HOMEOTRANSPLANTATION AND AUTOTRANSPLANTATION OF THE SPLEEN IN RABBITS.

III. Further Data on Growth, Permanence, Effect of Age, and Partial or Complete Removal of the Spleen.

By DAVID MARINE, M.D., and O. T. MANLEY, M.D.

(*From the Department of Experimental Medicine of Western Reserve University, Cleveland.*)

(Received for publication, February 27, 1920.)

We have been unable to find any references in the literature to the transplantation of fragments of spleen to parts of the body widely separated from the normal neurovascular field of this organ in addition to those referred to in 1917. At that time[1, 2] we reviewed the literature and reported our first experiments with spleen homeografts and autografts in fifteen rabbits. The present paper includes the data of further experiments with homeografts and with autografts, together with certain general physiological reactions relative to the spleen which this study has emphasized.

Method.

The method consists of transferring small fragments of the spleen roughly 2 by 2 mm. to the subcutaneous tissues of the abdomen under strict aseptic precautions. The tissue to be transplanted was kept in isotonic salt solution at temperatures varying between 36° and 39°C. Sometimes the tissue was transplanted within a few minutes after removal, while in others it was kept in salt solution as long as 3 hours. The fragment to be transplanted was rinsed in the salt solution, but no other attempt to remove contained blood was made.

[1] Manley, O. T., and Marine, D., The transplantation of splenic tissue into the subcutaneous fascia of the abdomen in rabbits, *J. Exp. Med.*, 1917, xxv, 619.

[2] Marine, D., and Manley, O. T., Influence of age on the permanence of subcutaneous autografts of the spleen in rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 123.

After making a transverse abdominal skin incision approximately 2 cm. in length, usually one on each side of the umbilicus, the subcutaneous fascia was lifted with fine forceps and an area free from blood vessels was punctured with a cataract knife, the tissue introduced, and the fascial opening closed with a ligature. The skin incision was sutured and sealed with celloidin. All operative procedures were carried out under ether anesthesia. All subsequent examinations were direct (autopsy or operation). If the examination was made by operation, ether anesthesia was used, and with aseptic precautions the skin was incised, the graft or its site was exposed, measurements were taken, the graft was removed for microscopic examination or left *in situ*, and the skin closed with suture and sealed with celloidin. The intervals between examinations are given in the tables. This technique has been employed in all our transplantation work with ductless gland tissues.

Both homeografts and autografts were studied in relation to taking and growth or regeneration, in relation to age of the animals, and in relation to partial and complete removal of the spleen and to permanence of the grafts.

Homeotransplantation.

The data of the experiments are given in Table I. Only the rabbits which had not been subjected to previous homeografts of any tissue are included in the table. This was done to avoid the untoward effect of active immunity which such grafts produce and to render conditions as favorable as possible for taking and growth. Notwithstanding this important precaution not a single graft was found active after the 30 day period, a period arbitrarily chosen, but somewhat longer than the time necessary for the full development and destructive effect of the active immunity. The experiments were not studied from the standpoint of determining how rapidly the grafts were destroyed, though by comparison they show very clearly that the destruction of spleen is much more rapid than that of thyroid tissue transplanted under the same conditions. Thus in a large number of routine homeotransplants of thyroid we have obtained about 10 per cent active at the end of 30 days, though ultimately (in the course of

60 to 90 days) these also might be destroyed. These observations indicate that spleen tissue produces a high degree of immunity more quickly than does thyroid.

The well established favorable factor of blood relation in the transplantation of certain tissues was also utilized in the hope that grafts might survive beyond the 30 day period, but with spleen we were unsuccessful. The antigenic power of spleen, then, is very high and on a level with that of other hematopoietic tissues. In a few instances in which the grafts were examined at the 7th, 8th, and up to the 16th day, active splenic tissue, well established blood supply, and evidence of growth were found. This is observed generally with homeografted tissues. Many literature reports of positive homeografts of tissues are misleading because they have emphasized or reported only this early stage. Effective immunity to foreign proteins in general does not become manifest until the 8th to 10th day, as shown by studies on anaphylaxis. Within this period homeografts usually take and often show growth, while after this period rapid destruction takes place. Different tissues show different rates of destruction. In our experience lymphoid (lymph gland and thymus) and splenic tissues undergo destruction more rapidly than thyroid, and thyroid cells more rapidly than cells rich in lipoids, as adrenal cortex and interstitial cells of the ovary and testis. Associated with this destruction there is the well known infiltration or invasion of the graft with lymphoid cells. Much importance has been assigned to this phenomenon and some authors, notably Murphy and his coworkers,[3] have claimed for it a primary importance in the death and destruction of homeografts. We cannot subscribe to this view. We believe that the primary injury is due to the action of antibodies and that the lymphoid cells are attracted to the site secondarily, and in response to a special low grade irritation which injured cells set up. Loeb[4] and Sittenfield[5] also have expressed the view that lymphocytes are not primarily concerned in the destruction of foreign proteins.

