THE TRANSPLANTATION OF SPLENIC TISSUE INTO THE SUBCUTANEOUS FASCIA OF THE ABDOMEN IN RABBITS.

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Plate 54.

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The reactions of certain structures of the spleen, for example the pulp cells and the endothelium of the sinuses, to many varieties of toxins have been extensively studied. On the other hand, little is known of the nature and function of the Malpighian bodies. While morphologically they resemble lymphoid tissue, the fact that this tissue rarely shows the reactions seen in other lymphoid tissues to bacterial toxins like typhoid, streptococcus, etc., may be of significance. In certain states, however, as in lymphatism, exophthalmic goiter, etc., this tissue, like the thymus, shares in the general lymphoid hyperplasia. Then too, its arrangement as an envelope about the arteries is not duplicated elsewhere in the body. The question of the relative importance of the structures in regeneration also has received little attention. Are the Malpighian bodies, the pulp cells, and the sinuses separate tissues with separate functions, or are they more interrelated functionally and morphologically than their anatomical appearances indicate? It occurred to us that transplantation, if this was possible, would throw some light on the subject of regeneration and possibly on the relative value of the tissues in this reaction. We have not been able to find any record of the transplantation of splenic tissue where the grafts were studied from these viewpoints or from the standpoints of the growth and permanence of the grafts.

TABLE I.

Transplantation of Spleen.

Rabbit No.	Sex.	Date of splenectomy.	Date of thymectomy.	Date of transplantation.		First examination.		Final examination.		Histological examination.
				Autotransplantation.	Homoiotransplantation.	Day.	Condition.	Day.	Condition.	
		1915	*1915*	*1915*	*1915*					
1	Male.	Aug. 26			Dec. 16	56	—	56	—	Negative (infected); infiltrated with pus cells and edematous.
2	"	" 26	Nov. 29		Nov. 29	15	—	15	—	No trace of spleen transplant found.
3	Female.	" 26	" 29		Dec. 16	65	—	65	—	Negative at examination; area not removed.
4	Male.	Nov. 5	" 29		Nov. 29	17	—	17	—	Negative; nearly absorbed; marked connective tissue reaction and invasion; persistence of the spleen trabeculæ; no lymph or pulp tissue present.
					Dec. 16	67	—	67	—	Complete absorption.
5	"	" 5	Dec. 2		" 18	63	—	63	—	Absorbed area with much yellow pigment in endothelial-like cells; some lymph tissue. This is clearly an atrophic spleen transplant with persistence of the endothelial elements.
6	Female.	" 5	" 2		" 2	14	+	14	+	Positive; marked connective tissue reaction and involution. Lymph and pulp tissues still present; highly vascular; in the process of absorption.
					" 16	65	—	65	—	Complete absorption. Several areas contain large numbers of phagocytic cells engorged with blood pigment.
7	Male.	" 5	" 2		" 16	65	—	65	—	Complete absorption.
8	"	" 5	" 3		" 16	67	—	67	—	Negative at examination; area not removed.
9	Female.	" 5	" 3		" 16	119	—	119	—	" " " " " "

10	Female.	Nov. 29	Nov. 29	Nov. 29		8	+	8	+	Positive; very well developed blood supply; marked connective tissue reaction.
11	Male.	Dec. 2	Dec. 2	Dec. 2		11	+	11	+	Positive; well organized blood supply; one large trabecula; both lymph and pulp cells present; well marked connective tissue reaction.
12	"	" 3	" 3	" 3		13	+	80	−	Negative at examination; area not removed.
13	"	" 3	" 3	" 3		13	+	13	+	Positive; in the process of absorption; marked connective tissue reaction, with ingrowth into transplant area; good blood supply.
					Dec. 16	67	−	67	−	Negative at examination; area not removed.
14	Female.	" 16	" 16	" 16		67	++++	325	++++	Transplant 4 by 2.5 by 2 mm.; whole transplant shows usual splenic vascularity; encapsulated; typical Malpighian body formation, trabeculæ, pulp, and sinus formation.
15	"	" 18	" 18	" 18		65	−	65	−	Complete absorption.

Many experiments of dislocation, often misnamed transplantation have been reported, as the experiments of Hédon[1] of pulling the organ through an abdominal wound with the blood supply intact, and suturing it into the subcutaneous tissues. Those of Lüdke,[2] of introducing bits of spleen into the spleens of alien species of animals, are not related to our problem, since heterotransplantation in mammals has never succeeded. The blood vessel suture experiments of Carrel[3] also have no bearing on the questions suggested above.

