Relation of Survival Time to Implantation Time of Second Set Skin Homografts in the Rat.* (21252)

JEROME W. LEHRFELD, A. CECIL TAYLOR, AND JOHN MARQUIS CONVERSE.

From the Departments of Surgery, New York University College of Medicine and of Anatomy, New York University College of Dentistry.

It is fairly well established that a second homograft of skin or other organs from the same donor will be rejected more rapidly than the first. Kidneys transplanted to guinea pigs previously immunized with emulsions of guinea pig kidney were rejected sooner than kidneys put into non-immune animals(1). Skin homografts in several human burn cases sensitized the patient to the extent that a subsequent homograft from the same donor caused an anaphylactic type reaction(2). More recently, it has been shown that second set skin homografts in human beings persist for a shorter time than do the first set grafts (3-5). An exception has been noted in a human case where the second set graft lasted nearly twice as long as the first set(12). Work on the rabbit(6-9), the guinea pig(10), and the dog(11) has demonstrated that a second skin homograft from the same donor does not persist as long as does the first. The so-called second set phenomenon is very strong evidence in favor of Medawar's theory of acquired immunity(6,7). However, several points remain to be cleared up. Medawar(6) and Lehrfeld and Taylor(13) showed that a first set skin homograft induces a systemic response which causes destruction of the graft. This response, induced by the first set graft, apparently destroys any ensuing grafts from the same donor. When this response first appears, when it reaches its peak and when it is no longer present are questions which need answering. This study proposes to answer these questions by implanting second set grafts while the first sets are still surviving, at the time of their rejection and at various times after rejection. Furthermore, very little work has been done on third set homografts. Studies on third, fourth and fifth set homografts used in the treatment of burn cases have shown that each successive graft has a survival time shorter than the one before(5). Third set corneal grafts onto the thoracic wall of rabbits have been shown to survive for the same length of time as do second set grafts(14).

Material and methods. All animals used were male albino rats, 6-8 weeks old, of the Sherman and Wistar strains. They were obtained from 2 different dealers in order to avoid the possibility of genetic similarity between the 2 animals of each homografted pair. The grafting procedure employed has been given in detail by Lehrfeld and Taylor (13). All animals were conditioned by a large first set homograft sewed into the upper dorsum. These grafts ranged in area from 100-225 sq mm and were removed at the level of, but not including the panniculus carnosus. In all cases, the same donor supplied the first and second set grafts. The second set homografts were added to the mid-dorsal region of the host simultaneously with the first set graft, or 5, 6, 7, 8, 9, 11, 12, 15, 18, 30 and 60 days after implantation of the first set graft. These second set grafts were either large grafts (100-225 sq mm) or pinch grafts (1-4 sq mm). In addition, third set grafts were placed on a number of animals after rejection of the second set grafts. Dressing changes and observations were performed daily with the animal under ether anesthesia, according to the technic of Taylor and Lehrfeld(15). Observations of the grafts were done through mineral oil under a stereoscopic dissecting microscope at 90 x magnification. The homograft was considered rejected at the time that hemal stasis was observed in the vascular system of the graft (15).

Results. The results of the experiment are illustrated in Table I. The mean survival time of the conditioning grafts was 8.14 days. Pinch grafts, when added simultaneously with the first set grafts, survived for 8.1 days. Second set grafts added 5 days after the first

* Supported by a grant from the Atomic Energy Commission.

TABLE I. Comparison of Survival Times of 1st and 2nd Set Skin Homografts.

No. of cases	Mean 1st set survival time, days	Interval between 1st & 2nd set graft, days	Type of 2nd set graft	Mean 2nd set survival time, days
10	8.1	0	P*	8.1
8	8.5	5	L (4)	3.0
	7.25	5	P (4)	2.25
12	7.75	6	L (6)	0
	8.0	6	P (6)	0
10	8.3	7	L (6)	0
	8.0	7	P (4)	0
6	8.2	8	L	5.0
2	8.5	9	L	5.0
14	8.3	11	L(10)	5.3
	8.25	11	P (4)	5.0
6	8.1	12	L	4.7
4	8.25	15	L	5.5
2	8.75	18	P	5.0
8	8.5	30	L	5.5
8	8.5	60	L	8.0

* P = Pinch; L = Large.

set implantation survived for 2.62 days. Second set grafts added 6 and 7 days after implantation of the first set never became revascularized and are considered to have a survival time of zero days. Grafts added 8, 9, 11, 12, 15, 18 and 30 days after implantation of the first set graft showed a mean survival time of 5.1 days and in no case did any of these grafts survive longer than 6 days or less than 4 days.

The survival time of second set grafts implanted 60 days after implantation of the first set graft was 8 days, which is a typical first set survival time, showing no accelerated breakdown.

In 6 rats, third set homografts were implanted 7 days after implantation of the second set graft. These survived 4.8 days as compared to 4.7 days for the second set graft.

Discussion. Two facts emerge from these results: 1. The survival time of second set skin homografts is definitely and consistently shorter than that of the first set graft. 2. The factor determining the length of survival time of second set homografts is the time relationship between implantation of first and second set grafts. Second set grafts implanted simultaneously with, or up to 5 days after implantation of the first or conditioning grafts, become revascularized in the same way as do the first set grafts. The rejection of these grafts is identical to, and occurs simultaneously with, the rejection of the first set grafts.