[3] Murphy, Jas. B., and Morton, J. J., The lymphocyte in natural and induced resistance to transplanted cancer. II, *J. Exp. Med.*, 1915, xxii, 204.

[4] Loeb, Leo, Multiple transplantations of the thyroid and the lymphocytic reaction, *J. Med. Research*, 1918–19, xxxix, 71.

[5] Sittenfield, M. J., The significance of the lymphocyte in immunity to cancer, *J. Cancer Research*, 1917, ii, 151.

TABLE I.

Homeotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used.		First examination.	Final examination.	Additional data.
					R.*	L.			
		days		*1917*	*1917*	*1917*			
1	4–46	26	M.	Jan. 18 Complete.		Jan. 18 3–155		L. 54–	Jan. 18. L. adrenalectomy. Thyroids and thymus enlarged.
2	4–48	33	F.	Jan. 25 Complete.		Jan. 25 3–157	L. 47±†	" 132–	Thymus atrophic. Jan. 25. L. adrenalectomy; homeograft of adrenal.
3	4–52	40	M.	Feb. 1 Complete.	Feb. 1 3–159		R. 40–	R. 125–	Feb. 1. L. adrenalectomy. Thymus present; thyroid large.
4	4–54	47	"	Jan. 30 Complete.	Jan. 30 3–161			" 42–	Jan. 30. L. adrenalectomy; homeograft of adrenal. Thymus large; thyroid normal.
5	4–64	54	F.	Feb. 6 Complete.	Feb. 6 3–163		R. 35–	" 121–	Feb. 6. L. adrenalectomy. Thymus atrophic; thyroid normal.
6	4–74	61	"	Feb. 13 Complete.	Feb. 13 3–165		" 58–	" 114–	Feb. 13. L. adrenalectomy; homeograft of adrenal. Thymus large; thyroid normal.
7	4–94	90	M.	Mar. 22 Complete.		June 7 3–184		L. 98–	Mar. 22. L. adrenalectomy. Thymus cellular; thyroid normal.
				1915		*1915*			
8	4–17	123	"	Dec. 3 Complete.		Dec. 16 1–320		" 67–	Spleen was enlarged; thymus small; adrenals small. Dec. 3. Thyroidectomy.
				1917		*1917*			
9	4–78	168	"	Feb. 15 Complete.		Feb. 15 3–169		" 56–	Feb. 15. L. adrenalectomy. Chronic nephritis; thymus atrophic; thyroid and parathyroids enlarged.

				1915		*1915*			
10	4–2	Adult.	M.	Aug. 26 Complete.		Nov. 29 1–313		L. 15—	Nov. 29. Thyroidectomy.
11	4–3	"	F.	Aug. 26 Complete.		Dec. 16 1–320		" 65—	" 29. "
					1915				
12	4–4	"	M.	Nov. 5 Complete.	Dec. 16 1–320	Nov. 29 1–313		R. 67— L. 17—	" 29. "
13	4–5	"	"	Nov. 5 Complete.	Dec. 18 1–321			R. 63—	Dec. 2. "
14	4–6	"	F.	May 11 Complete.		Dec. 2 1–315 Dec. 16 1–320		L. 14±(Dec. 2). " 65—(" 16).	" 2. "
15	4–	"	M.	Nov. 5 Complete.		Dec. 16 1–320		L. 65—	" 2. "
16	4–8	"	"	Nov. 5 Complete.		Dec. 16 1–320		" 67—	" 3. "
17	4–9	"	F.	Nov. 5 Complete.		Dec. 16 1–320		" 119—	" 3. "
				1916		*1916*			
18	4–42	"	"	Dec. 12 Complete.		Dec. 12 3–151		" 43—	" 12. " L. adrenalectomy.
					1917				
19	4–44	Old adult.	M.	Dec. 19 Complete.	Feb. 20 1–411			R. 23—	Dec. 12, 1916. Thyroidectomy; L. adrenalectomy.
				1917		*1917*			
20	4–93	52	F.	Mar. 20 ½ cm. Apr. 17 1 cm.		Apr. 17 3–179		L. 52—	Mar. 20. L. adrenalectomy. Thymus atrophic; thyroid normal.

* R. indicates right; L., left.

† The condition of the transplants at the different examinations is expressed by plus or minus signs instead of the actual measurements obtained in order to facilitate comparison. The figures express the intervals in days.