Brief mention of our first observations with spleen transplantation was made in a previous paper.[4] At that time only negative results with both auto- and homoiotransplantations had been obtained.

EXPERIMENTAL.

We now wish to report the end-results of a series of transplantations made more than a year ago. Twelve attempts at homoiotransplantation and six attempts at autotransplantation were made on fifteen rabbits. The more important data relating to each experiment are given in Table I.

Method.

The method employed is the same as that used by us in the transplantation of ductless gland tissues, and consists of transferring small sections of the spleen of about 2 mm. in their greatest dimension under strict aseptic precautions. Alcohol and bichloride of mercury were used instead of iodine for skin sterilization, because it is necessary to control the intake of iodine whenever the thyroid may be involved directly or indirectly in the experiment. After making a transverse abdominal skin incision about 2 cm. in length, the subcutaneous fascia is lifted with fine forceps, punctured with a cataract knife, the tissue introduced, and the fascial opening closed with a black silk ligature, which also serves to mark the site for subsequent examinations.

[1] Hédon, E., Transplantation sous-cutanée de la rate, *Compt. rend. Soc. biol.*, 1899, vi, 560.

[2] Lüdke, H., Ueber Milztransplantationen, *Münch. med. Woch.*, 1909, lvi, 1469.

[3] Carrel, A., Remote Results of the Reimplantation of Kidney and Spleen, *J. Exp. Med.*, 1910, xii, 146.

[4] Manley, O. T., and Marine, D., Transplantation of Ductless Glands with Reference to Permanence and Function, *J. Am. Med. Assn.*, 1916, lxvii, 260.

The spleen was completely removed at the time of transplantation in the group of autotransplants, while in all the homoiotransplants it was removed some time (from 112 to 24 days, in the majority about 40 days) before transplantation. This is probably too long a time interval to include a possible advantage to the transplant that might accrue from a splenic insufficiency, since Musser and Krumbhaar[5] and others[6] have shown that certain animals (dogs) usually begin to recover from the systemic effect of splenectomy, as indicated by the erythrocyte counts, in 3 to 4 weeks.

It has been definitely established that a physiological insufficiency markedly influences the growth of autotransplants of the ductless glands, but it does not influence to any extent the taking of the transplants. Likewise with homoiotransplants there is no evidence that an induced physiological insufficiency modifies the taking. Sex also does not influence the taking, growth, or rate of destruction of the transplanted tissue. The thyroid was removed in every instance but one at the time of, or shortly before transplantation without any influence on the taking or growth of the spleen grafts. Removal of the thyroid, ovaries, and spleen at one time, with immediate autotransplantation in one animal, was without effect.

Homoiotransplantation.

Direct and microscopic examinations were made in three cases on the 14th, 15th, and 17th days, while in the remaining ones it was delayed for 2 or more months. Only in one instance, the 14 day transplant, was there definite splenic tissue remaining, and in this only the lymphoid tissue and trabeculæ could be recognized. The one outstanding difference between the spleen and other tissue transplants is the marked early connective tissue reaction resembling

[5] Musser, J. H., Jr., and Krumbhaar, E. B., The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. VI. The Blood Picture at Various Periods after Splenectomy, *J. Exp. Med.*, 1913, xviii, 487.

[6] Sollberger, H., Beiträge zur Physiologie der Drüsen. XIX. Fortgesetzte Beiträge zur Lehre von der Funktion der Milz als Organ des Eiweissstoffwechsels. Über die Kompensationsvorgänge nach Milzexstirpation, *Biochem. Z.*, 1913, lv, 13.

the granulation tissue formation seen in chronic inflammation. Infection from organisms in the spleen, however, is not probable, as only normal spleens from rabbits with no evidence of acute infection were used. It is more probable that the splenic tissue when transferred to the subcutaneous fascia is an active irritant. The studies of Carrel[7] on the effect of tissue extracts on the growth of connective tissue *in vitro* are of interest in this connection. He found that adult spleen extracts, thyroid extracts, and the Rous chicken sarcoma extract markedly accelerated the proliferation of connective tissue. Our observations on the irritant effect of spleen grafts confirm his observations. The effect is present in auto- as well as homoiografts, though perhaps the homoiografts excite a slightly greater connective tissue reaction. Carrel also found that thyroid extracts excite in the living animal an even more marked connective tissue proliferation both in healing skin and periosteum wounds. We have been unable to detect an excessive connective tissue proliferation around thyroid grafts. It would seem, as suggested by Carrel, that spleen extracts could be used to promote the granulation of wounds.

Autotransplantation.

