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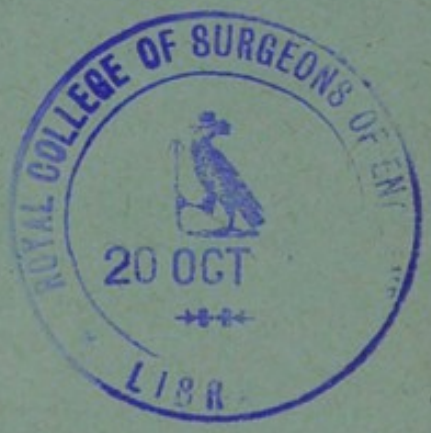

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TISSUE TRANSPLANTATION INTO DIFFERENT SPECIES.

By LEO LOEB and W. H. F. ADDISON.

(From the Laboratory of Experimental Pathology and the Anatomical Laboratory, University of Pennsylvania.)

The following investigations represent one part of a connected series of researches into the condition of tissue growth.[1] Former studies demonstrated the importance of the character of the body fluids for the life and growth of transplanted tissues.[2] We expected that a systematic investigation into the transplantation of one kind of tissue of a certain species into a variety of other animal species might throw additional light on the problems of tissue growth. We used for this purpose the skin of the guinea-pig, and carried out series of transplantations into the guinea-pig, the rabbit, dog, pigeon and frog. Pigmented skin of the guinea-pig ear was used in all cases; it was first thoroughly washed with soap and water, then cleaned with alcohol and bichloride of mercury. The skin was transplanted into pockets in the subcutaneous tissue of the various animals. The pieces were removed after various periods and most of them cut into serial sections.

We know of only two investigators who transplanted skin into animals of different species. Beresowsky[3] covered defects of the skin

[1] Leo Loeb, Beiträge zur Analyse Gewebewachstums I, Arch. f. Entwicklungsmechanik, 1907, vol. xxiv.

[2] Leo Loeb and Samuel Leopold, on the Difference in the Results Obtained after Inoculation of Tumors into the Individual in which the Tumors had Developed, etc., Jour. of Med. Research, vol. xvii, No. 3.

[3] Ueber d. Histolog. Vorgänge bei der Transplantation von Hautstücken auf Thiere einer anderen Species, Ziegler's Bieträge, 1893, Band xii.

of dogs with flaps of frog skin; the transplanted frog skin became necrotic, was infiltrated by leukocytes, and cast off. A similar fate befell pieces of dog skin transplanted upon wounds in the guinea-pig. Here the skin remained preserved somewhat longer, but at no time were mitoses or any progressive changes noticeable. The technique in the experiments of Beresowsky differed somewhat from our own. H. Ribbert,[1] on the other hand, used the technique employed in our investigations. This author transplanted pieces of guinea-pig skin and human skin into the subcutaneous tissue of the rabbit. He summarizes his results as follows:

In the first three days proliferation of the transplanted epithelium takes place. The epithelium grows partly upon the connective tissue of the rabbit and of the guinea-pig. All these proliferative phenomena, however, are not as marked as after transplantation into the same species. After three days the process of growth ceases. The layers of epithelium are still preserved on the fourth and fifth day; but at this period the nuclei no longer stain well, and the cells swell. On the eighth day the pieces are seen degenerating. His explanation of these phenomena is as follows:

In the beginning the cells have nourishment which is sufficient for proliferation. The host supplies merely indifferent food substances, as oxygen and water. The transplanted cells cannot use the food substances of the host, therefore they all die after some time. We notice that Ribbert does not differentiate between the behavior of the skin of the guinea-pig and that of the human being when transplanted into the rabbit. From his brief description we may judge that he bases his conclusions mainly on the study of the guinea-pig skin transplanted into the rabbit. We aimed, on the other hand, especially at comparing the results of the transplantation of guinea-pig skin into species nearly related to the guinea-pig, and into others farther removed, as the pigeon and the frog. We carried out experiments as shown in the accompanying table:

[1] Ueber Transplantation auf Individuen anderer Gattung. Verhandl. d. pathol. Gesellschaft, Breslau, 1904.

TABLE OF TRANSPLANTATION OF GUINEA-PIG SKIN INTO DIFFERENT SPECIES.

		Days									
		1	2	3	4	5	6	7	8	9	10
Guinea-pig	(*a*)	10	8	8	5	16	4	7	5	..	7
	(*b*)	9	8	7	5	13	4	7	5	..	6
	(*c*)	9	8	5	5	11	4	6	5	..	4
		(2 m.)	(6 m.)	(5 m.)	(5 m.)	(10m.)	(1 m.)				
Rabbit	(*a*)	2	12	14	4	10	3	7	2	..	8
	(*b*)	1	6	8	..	2	..	3	2	..	2
	(*c*)	..	2	5	..	1	..	2	1		
				(3 m.)		(1 m.)		(2 m.)	(1 m.)		
Dog	(*a*)	6	9	10	4	18	6	8	..	..	8
	(*b*)	1	6	3	3	6	4	1			
	(*c*)	..	2	1	1	3	2	1			
			(2 m.)	(1 m.)	(1 m.)	(3 m.)	(2 m.)	(1 m.)			
Pigeon	(*a*)	4	7	13	10	16	..	10	..	1	15
	(*b*)	4	7	7	6	10	..	2	..	..	2
	(*c*)	1	4	6	5	5					
			(2 m.)	(5 m.)	(3 m.)	(4 m.)					
Frog	(*a*)	6	1	3							
	(*b*)	1	0	0							
	(*c*)	0	0	0							

(*a*) Shows number of pieces transplanted for each period.