TABLE I—*Continued.*

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used. R.*	Date transplanted. Spleen used. L.	First examination.	Final examination.	Additional data.
		days		*1917*	*1917*	*1917*			
21	4–104	80	M.	Apr. 17 ½ cm.		Apr. 17 3–179	L. 53—	L. 163—	All organs intact; same litter as Nos. 4–105 and 4–106; thyroid slightly enlarged; thymus present.
22	4–105	80	"	Apr. 17 1 cm.		Apr. 17 3–179		" 53—	All organs intact.
23	4–106	80	"	Apr. 17 1 cm.		Apr. 17 3–179	L. 53—	" 163—	" " " thymus small.
24	4–108	80	"	Apr. 17 ⅔ cm.		Apr. 17 3–192		" 52—	" " "
25	4–98	104	F.	Apr. 10 ½ of spleen.		Apr. 10 3–177	L. 58±	" 169—	Thyroid lobes large, vascular; thymus normal.
26	4–101	60	M.		Apr. 10 3–177			R. 56—	June 26, 1916. R. adrenalectomy. Oct. 10. Gonadectomy. Mar. 13, 1917. Thyroidectomy.
		mos.							Spleen small; thymus very large.
27	4–65	4	"			Feb. 6 3–163		L. 23—	Feb. 6. Thyroidectomy; much enlarged. H_2SO_4.
28	4–49	4	F.			Jan. 25 3–158		" 21—	Parathyroids very large; thymus large.
									Jan. 25. Thyroidectomy; H_2SO_4.
29	4–50	4	"			Jan. 25 3–157		" 40—	" 25. " H_2SO_4.
									Spleen small; parathyroids very large.
30	4–55	4	"		Jan. 30 3–162		R. 14++	R. 35—	Jan. 30. Thyroidectomy; H_2SO_4.
									Spleen enlarged; parathyroids very active.

31	4–56	4	F.		Jan. 30 3–162			R. 30–	Jan. 30. Thyroidectomy; H_2SO_4. Spleen normal; thymus active.
32	4–57	4	"		Jan. 30 3–162			" 30–	Jan. 30. Thyroidectomy; H_2SO_4. Adrenals and spleen normal; thymus atrophic.
33	4–58	4	M.		Jan. 30 3–162			" 30–	Jan. 30. Thyroidectomy; KOH.
34	4–59	4	"		Feb. 1 3–159			" 28–	Feb. 1. " H_2SO_4. Spleen and thymus normal.
35	4–60	4	"		Feb. 6 3–159			" 28–	Feb. 6. Thyroidectomy; H_2SO_4 and KOH. Spleen small; thymus very large.
36	4–61	4	F.			Feb. 6 3–163		L. 23+	Feb. 6. Thyroidectomy; H_2SO_4. Thymus atrophic.
37	4–76	5	M.			Feb. 13 3–166	L. 16+	" 42–	Feb. 13. Thyroidectomy; KOH.
38	4–62	5	F.			Feb. 6 3–163		" 23–	" 6. " KOH.
39	4–75	Adult.	"			Feb. 13 3–165	L. 16+	" 42–	" 13. " H_2SO_4 and KOH.
40	4–81	"	"			Feb. 15 3–169		" 8+	Jan. 23. Thyroidectomy.
41	4–82	"	"		Feb. 15 3–174			R. 42–	" 23. "
42	4–99	"	M.		Apr. 10 3–177			" 7+	Sept. 28, 1916. R. adrenalectomy. Jan. 16, 1917. L. adrenalectomy. Mar. 3. Thyroidectomy; H_2SO_4. Thymus atrophic; spleen small.
43	4–100	"	"		Apr. 10 3–177			" 58–	Sept. 22, 1916. R. adrenalectomy. Jan. 14, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus atrophic; spleen small.

TABLE I—*Concluded.*

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used. R.*	Date transplanted. Spleen used. L.	First examination	Final examination.	Additional data.
					1917				
44	4–102	Adult.	M.		Apr. 10 3–177			R. 7+	May 25, 1916. R. adrenalectomy. June 1. L. adrenalectomy. Dec. 7. Thyroidectomy; gonadectomy. Spleen small; thymus atrophic.
45	4–109	"	"		Apr. 10 3–177			" 58—	Sept. 26, 1916. R. adrenalectomy. Jan. 11, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus large; spleen normal; L. adrenal much enlarged.
46	4–40	"	"		Jan. 25 3–158			" 26—	Dec. 26, 1916. Thyroidectomy; H_2SO_4.
						1916			
47	4–21	"	"			Dec. 19 3–154		L. 34—	Mar. 11, 1916. R. adrenalectomy. Dec. 19. L. adrenalectomy. Jan. 9, 1917. Gonadectomy. Thyroid normal; thymus very large.
48	4–39	"	"		Jan. 25 3–158			R. 26—	Jan. 2. Thyroidectomy.
49	4–43	"	"		Jan. 25 3–158			" 26—	" 2. " Previous homeografts.
						1917			
50	4–87	Old adult.	F.			Feb. 27 3–167		L. 23—	Jan. 22. Thyroidectomy. Many previous homeografts.