Four transplants were examined on the 8th, 11th, and 13th days, respectively. All were positive and seemingly active. The 8th, 11th, and one 13th day transplants were removed for histological examination. In contrast with the homoiotransplants, examined after approximately the same period, all were positive with well established blood supplies and central necrosis, and the peripheral zone of pulp, lymphoid, and trabecular tissues was distinct. There was also the same connective tissue reaction seen in the homoiotransplants.

Of the three transplants examined at the 65th, 67th, and 80th days, complete absorption leaving large white scars at the sites had occurred in two, while in the third (Rabbit 14) a dark red, sharply circumscribed mass appeared, the size of a small wheat kernel. There were several large vessels entering it, and except for its darker color it could

[7] Carrel, Artificial Activation of the Growth *in Vitro* of Connective Tissue, *J. Exp. Med.*, 1913, xvii, 14.

easily have been mistaken for an autothyroid transplant. We did not remove it, and it was recovered at autopsy 325 days after transplantation, the rabbit having died of acute pneumonia and pleurisy. Grossly, the transplant measured 4 by 2.5 by 2 mm., and was dark bluish red in color. It had evidently reached its maximum growth before the first examination on the 67th day, since it was not noticeably larger on the 325th day than at the first examination. This could be interpreted as evidence that its growth had occurred in response to a physiological insufficiency during the time when other tissues were assuming the function lost through removal of the spleen.

Microscopic examination reveals typical congested splenic tissue embedded in striped muscle and mammary gland. The capsule is very thick as compared with transplants of ovary, parathyroid, thyroid, or adrenal, and several connective tissue bands extend from the capsule to the surrounding muscle fascia. Many large vessels run in the outer layer of the capsule. Small trabeculæ also extend throughout the gland parenchyma. Their numbers are the same that one finds in the normal spleen, while their size is proportional to the size of the organ. No attempt was made to demonstrate the presence or absence of smooth muscle fibers in the capsule and trabeculæ and no studies on the reticulum have been made. In some sections one can see as many as sixteen well defined Malpighian bodies with the characteristic central artery and radial capillary system. These Malpighian bodies are surrounded by typical splenic pulp with large highly congested sinuses. In places there are deposits of the yellowish brown blood pigment usually seen in the normal spleen pulp. We have, therefore, to deal with a small newly formed spleen developed in an entirely foreign field as regards its location, nerves, and blood vessel relations. It has all the characteristics of the normal spleen in as far as these have been investigated, both as to the number of its component structures, capsule, trabeculæ, Malpighian bodies, pulp, sinuses, and blood pigment, and their relation to each other. The trabeculæ are proportional to the size of the organ, while all the other structures are of normal proportions, suggesting that the trabeculæ play a purely mechanical part. The capsule is still relatively much thicker than that of the normal spleen,

and it seems probable that this is due to the enormous proliferation of the connective tissue which takes place around the graft very early (within 2 weeks), and also that this tends to return to relatively more normal proportions through absorption as the connective tissue becomes adjusted to its new neighbor. One of the striking facts, then, is that the regeneration involves all the structures in such a way that both their normal proportions and arrangements are wholly retained. This would suggest a fairly uniform vitality for all the component parts as well as a close functional interrelation. It may be recalled that in the adrenal the cortical cells readily survive, while the medullary cells invariably die, at least in our experience. When one recalls that in the thyroid only a narrow peripheral zone of three or four cells in thickness survives the transfer and that the whole interior portion undergoes necrosis, it is of much more significance that a fully regenerated organ should develope after transplantation of splenic tissue than in the case of such tissues as the thyroid or parathyroid which contain but a single specialized tissue.

The regeneration of Malpighian bodies is also of interest. Why does the lymphoid tissue not regenerate around the regenerated arteries running in the capsule and trabeculæ as well as in those of the interior of the lobule? The best studies on the taking of engrafted tissues indicate that the host supplies the new blood vessels to the transferred tissue rather than that it utilizes the possible surviving fragments in the graft. It is difficult to harmonize this with the known fact that the intralobular arteries of even a regenerated spleen develop this envelope of lymphoid-like cells. Either the vessels must be specific, which does not seem probable, or the lymphoid-like cells are specific in that they control and determine the blood vessel arrangement. If this is the case, we have proof that the Malpighian bodies represent a different type of lymphoid tissue, functionally as different from ordinary lymph node tissue as is thymic tissue.

The same questions must also be raised in connection with the development of the spleen sinuses as have been discussed in connection with the formation of the Malpighian bodies. The careful study of an appropriate series of these grafts will doubtless give valuable data, both as to the normal development and function of this peculiar

organ. The survival and growth of the pulp cells are more easily understood and the process is probably similar to that of other single type tissues.