(*b*) Shows number of pieces which have living epithelium.

(*c*) Shows number of pieces which have large amount of living epithelium or mitoses. The number in brackets shows in how many of these pieces mitoses have been observed.

SUMMARY.

1. After transplantation of guinea-pig skin into animals of another species the epithelium does at no time grow as actively as after transplantation into the guinea-pig.

2. The period of active proliferation as evidenced by the presence of mitoses is considerably greater than found by Ribbert. Instead of three days, we found growth taking place in the guinea-pig skin, in the rabbit as late as eight days, in the dog seven days, and in the pigeon five days after transplantation.

3. If we consider, however, the different series of transplantations as a whole, the difference between the results of transplantations into

the guinea-pig, on the one hand, and into other species, on the other hand, becomes more marked at later periods after transplantation, no active proliferation having been noticed in any animal except the guinea-pig later than eight days.

4. There is a distinct difference in the energy of growth of transplanted pieces according to the species into which the guinea-pig skin has been transplanted. The proliferative energy manifests itself longest in the rabbit, namely, eight days. Not quite so favorable as the rabbit is the dog, in which the growth ceases after seven days. In the pigeon no growth takes place after five days. In the frog no growth takes place at any time, as might be expected if we consider the body temperature of this animal, which is not sufficiently high to permit growth phenomena in mammalian tissues. But the conclusion that the frog is a very unfavorable soil for the transplanted guinea-pig skin is also suggested by the results of the re-transplantation of the guinea-pig skin from the frog into the guinea-pig, inasmuch as pieces which had been kept longer than three and a half hours in the frog did, in no case, grow after re-transplantation into the guinea-pig, and in these the growth was very insignificant.

5. The differences between the growth of guinea-pig skin transplanted into the rabbit, on the one hand, and into the pigeon, on the other hand, would, in all probability, have been still more striking but for the interference of another factor, that of bacterial infection. Bacteria which cannot be entirely eliminated from the guinea-pig skin cause least interference with the growth of the epithelium after transplantation into the guinea-pig, nor do they become very active in the pigeon. But they are a factor seriously interfering with the result of the transplantation into the rabbit and also into the dog. The collections of leukocytes found in the different species after transplantation of the skin can probably be used as an indicator of the growth and toxic action of bacteria transferred with the skin. In many cases the negative results after transplantation of skin into rabbit and dog can be brought into causative relation with the presence of bacteria as indicated by leukocytic infiltration. The small importance of this complicating factor in the pigeon causes the skin of the guinea-pig to be under relatively much more favorable conditions in the pigeon, and permits comparatively many pieces of skin

to grow in the first five days. Since, notwithstanding the absence of infection, the pieces suddenly cease to grow in the pigeon after five days, although they continue to grow for a longer period in a certain number of cases in the dog and in the rabbit, we may conclude that the body fluids of the pigeon are more injurious to the guinea-pig skin than those of the rabbit and of the dog.

6. Although collections of leukocytes are almost absent around the transplanted guinea-pig skin in the frog, they appear usually very abundantly after re-transplantation of the guinea-pig skin into the guinea-pig. This fact indicates an increased growth or an increased virulence of the bacteria after re-transplantation into the guinea-pig. Notwithstanding the existence of this complicating factor, the fact that after a presence of more than three and a half hours in the frog, the guinea-pig skin has, after re-transplantation into the guinea-pig, lost its entire proliferative energy, renders it very probable that the body fluids of the frog have a direct injurious influence on the epithelium of the guinea-pig.

7. From these experiments and considerations the conclusion seems justifiable that there exists a difference between the adequacy of different species as a soil for epithelium of the guinea-pig, and that the more nearly related two species are, the better will be the growth of the transplanted epithelium. We may, therefore, arrange the different species in the following order, indicating the gradual decrease in the adequacy of the soil for transplanted guinea-pig skin: (1) Guinea-pig; (2) rabbit; (3) dog; (4) pigeon; (5) frog.

8. If we leave out of consideration the factor of bacterial activity, which causes the necrosis of a relatively large number of pieces of skin, we observe the following processes leading to the death or disappearance of the transplanted pieces.

(*a*) The signs of active proliferation cease, the cells remain alive for some time, and in certain instances active keratinization takes place. They are finally destroyed under the influence of the ingrowing connective tissue.