Effect of Age.—This factor has no appreciable influence. In this series rabbits of known age and parentage, varying from 26 to 168 days, as well as a large number of adults of different but known ages, were used. In no instance did the graft survive the 30 day period. Variations in the rate of destruction depending on age must be expected, but their detection in the case of spleen would require frequent examinations within the 30 day period. Further attempts were made to determine whether splenectomy, partial or complete, had any effect. No noteworthy difference in the outcome even in young rabbits was detected whether the spleen was intact, or partially or completely removed at the time of, before, or after transplantation. The experiments do not eliminate the possibility of an advantage accruing to the transplant from induced splenic insufficiency, but indicate that if splenectomy is of aid it must be looked for by frequent examinations within the first 30 days, since by that time the developed immunity has destroyed any possible evidence.

Thyroidectomy and partial or complete adrenalectomy combined or separate likewise have no noteworthy effect on delaying destruction of the grafts and, therefore, probably on the degree of immunity developed. In this connection it may be pointed out that Gates[6] was unable to detect any noteworthy difference in the degree of immunity to sheep erythrocytes and typhoid bacilli obtained in guinea pigs with and without partial removal of the adrenal glands. We have found[7] that thyroidectomy and splenectomy alone or combined have no marked effect on the antibody formation following injection of sheep erythrocytes in rabbits. Bullock and Rohdenburg[8] find that splenectomy has no influence on the immunity to transplanted tumors.

[6] Gates, F. L., Antibody production after partial adrenalectomy in guinea pigs, *J. Exp. Med.*, 1918, xxvii, 725.

[7] Unpublished results.

[8] Bullock, F. D., and Rohdenburg, G. L., Splenectomy exerts no appreciable influence upon immunity against transplanted tumors, *J. Cancer Research*, 1917, ii, 465.

Autotransplantation.

The data concerning these experiments are given in Tables II and III. Usually two fragments were transplanted, one to the right and the other to the left of the umbilicus, and designated as right and left transplants. In the six experiments reported in 1917 one failed and in the present series one failed. This was due to infection. Failure of autografts to take always indicates some gross technical error. Some of these rabbits had been used for homeotransplantation of other tissues—adrenal, thyroid, and sex glands. Previous or coincident homeografts have no demonstrable effect on the taking or growth of autografts. The subsequent course of these autografts was followed and it was found to be modified by several factors, the most striking of which are age and partial or total removal of the spleen.

Effect of Age.—The experiments have been arranged in Tables II and III according to the age at the time of transplantation. The youngest rabbit used was 26 days old and the oldest whose age was definitely known was 320 days; one other is listed as 586+ days because it had been in the laboratory for this length of time before being utilized for this purpose. A maximum of three examinations is given in the tables, although in several instances five, six, and more examinations have been made. A consideration of the series as a whole shows a distinct decrease in the amount of growth of the transplants as one passes from the youngest to the oldest rabbits of the series, although other factors remained as constant as possible. The greatest and most rapid growth of transplants occurred in the youngest rabbits, also the least growth in the oldest. While this decrease with age in the rate and amount of growth is gradual, the differences become striking about the 4th to 5th month. In our series the first instance of failure to obtain marked growth was in a rabbit 132 days old. The remaining ten rabbits, 167 or more days old at the time of transplantation, failed to show marked growth. This change in the rate of growth corresponds roughly with the time of sexual maturity or early adult life. There is a suggestion that in adult rabbits transplants that in the 1st month showed some growth tended to involute or undergo atrophy later. This tendency to atrophy or

involution was not seen in young rabbits with transplants of similar duration. As suggested in a previous note[2] two factors (possibly others) may be considered. The first is that growth of the transplants is a part of the normal growth of the animal and ceases when physical growth is complete. This possibility is disproved by the fact that such transplants fail to show marked growth even in the young if the spleen is not removed. The second factor would presuppose that the value of the spleen to the organism decreases with age and that after adult life whatever functions the organ normally has may be assumed by other tissues, possibly lymph glands and bone marrow. This view is supported by the results obtained by the removal of the spleen which follow.

