PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1132–1140 (1975)

Electrocardiographic Recordings for Assessing Survival of Cardiac Allografts in the Hamster Cheek Pouch[1] (38703)

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(Introduced by F. Homburger)

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The implantation of denervated, nonwork-loaded hearts into various anatomical sites has been reported for the hamster cheek pouch (1–3), mouse ear (4, 5), and chick embryo (6). While these transplanted hearts lacked direct innervation and hemodynamic relationships with the host, they were deemed to be functional based upon the direct visual observation of pulsatile and contractile activity as well as electrical potentials resembling ventricular depolarization (5, 6).

The present research was designed to study by ultrasensitive bioelectrical recording techniques the voltages generated by the contraction of cardiac tissue grafted into the cheek pouch. The specific aims of this experimentation were: (a) development of a highly sensitive recording technique; (b) application of this technique to the assessment of allogeneic cardiac grafts in two strains of inbred hamsters with defined histocompatibility patterns. Such an investigation was made possible by the availability of highly inbred hamster lines and Handler and Cosman's demonstration of strain-specific differences in immunological tolerance (7).

Methods and Materials. Hamsters of the BIO 2.4 and 15.16 inbred strains (40 and 29 generations of brother × sister mating, respectively) were chosen for these studies. Based upon reported results of skin allograft exchanges, the BIO 2.4 line would be expected to promptly reject grafts from BIO 15.16 donors. Conversely, the BIO 15.16 line would be expected to tolerate allografts from BIO 2.4 donors (7). Neither strain is known to be a carrier of congenital disorders which would affect the experimental results. Two- to 3-mo-old animals from each of the strains were purchased from TELACO (Trenton Experimental Laboratory Animal Company), Bar Harbor, Maine.

Whole fetal hearts (14–15 days of gestation) were grafted into hamster cheek pouches unilaterally using previously described techniques (8).

Weekly observations were made by simultaneous visual and electrical measurements which permitted correlation of the electrical waveforms with the observed pulsatile activity. All observations were carried out under Nembutal (Abbott–6 mg/100 g) anesthesia. The cheek pouch was everted and pinned onto a cork board to facilitate direct observation and ECG recording.

Implants in cheek pouches were examined under 2 × magnification for the determination of pulsatile activity and under 50 × magnification for assessment of vascularization, graft condition and host response.

Bioelectrical recordings were made, as suggested by Judd (5), but using modified recording techniques. Platinum pin electrodes were placed in the connective tissue that separates the epithelium of the cheek pouch in such a manner as to place the longitudinal axes of the two electrodes in parallel position; the transplant was then positioned between the two electrodes. In addition to the implant ECG, a host ECG was recorded simultaneously, using sc platinum electrodes placed in the conventional lead configuration (Fig. 1).

The recording system used in this experiment was a model 7 Grass polygraph recorder equipped for simultaneous display of four physiological parameters. All "driver" amplifiers were conventionally standardized. Three channels were used to display leads I, II and III of the host ECG. The preamplifier (model 7P6B) calibration for these channels uses a sensitivity

[1] This work was supported by USPHS Research Support Grant No. S01-RR-05525 (Division of Research Facilities and Resources).