Second set grafts implanted on the 6th and 7th days after implanting the first set, a time at which the first set graft is still surviving, never became revascularized and appear to have a survival time of zero days. This occurs even with very small pinch grafts which are capable of becoming revascularized within 24 hours(13). Second set grafts implanted from 8-30 days after implantation of the first set graft survive for a mean time of 5.1 days, while those implanted 60 days after the first set, had returned to a normal first set survival time of 8 days. There was no significant difference between the survival time of second and third set grafts when the third set graft was implanted 7 days after the second.

The systemic response invoked by the conditioning graft apparently functions in the destruction of any second set graft implanted before the rejection of the first set graft. This response appears to reach maximum effectiveness at the 6th and 7th days as shown by the fact that second set grafts implanted during this time do not become revascularized, although if implanted 24 hours earlier or later they develop an active hemal circulation before their rejection.

The 5-day survival time exhibited by second set grafts implanted between 8 and 30 days after the first set grafting indicates that the systemic response elicited by the first graft has diminished in intensity to a point where it cannot destroy the second graft immediately. The temporal sequence in the accelerated breakdown of these second set grafts is suggestive of an anamnestic type response by which a mechanism, having already been primed by contact with the first set graft, becomes effective enough to destroy the second set graft in only 5 days. Somewhere between 30 and 60 days, this type of response is exhausted and second set homografts implanted at this time survive for 8 days, which is normal first set survival time.

Summary and conclusion. 1. Relation of

survival time to implantation time of second set skin homografts in the rat was studied. 2. Second set homografts implanted simultaneously with, or 5 days after the first set graft, develop an active hemal circulation and are rejected simultaneously with first set graft. 3. Second set homografts implanted 6 or 7 days after first set graft never become revascularized and are considered to survive for zero days. 4. Second set homografts implanted 8-30 days after first set graft develop an active hemal circulation and survive for a mean time of 5.1 days. 5. Second set homografts implanted 60 days after first set graft show a normal first set survival time of 8 days. 6. There is no significant difference in survival time of second and third set grafts when third set is implanted 7 days after the second set graft. 7. It is concluded that temporal sequence in accelerated breakdown of second set skin homografts is suggestive of an immunological mechanism triggered by the host's contact with the first set graft. This is in agreement with Medawar's theory of acquired immunity.

1. Fleisher, M. S., *J. Med. Res.*, 1918, v38, 191.
2. Holman, E., *Surg. Gyn. and Obs.*, 1924, v38, 100.
3. Gibson, T., and Medawar, P. B., *J. Anat. Lond*, 1943, v77, 299.
4. Longmire, W. P., Jr., Stone, H. B., Daniel, A. S., and Goon, C. D., *Plast. and Reconst. Surg.*, 1947, v2, 419.
5. Baxter, H., and Entin, M. A., *Am. J. Surg.*, 1951, v81, 285.
6. Medawar, P. B., *J. Anat. Lond.*, 1944, v78, 176.
7. ———, *ibid.*, 1945, v79, 157.
8. ———, *Brit. J. Exp. Path.*, 1946, v27, 9.
9. Dempster, W. J., Lennox, B., and Boag, J. W., *ibid.*, 1950, v31, 670.
10. Sparrow, E. M., *J. Endocrinol.*, 1953, v9, 101.
11. Dempster, W. J., *Brit. J. Plast. Surg.*, 1952-53, v5, 228.
12. Dogo, G., *Plast. and Reconst. Surg.*, 1953, v11, 475.
13. Lehrfeld, J. W., and Taylor, A. C., *ibid.*, 1953, v12, 432.
14. Billingham, R. E., and Boswell, T., *Proc. Roy. Soc. ser. B.*, 1953, v141, 392.
15. Taylor, A. C., and Lehrfeld, J. W., *Plast. and Reconst. Surg.*, 1953, v12, 423.

Received July 23, 1954. P.S.E.B.M., 1954, v86.

Biosynthesis of Ascorbic Acid and Prevention of Glycogen Depletion in Liver and Muscle. (21253)

M. C. NATH AND C. H. CHAKRABARTI.

From the University Department of Biochemistry, Nagpur, India.

We previously reported that gradual accumulation of fat metabolites (like acetoacetate and β-hydroxybutyrate in the systems of rabbits and guinea pigs) is responsible not only for the onset of hyperglycemia following a hypoglycemia but also for causing decreased glucose tolerance, depletion of reduced glutathione of blood and other diabetic symptoms (1-6). Tidwell and Nagler(7) observed that fasting blood sugar level of rats were significantly depressed by repeated daily injections of acetoacetate, without showing its eventual rise as in rabbits. They referred to the work of Parnes and Wertheimer(8) to explain such effect on blood sugar levels of rats injected with acetoacetate on the basis of decreased glycogenolysis or increased glycogenesis. We have failed to coroborate such observations. They reported, on the other hand, that acetoacetate markedly increased glycogenolysis(9). That acetoacetate depresses glycogenesis is also evident from the observation of Chari and Wertheimer(10) that acetoacetate has a specific effect on glycogen synthesis. Tidwell and Nagler(7) also referred to our work(11) stating that acetoacetate markedly depletes glycogen storage in liver and muscle of rats. This statement also seems inaccurate. Most of our work was done on rabbits. We never reported that sodium acetoacetate depletes liver and muscle glycogen of rats. It is likely that species difference might play an import-