Effect of Partial and Complete Removal of the Spleen on Autografts.—The data of these fifteen experiments are given in Table III. The amount of spleen removed and the dates are given in the table. Usually less than 0.5 cm. of the anterior portion was removed at the time of transplantation.[9] The youngest rabbit with partial removal of spleen and transplantation was 52 days old and the oldest 124 days. The same differences in the growth of transplants, dependent upon age and independent of amount of spleen removed, are noted as in Table II. Thus in No. 1, a 52 day old rabbit in which one-fourth of the spleen was removed, the transplants were ++ at 80 days and +++ at 177 days, while in No. 15, a 124 day old rabbit in which one-eighth of the spleen was removed, no growth took place in 132 days. Between these extremes there is the same evidence of gradation of growth dependent upon age as shown in Table II. There is no instance of a very young rabbit with a minimum amount of spleen removed. Such experiments would show whether in the absence of splenic deficiency marked growth could occur. This question, however, is clearly answered in the negative for 52 day old rabbits by Experiments 1, 2, and 3, in which all were from the same litter. Two had about one-fourth of the spleen removed at the time of transplantation and at the first examinations 80 and 52 days later both were ++, while the third, which had only one-eighth of the

[9] There are considerable variations in the size of the spleen in apparently healthy rabbits, just as in other animals, which cannot be accounted for with our present knowledge of physiology and pathology.

TABLE II.

Autotransplantation of Spleen (Complete Removal of Spleen).

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
1	4–45	26	330	M.	Jan. 18	Jan. 18	R. 54+++ L. 54+++	R. 145+++ L. 145+++	R. 278+++ L. 278+++	Jan. 18. L. adrenalectomy. May. 1. Thyroidectomy.
2	4–46	26	315	"	" 18	" 18	R. 54+++	R. 139+++	R. 141+++	Jan 18. L. adrenalectomy.
3	4–47	33	575	"	" 25	" 25	" 47++ L. 47++	" 96+++ L. 96+++	" 230+++ L. 230+++	" 25. " " H_2SO_4. May 1. Thyroidectomy.
4	4–48	33	505	F.	" 25	" 25	R. 47++	R. 132+++	R. 134+++	Jan. 25. L. adrenalectomy.
5	4–51	40	330	"	Feb. 1	Feb. 1	" 40++ L. 40++	" 110++++ L. 110+++	" 224++ L. 224++	Feb. 1. " " H_2SO_4. May 1. Thyroidectomy; transplants slightly decreased.
6	4–52	40	415	M.	" 1	" 1	" 40++	" 125++	" 232+++	Feb. 1. L. adrenalectomy. Thyroids markedly enlarged at autopsy.
7	4–53	47	405	F.	Jan. 30	Jan. 30	R. 42++ L. 42++	R. 226++++ L. 226++++	R. 384++++ L. 384++++	Jan. 30. L. adrenalectomy; H_2SO_4. May 1. Thyroidectomy.
8	4–63	54	410	"	Feb. 6	Feb. 6	R. 35++ L. 35++	R. 84+++ L. 84+++	R. 121+++ L. 121+++	Feb. 6. L. adrenalectomy; KOH. May 1. Thyroidectomy.
9	4–64	54	310	"	" 6	" 6	" 35++	" 121++	" 133++	Feb. 6. L. adrenalectomy. Thyroids small.

10	4–73	61	555	F.	Feb. 13	Feb. 13	R. 58++++ L. 58++++	R. 77++++ L. 77++++	R. 119++++ L. 119++++	Feb. 13. L. adrenalectomy; KOH. May 1. Thyroidectomy.
11	4–94	61	595	"	" 13	" 13	" 58+++	" 114+++	" 115+++	Feb. 13. L. adrenalectomy. Thyroid normal.
					1916	*1916*				
12	4–38	66	1,275	M.	Dec. 7	Dec. 7	" 36+	" 82++	" 183++	Dec. 6. L. adrenalectomy; phosphoric acid.
13	4–37	66	875	F.	" 7	" 7	" 36+	" 56++	" 82+++	Dec. 6. L. adrenalectomy; phosphoric acid.
14	4–36	66	1,225	M.	" 7	" 7	" 36+	" 56+	" 181++	Dec. 6. L. adrenalectomy; phosphoric acid. Feb. 27, 1917. Thyroidectomy.
					1917	*1917*				
15	4–86	75	790	"	Feb. 27	Feb. 27	R. 44++ L. 44++	R. 63+++ L. 63+++	R. 197++++ L. 197++++	Feb. 27. L. adrenalectomy; KOH. May 1. Thyroidectomy.
					1916	*1916*				
16	4–26	83	1,620	"	Dec. 5	Dec. 5	" 38++	" 290+++	" 1,181+++	Dec. 5. L. adrenalectomy; phosphoric acid; double ligation of vas deferens. Apr. 5, 1917. L. gonadectomy; partial thyroidectomy.
17	4–25	83	1,450	F.	" 5	" 5	" 38+	" 84+++	" 185+++	Dec. 5. L. adrenalectomy; phosphoric acid; partial thyroidectomy.
					1917	*1917*				
18	4–110	84	1,050	"	May 8	May 8	R. 31++ L. 31++	R. 141++++ L. 141++++	R. 679+++ L. 679+++	Pregnant; thyroids normal.

