina, and masses of disintegration products. It is interesting to note that in this case the dermis grew over the graft along with the epidermis. This was the only indication observed in any of the cases, that the dermis might be comparable to the epidermis in activity.

Strictly speaking, case 16, page 24 & 25, does not come within the field of this thesis, but it illustrates some interesting points from the larger field which should be mentioned. The left eye had atrophied, and as a result the connected parts of the central nervous system exhibit hypoplasia. The optic chiasma on the left side is almost non-existant, and the right side of the mid brain is much underdeveloped. Similar effects may be produced by the grafting of organs that are out of scale with the host.

The Nature of the Reacting Substances

The observable methods whereby host and graft tissues interact, and by which the graft tissue is displaced by host tissue, have been discussed rather fully. The reason for this reaction, and the stimulus producing and inducing substances which initiate and the interaction have not been discussed. This is beyond the field of the present experimentation, but a short consideration of these factors will put the field under discussion in better perspective. The opinions expressed are those of Leo Loeb.

The introduction of parts of organs or tissues which originated in a strange individual causes disturbances which lead to changes similar to those found as a result of the action of toxic substances. These substances act, not unlike those given off by certain microorganisms, as for instance the tubercle bacillus. It has been pointed out that the action of these substances is graded in accordance with the relationship between donor and host. Thus in homoiotransplantation, the reaction may be due to products of metabolism given off by the introduced tissue, which act as homoiotoxins. It is as yet doubtful, how far these disturbing substances are those given off in the normal metabolism of the transplanted cells - substances which arettoxic merely because they act on a strange host - and how far they are the product of an abnormal metabolism of

the introduced cells, the pathological change being due to the action of the body fluids of the host upon the strange cells. The latter seems true in many cases, for example, in man certain groups of individuals can be distinguished according to the interaction of blood cells against agglutinins preformed in the blood. The substance in the body fluid which reacts with the homoiodifferential of the tissue, may be called the supplementary homoiodifferential.

It is interesting to note that, in fertilization homoiotransplantation is the normal occurance, and no reaction occurs against the homoiodifferential, even in the higher animals, just as in the case of more primitive tissues, a reaction of incompatibility occurs after heterofertilization. In both heterotransplantation and heterofertilization, reciprocal relations may lead to very divergent results.

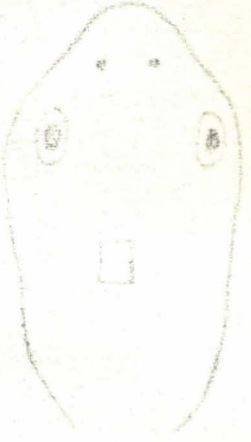
The genetic composition of the homoiodifferential is as yet very imperfectly known. It has been established that the differential is genetically composed of multiple factors, the number of which is not definitely known. It is possible that all except the sex chromosomes participate in the composition of the differential.

Leo Loeb believes "We have every reason to assume that the homoiodifferential is a proteid substance, or at least that it occurs only in combination with proteid substances."

CONCLUSIONS

Herbert Rand and Madelene E.Pierce of Radcliffe College have done some interesting transplantations on frog tadpoles. Their animals were Rans calamitans and a few Rana palustris. Their results are based, for the most part, on 25 grafts ofwwhich 20 were autoplastic and 5 homoioplastic. All of these were skin transplantations from the ventral to the dorsal surface.

They found that all the grafts either retained their specificity, or else the graft area became pigmented in a manner similar to that of the host. All the grafts which retained their specificity were autoplastic. In those cases that lost their specificity, they found that the pigment a appeared progressively from the edge of the graft, advancing centripetally until in 3 or 4 weeks, the entire graft region was pigmented. In order to investigate the nature of this advance, they devised the "barrier" experiment, in which a strip of shell membrane of the hen's egg was interposed between the host skin and the graft skin as illustrated in the diagram.



not become regulated, but rather becomes necrotic and displaced by the host tissue. Their ingeneous 'barrier' experiment did greatly clarify the manner in which invasion by the host tissue takes place. They did not do any transplantation from dorsal to ventral surface, however, nor did they do transplantations of any other organs, both of which gave valuable clues regarding the nature of the interaction in the experiments with Rana catesbeiana.

Loeb defines the reaction against homoiotransplantation as 'homoiodifferential! He stated (1921) that the first indication of homoiodifferential in the phylogenetic series occurs in adult Amphibia. These experiments and those of Rand and Pierce both indicate that there is a homoiodifferential in larval Amphibia as represented by frog tadpoles of at least three species.

APPENDIX

There is limitless opportunity for individual initiative in devising new and correlating established types of experiments and methods. The following are some suggestions, which if followed, would have greatly increased the value of the present work.

It will be noticed that many of the transplantations were made to the tail region. It was intended that the animals should be induced to metamorphose by chemical treatment, and the fate of the grafts recorded. Due to limitations of time this was not done.

In the present record there is only one case of transplantation from dorsal to ventral side. Many more would have been a valuable aid in establishing the pattern of interaction in skin transplants.

Autoplastic transplantations, and "barrier" experiments would have added greatly to the value of the work. A combination of the "barrier" experiment with the reciprocal transplantation should yield interesting and useful results.

Because the reaction between host and graft was so slow, most of the host animals were allowed to live as long as they would, in order to observe the maximum change. They should have been killed and fixed at various time intervals after the operation, in order to follow the progress of the invasion.

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