The general features of the transplant are shown in the accompanying photomicrographs (Figs. 1, 2, and 3).

SUMMARY.

We have not found in the literature a report of an instance of permanent homoio- or autotransplantation of the spleen, or of the probably closely related spleno- and hemolymph glands. Spleen autotransplants with considerable difficulty as compared with thyroid, parathyroid, ovary, or adrenal cortex. This may be due to its complex anatomical structure. An instance of a permanent autotransplant has been observed. None of our attempts to homoiotransplant it were successful beyond the usual taking and persistence for 2 or 3 weeks, common to all homoiografts. The successful permanent subcutaneous autotransplantation had all the morphological characteristics of a fully differentiated and functionally active spleen. This method of transplantation would seem to offer a means of learning more of the normal development, regeneration, and function of this complex tissue.

EXPLANATION OF PLATE 54.

FIG. 1. Photomicrograph of the 325 day spleen transplant (Rabbit 14), showing adjacent abdominal muscle, capsule, and general characteristics. × 20.

FIG. 2. Photomicrograph of the same transplant, showing the structure. × 100.

FIG. 3. Photomicrograph of the same transplant, showing trabeculæ, pulp, and a Malpighian corpuscle. × 100.

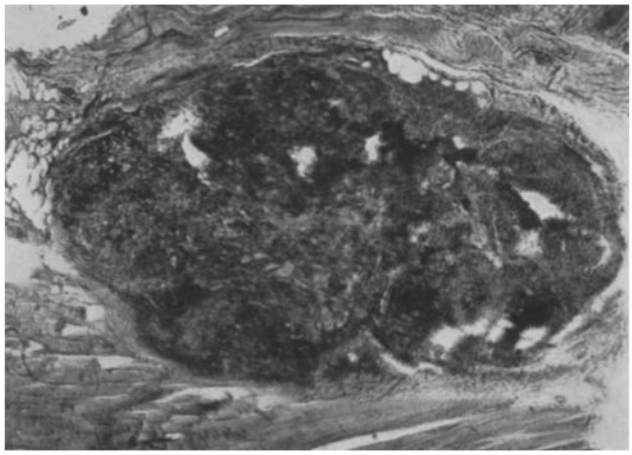

FIG. 1.

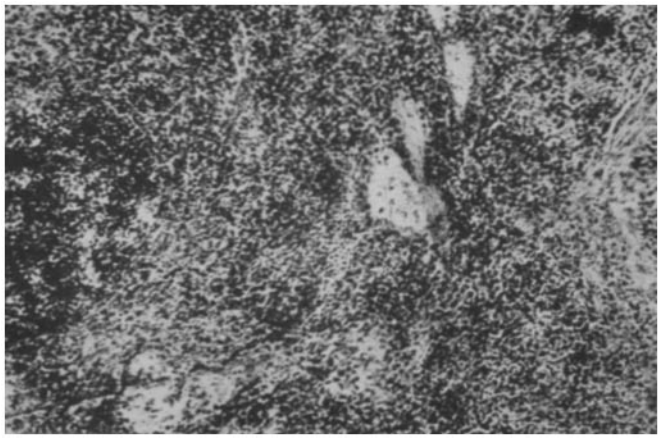

FIG. 2.

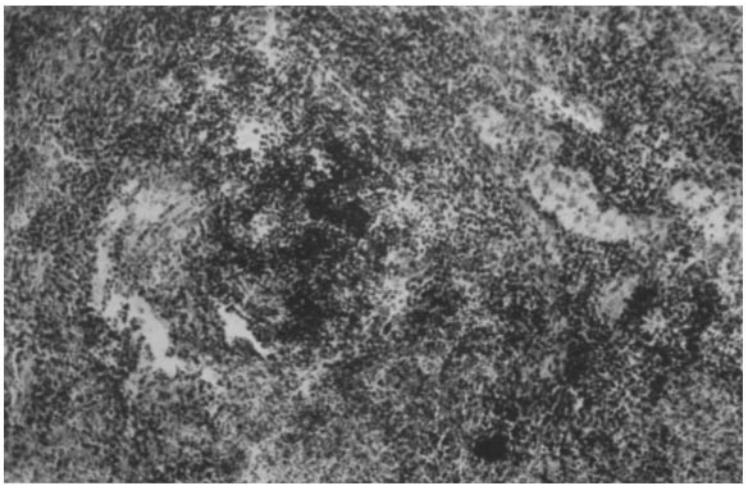

FIG. 3.

(Manley and Marine: Transplantation of Splenic Tissue.)