TABLE II—*Concluded.*

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
19	4–112	84	1,090	M.	May 8	May 8	R. 31+ L. 31+		R. 141+ L. 141+	Thyroids normal; lack of growth of transplants; large accessory spleen.
20	4–113	84	1,225	"	" 8	" 8	R. 31++ L. 31++	R. 141+++ L. 141+++	R. 475++++ L. 475++++	Example of acute enlargement of spleen transplant with pneumonia. White blood corpuscles 30,800.
21	4–94	90	1,195	"	Mar. 22	Mar. 22	R. 77+ L. 77+		R. 175++ L. 175++	Mar. 22. L. adrenalectomy.
22	4–95	97	1,200	F.	Apr. 3	Apr. 3	R. 64++ L. 64++	R. 176+++ L. 176+++	R. 1,062+++ L. 1,062+++	
23	4–97	104	1,265	M.	" 10	" 10	R. 58+++ L. 58+++		R. 169+ L. 169+	Spleen much enlarged at time of removal; evidence that transplants grew more rapidly, then decreased with recovery of animal.
24	4–103	111	1,275	"	" 17	" 17	R. 51++ L. 51++		R. 163++ L. 163++	
25	4–119	132	2,240	"	June 7	June 7			R. 111+ L. 111+	Failure to obtain growth with spleen removed.
26	4–77	167	3,025	"	Feb. 15	Feb. 15	R. 56+ L. 56+	R. 75+ L. 75+	R. 209+ L. 209+	Feb. 15. L. adrenalectomy; KOH. May 1. Thyroidectomy.

27	4–78	167	3,445	M.	Feb. 15	Feb. 15	R. 56–		R. 113–	Feb. 15. L. adrenalectomy; enlarged thyroid lobes. Failure to take probably due to error in technique.
28	4–84	173	2,575	F.	" 22	" 22	" 49+ L. 49+	R. 68+ L. 68+	" 106+ L. 106+	Feb. 22. L. adrenalectomy; KOH; thyroidectomy.
29	4–85	173	3,425	"	" 22	" 22	R. 49+ L. 49+	R. 68+	R. 202+	Feb. 22. L. adrenalectomy; KOH.
30	4–79	320	4,835	"	" 15	" 15	R. 56++ L. 56++		" 61++ L. 61++	Died; pneumonia.
31	4–80	320	4,435	"	" 15	" 15			R. 23+ L. 23+	" "
32	4–83	320	2,940	M.	" 20	" 20	R. 51+		R. 107+	Feb. 20. L. adrenalectomy; thyroidectomy. Apr. 3. Gonadectomy.
33	4–68	396	2,525	"	" 8*	" 8	" 33+ L. 33+	R. 114+ L. 114+	" 216+ L. 216+	June 22, 1916. R. adrenalectomy. Oct. 10. L. gonadectomy. Apr. 11, 1916. Thyroidectomy.
34	4–71	396	2,790	"	" 8	" 8	R. 33+ L. 33+		R. 118+ L. 118+	Feb. 12, 1916. Thyroidectomy. Nov. 3. R. adrenalectomy.
35	4–72	586+	3,150	"	" 8	" 8	R. 33+ L. 33+		R. 118+ L. 118–	Apr. 5. Gonadectomy. Oct. 25, 1915. Thyroidectomy.

*Small accessory spleen left.

TABLE III.

Autotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy.	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	*gm.*		*1917*	*1917*				
1	4–92	52	720	M.	Mar. 20. ¼ removed.	Mar. 20	R. 80++ L. 80++		R. 177+++ L. 177+++	Mar. 20. L. adrenalectomy. Thyroid normal.
2a	4–93	52	700	F.	Mar. 20. ¼ removed.	" 20	R. 52++ L. 52++		R. 177+++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid normal.
b					Apr. 17. ¼ removed.	Apr. 17	R. 52++		R. 150++	
3a	4–90	52	635	M.	Mar. 20. ⅛ removed.	Mar. 20	" 80+ L. 80+			Mar. 20. L. adrenalectomy.
b					June 9. Remainder removed.				" 177++++ L. 177++++	Very little growth in first 80 days; marked after removal of spleen; compensatory.
4a	4–88	64	1,050	"	Mar. 20. ⅛ removed.	Mar. 20	R. 49+ L. 49+			Mar. 20. L. adrenalectomy.
b					May 8. Remainder removed.			R. 79+++ L. 79+++	R. 177+++ L. 177+++	Thyroid slightly enlarged; very little growth in first 80 days; marked after removal of spleen.
5a	4–89	64	985	"	Mar. 20. ⅛ removed.	Mar. 20	R. 49+ L. 49+			Mar. 20. L. adrenalectomy.
b					May 8. Remainder removed.			R. 79++ L. 79++	R. 177+++ L. 177+++	Thyroid vascular; slightly enlarged. Rapid growth after removing spleen.