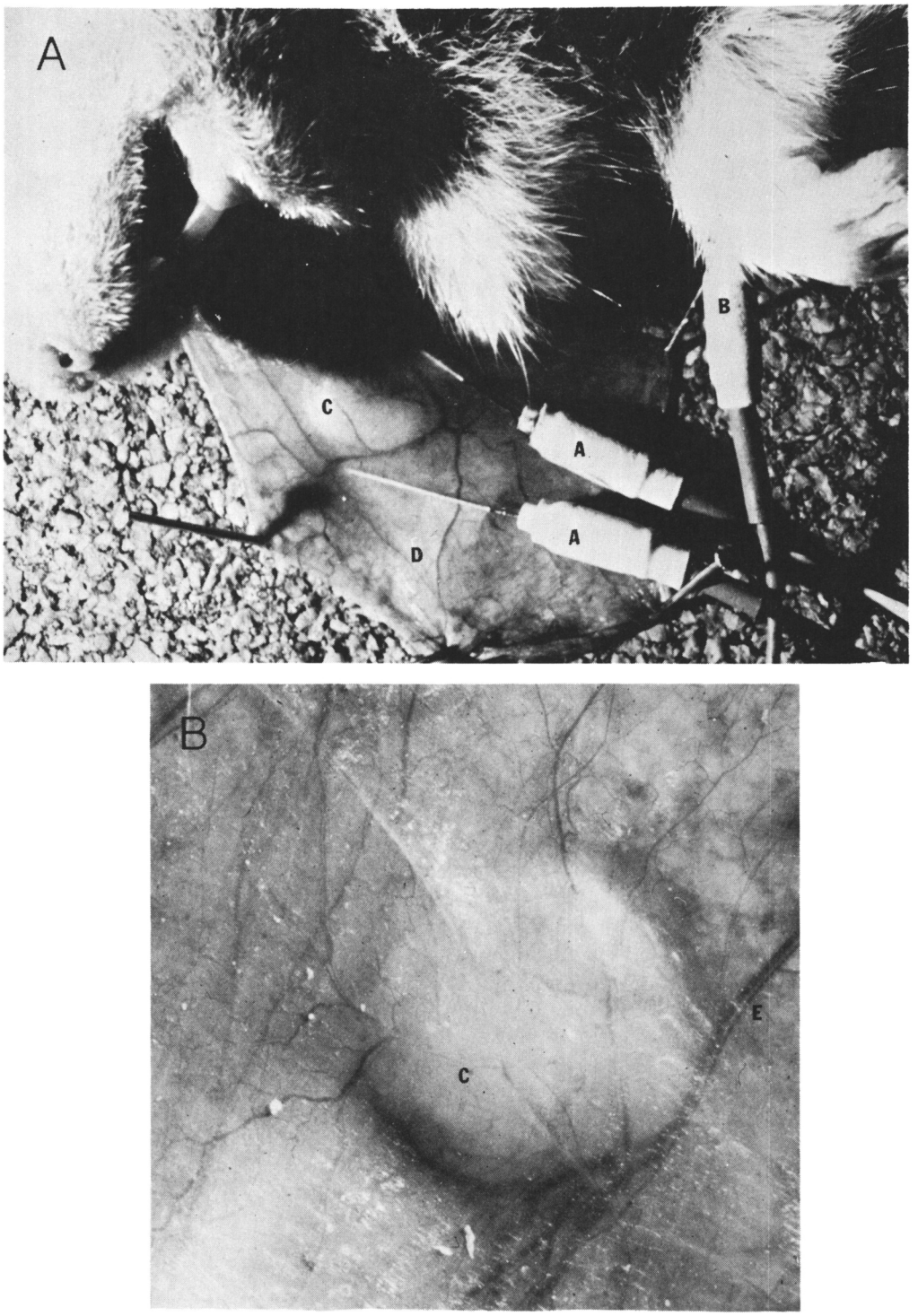

FIG. 1. Hamster with everted cheek pouch. This demonstrates (A) electrodes for recording of implant ECG, (B) subdermal electrode in left foreleg (one of four used to record host ECG), (C) implant, (D) stretched cheek pouch. In the insert, the graft (C) and the vascular bed (E) are clearly shown.

of 0.5 mg/cm, while the high-gain preamplifier (model 7P3B) used to record the implant ECG is capable of detecting and displaying electrical activity as low as 10 μv/4 cm. In addition to the physiological recording channels, the instrument is equipped with a time event marker that makes a mark every second and can also be used to mark individual specific events. Through the use of the event marker, it is possible to correlate the visual observations of implant contractions with the electrical recording of transplant activity.

Prior to each recording, validation calibration marks were made to permit eventual reduction of the variable voltages to comparable units of electrical activity. The individual recordings were analyzed for the rate of both host and transplant beat as well as for the amount of electrical voltage generated by both transplant and host cardiac tissue.

Results. Direct submicroscopic observations of the graft showed a pattern of survival and subsequent rejection similar to that previously described by Handler (8). The time course for vascularization and establishment of the graft was found to be similar in the two strains. Within 1–2 days following surgical implantation, vascularization began and was completed within 6–7 days. Upon vascularization, the implant took on a healthy pinkish color and the viable cardiac tissue displayed visible contractile activity. Based upon direct observations, the time course for the ultimate rejection or disorganization of the grafted tissue differed according to the source of the tissue and the host into which the tissue was implanted. The major grossly visible characteristic of rejection was fatty infiltration. Microscopically, there were marked chronic inflammation, stromal fatty infiltration, and necrosis. The rejection process started early in the case of 15.16 hearts transplanted to BIO 2.4 hosts. There was no apparent effect of host sex upon the rejection sequence. Fatty infiltration was significantly delayed in the combination of BIO 2.4 hearts transplanted into BIO 15.16 hosts (Table I, Fig. 2).