(*b*) The cells still show signs of proliferation, but the growing connective tissue of the host surrounds more and more the transplanted piece, and begins to press upon it. Small round cells, migrating from the connective tissue of the host, invade the transplanted epithe-

lium. The transplanted epithelium cannot resist the pressure and invasion for any length of time, and will gradually disappear. One of us[1] described similar phenomena in the guinea-pig in cases in which, especially after consecutive serial re-transplantations of the same piece of skin, the epithelium was unable to form a cyst. Under such conditions the epithelium will even disappear in the guinea-pig. After transplantation into individuals of another species the cell growth is in no case sufficiently strong to permit the cells to close into a cyst. They will, therefore, in each case die ultimately under the influence of the surrounding host connective tissue, even if the pieces have not become necrotic at an earlier period.

(*c*) It is very likely that a certain number of pieces become directly necrotic under the influence of the body fluids of animals of another species, although we cannot with certainty exclude the possibility that bacterial toxins are, in most cases, responsible for this kind of cell death.

There is one point which deserves still an especial mention, that is, the different degrees of liability of infection which we find in different species of animals toward the tissue of a certain species. These findings are not accidental, not due to mistakes of technique, but are evidently caused by a determined relation between certain organisms found on the skin, and certain species of animals. It is intended to study these relations in later investigations.

As to the theoretical interpretation of these results, and as to their bearing upon the problems of tissue growth, it is quite certain that the interpretation of Ribbert is inadequate. He assumes that the cells live on their stored up food material and are unable to assimilate the food of the host. Such a food was, afterward, designated by Ehrlich as X substance, and an increased avidity for this substance was declared by this author to distinguish tumor cells from ordinary tissue cells. Because the foodstuffs of different species differ, tissues transplanted into individuals of another species can, therefore, live and grow only until their own stored-up food-substance has been used. Such an explanation might have been found satisfactory, if it had been shown that the tissues behave alike in individuals of different species,

[1] Beiträge zur Analyse d. Gewebewachstums I, Archiv f. Entwicklungsmechanik, 1907, vol. xxiv.

without consideration as to whether the species of the animal from which the piece was taken and the species of the individual into which it was transplanted were nearly related or were very distant. That such a relationship exists is, however, made very probable through our experiments. Such an hypothesis is furthermore not able to explain a fact emphasized by one of us in former publications,[1] namely, that there exist even differences in the life and growth of tissues after transplantation into the individual in which the tissue had taken its origin, and into other individuals of the same species.

In order to explain these facts we have to assume either (1) the existence of a number of growth substances necessary for the full development of the tissues—these would be partially present in other individuals of the same species, present in smaller number in individuals of a different species, and absent in individuals of distant classes of animals—or we have to assume (2) that the more distantly two species are related, the more substances are present which inhibit the growth of the tissues of other species. That directly injurious substances are active even after transplantation in the same species seems to be indicated by the fact that even after transplantation of skin into an individual of the same species the hair follicles are frequently the only tissues preserved and, that they are the favorite seat for mitoses to occur. Surrounded on most sides by connective tissue, the hair follicles seem to be less accessible to the injurious influence of certain substances after transplantation. Injurious agencies of a chemical or physico-chemical character seem to be especially active in certain species. We noticed, for instance, especially after transplantation into the pigeon, that in the course of the first few days a swelling of the nuclei and cytoplasm, with consecutive washing out of the cytoplasm and karyolysis, is found to occur. In other species this change was much less frequent.

Whatever might be the ultimate conclusion as to the presence of injurious substances or to the absence of the necessary growth substances in more distant species, or as to the importance of both of these factors, we certainly find, here, indications of a specific physico-

[1] Leo Loeb, Further Investigations in Transplantation of Tumors, Jour. of Med. Research, 1902, vol. viii. Leo Loeb and Samuel Leopold, On the Difference in the Results Obtained after Inoculation of Tumors into the Individual in which the Tumors had Developed, etc., Jour. of Med. Research, xvii, No. 3.

chemical adaptation between cells and body fluids in one and the same species, or even in one and the same individual. An analogous specific adaptation has been shown by one of us to exist between certain cell constituents, namely the tissue coagulins and the fibrinogen, a constituent of the blood plasma.[1]

From the growth substances referred to here, which are active in individuals at all times, we have to distinguish certain temporarily active or intermittent growth substances, such as the ones secreted at certain periods by the ovaries, causing the growth of decidual new formations,[2] or substances which are active at later periods of pregnancy, causing the growth of the mammary gland and of certain mammary tumors.[3]

[1] Leo Loeb. Versuche ueber einige Bedingungen d. Blutgerinnung, inbesondere d. Specificität, etc., Vichow's Arch., 1904, Band clxxvi.

[2] Ueber d. exper. Erzeugung von Knoten von Deciduagewebe, Centralblatt f. allgem. Pathologie, 1907, Band xviii.

[3] Further Investigations in Transplantations of Tumors, Jour. of Med. Research, 1902, vol. viii.