6	4–104	80	960	M.	Apr. 17. $\frac{1}{8}$ removed.	Apr. 17	R. 53+		R. 163+	No growth with spleen intact.
7	4–105	80	1,055	"	Apr. 17. $\frac{1}{8}$ removed.	" 17	" 53+		" 196+	No growth with spleen intact.
8	4–106	80	1,030	"	Apr. 17. $\frac{1}{8}$ removed.	" 17	" 53+		" 163+	No growth with spleen intact.
9	4–114	84	1,260	F.	May 8. $\frac{1}{8}$ removed.	May 8	" 31+ L. 31+		" 141+ L. 141+	No compensatory growth of transplants.
10	4–115	84	1,075	"	May 8. $\frac{1}{8}$ removed.	" 8	R. 31+++ L. 31++		R. 141++ L. 141+	Thyroid vascular and enlarged.
11	4–107	87	1,110	"	Apr. 24. $\frac{1}{8}$ removed.	Apr. 24	R. 52+ L. 52+		R. 163+ (very small). L. 163+ (very small).	Failure to obtain growth without spleen removal.
12	4–108	87	1,010	M.	Apr. 24. $\frac{1}{8}$ removed.	" 24	R. 52+		R. 198+	Failure to obtain growth without spleen removal.
13	4–96	97	1,245	"	Apr. 3. $\frac{1}{4}$ removed.	" 3	" 64+ L. 64+		" 176+ L. 176+	No compensatory hyperplasia.
14	4–98	104	985	F.	Apr. 10. $\frac{1}{2}$ removed.	" 10	R. 58+ L. 58+		R. 169+ L. 169+	Decreased with recovery of animal. Partial removal failed to produce growth.
15	4–118	124	1,625	M.	May 17. $\frac{1}{8}$ removed.	May 17	R. 21+ L. 21+		R. 132+ L. 132+	Failure to obtain growth of transplant with spleen intact.

spleen removed, was only + at the first examination 80 days later. The remainder of the spleen in this rabbit was then removed and at 177 days the transplants were ++++, while the first two which did not have the remainder removed were only +++ at 177 days. The same reaction is seen in Experiments 4 and 5, little if any growth occurring until the spleen was removed, when a marked growth promptly took place. The effect of splenic deficiency in stimulating the growth of transplants is striking, as is shown either by comparing animals of similar ages with and without partial removal or by comparing the growth of the transplants before and after total splenectomy in the same animal. The stimulus for this increased growth must be chemical in nature and must operate through the blood stream. The compensatory hyperplasia of the stump following partial removal of the gland cannot be separated from a possible nerve influence. The method of transplantation clearly demonstrates that specific nerves are not necessary for this reaction. Some investigators have not been able to obtain compensatory hyperplasia even of the stump *in situ* following partial removal, or of the thyroid, whose functions so far as we know cannot be assumed by any other tissue, and have doubted its occurrence, while others have no difficulty in demonstrating the effect even in transplants. In the work of Halsted[10] and Hunnicut[11] on the thyroid their failure to obtain compensatory hyperplasia was probably due to their failure to induce a thyroid insufficiency, in the production of which two factors of the utmost importance are involved; *viz.*, the amount of thyroid removed and the presence of available iodine. Loeb[12] recently published his results on compensatory hypertrophy of the thyroid following partial removal in guinea pigs. He found that compensatory hypertrophy occurs, though it is necessary to remove nearly all the gland. We have found that in rats, rabbits, and guinea pigs it is necessary to

[10] Halsted, W. S., Hypertrophy of the thyroid gland. Revision of experiments made 25 years ago, *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 111.

[11] Hunnicut, J. A., The absence of hyperplasia of the remainder of the thyroid in dogs after piecemeal removal of this gland. Auto-transplantation of the thyroid in partially thyroidectomized animals, *Am. J. Med. Sc.*, 1914, cxlviii, 207.

[12] Loeb, Leo, Studies on compensatory hypertrophy of the thyroid gland. I, *J. Med. Research*, 1919, xl, 199.

remove relatively more thyroid in order to obtain compensatory hyperplasia of the remaining stump than in cats and dogs, and in all cases available iodine must be excluded because of its inhibitory effect.[13] We believe that Loeb would have obtained more constant results had he in all instances allowed an interval of 30 days for compensatory hyperplasia to take place.

Histology of the Transplants.—This has been described elsewhere[1] and only brief mention of certain features need be made. Many of the transplants have reached 5 or 6 mm. in diameter (intracapsular measurements). They have all the general characteristics of normal spleen, both as to the number of component structures—capsule, trabeculæ, lobules, Malpighian bodies, pulp, sinuses, blood pigment—and their relation to each other. No attempt has been made to demonstrate the presence or absence of smooth muscle fibers in the capsule and trabeculæ. Apart from this the spleen is capable of complete regeneration. These studies indicate that while anatomically the spleen is very complex, biologically all the major elements are simple and endowed with uniform and marked regenerative capacity.

Permanence of Spleen Grafts.—Most of the experiments were terminated within a year. Two rabbits (Nos. 16 and 22) have been allowed to survive and at the examination on March 1, 1920 the transplants in each were found active and very vascular—1,181 and 1,062 days respectively. They are possibly slightly smaller than at the second examination 290 and 176 days after transplantation. Both rabbits were young at the time of transplantation and in both complete splenectomy was performed, thus insuring growth of the transplants. Both rabbits are still strong and active. One can conclude, therefore, that spleen autografts made under conditions which insure good initial growth are permanent. There appears, however, to be a slight involution or atrophy with age even in splenectomized rabbits, and, as already pointed out, transplants made in old rabbits without splenectomy may in time (several months) undergo complete atrophy. Our experiments with autotransplan-

[13] Marine, D., and Lenhart, C. H., Colloid glands (goiters): their etiology and physiological significance, *Bull. Johns Hopkins Hosp.*, 1909, xx, 131.

tation of the spleen and also of the thyroid show that at least an anatomical deficiency is not necessary in order that transplants may take and remain active for several months. Growth of these grafts, however, usually does not occur unless there is a physiological insufficiency which may exist independent of the amount of functionally active organ. Loeb and Hesselberg[14] have also shown that the taking of transplants is independent of a physiological insufficiency. Halsted,[15] working with the parathyroid, concluded that it was necessary to induce a physiological insufficiency in order to obtain successful transplants.

Reaction of the Grafts in Acute Infections.—No experiments have been made relative to this point. In a few instances in which the rabbits died of pneumonia, the transplants were markedly congested, and in one rabbit (Experiment 19) which died of pneumonia, the transplants at autopsy were soft, engorged with blood, and microscopic examination showed increase in pulp cells. In the ordinary sporadic cases of pneumonia in rabbits the reaction of the spleen is so variable and even in healthy rabbits there are such variations that it would be necessary to carry out a series of experimentally controlled infections to obtain definite data.

SUMMARY.

No instance of survival of spleen homeografts beyond the usual taking and persistence for 1 or 2 weeks common to most homeografts has been observed, although the possible advantages of consanguinity, age, and splenectomy were fully utilized. This is in sharp contrast to thyroid, sex gland, and adrenal cortex homeografts, with which one may expect 10 per cent to survive the 30 day period. It suggests that spleen is a stronger antigen and excites a greater degree of immunity more quickly. With autografts survival and growth are the rule, and failures are due to technical errors. Age is an important factor in the growth of autografts. The younger the rabbit the

[14] Loeb, Leo, and Hesselberg, C., Studies on compensatory hypertrophy of the thyroid gland. II, *J. Med. Research*, 1919, xl, 265.

[15] Halsted, W. S., Auto- and isotransplantation, in dogs, of the parathyroid glandules, *J. Exp. Med.*, 1909, xi, 175.

more growth is aided. This beneficial effect decreases gradually and becomes negligible after sexual maturity. Removal of the spleen is a powerful stimulus to the growth of transplants. The effect varies inversely with the age and usually is negligible after sexual maturity. The influence of age and splenectomy suggests that the spleen is most important in early life and after sexual maturity is either unimportant or its functions may readily be assumed by other tissues (hematopoietic). Anatomically the spleen is a highly complex structure, but biologically all the major elements of the spleen are simple as indicated by the uniform and marked regenerative capacity. There is a tendency for grafts to involute or atrophy with age, and grafts made in old rabbits without removal of the spleen may undergo complete atrophy. Grafts made in young rabbits, accompanied by splenectomy, have been observed for more than 3 years and may be said to be permanent. There is some evidence that subcutaneous autografts react to infections in the same way as the intact spleen.