Physiological recordings made simultaneously and separately of graft and host hearts confirmed the direct observations in regard to the visually observed strain differences in contractile activity of the graft. The frequency of the implant beat was highly variable, but approximated about 25% of the host heart rate. From the recordings, there was no indication of host control over frequency of the graft beat, nor was there any evidence of nervous control or coupling of the graft contraction with that of the host heart. The variables that influence contraction of the grafted heart are at present unknown. The contractile activity of implanted cardiac tissue was easily distinguished from the contraction of the cheek pouch smooth muscle evident in some preparations.

As indicated by serial recordings from all animals bearing transplants (Table II), the frequency of contraction of BIO 15.16 hearts implanted into the BIO 2.4 host started at a rate lower than that of the BIO 2.4 hearts implanted in BIO 15.16 hosts. The contractile frequency of BIO 15.16 implants remained lower than that of BIO 2.4 implants, with the exception of the third week (which may have been due to sampling errors). The data after 8 wk are indicative of only one viable fragment remaining after the major portion of the implant had been rejected by the BIO 2.4 host. The pulsatile frequency of the BIO 15.16 implant declined to zero by the sixth week, whereas the BIO 2.4 implant showed good activity through the ninth week before beginning to decline. While not illustrated in Fig. 3, the frequency of contractions of the BIO 2.4 implants in BIO 15.16 cheek pouches remained at about the level of the 13-wk frequency for as long as 1.5 yr.

Ninety-five percent of isografts of prenatal hamster hearts into cheek pouches exhibited pulsatile activity, which persisted for long periods of time (1.5 yr for BIO 15.16 hearts grafted into BIO 15.16 hosts). The BIO 2.4 prenatal heart grafted into the BIO 15.16 host demonstrated good contractile activity for the duration of the experiment. Limited information on animals retained from the pilot studies indicated that contractile activity persisted far beyond the observation period.

TABLE I. SUMMARY OF VISUAL OBSERVATIONS ON CARDIAC ALLOGRAFTS.

	2.4 Host:15.16 Implant			15.16 Host:2.4 Implant		
No. weeks implanted	No. animals observed	No. hearts beating	%	No. animals observed	No. hearts beating	%
1	9			9		
2	9	5	55.6	9	8	88.9
3	8	2	25.0	9	7	77.8
4	8	3	37.5	9	8	88.9
5	8	2	25.0	9	9	100.0
6	8	2	25.0	9	8	88.9
7	8	1	12.5	9	9	100.0
8	8	1	12.5	9	9	100.0
9	8	1	12.5	9		
10	8	1	12.5	9	9	100.0
11	8	1	12.5	9	9	100.0
12	8	1	12.5	9	9	100.0
13	8	1	12.5	9	9	100.0

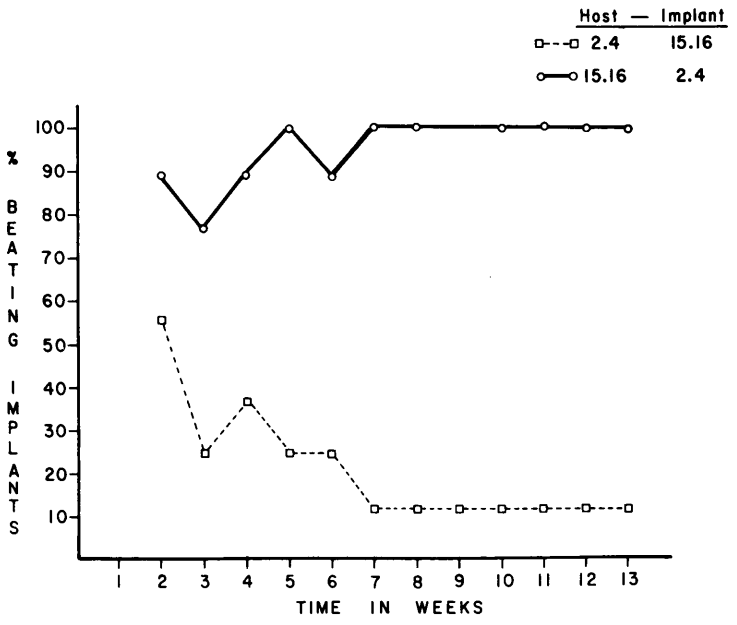

FIG. 2.

The host heart ECGs, as recorded for limb leads I, II and III, showed the expected strain differences in waveform configuration (Fig. 4), and clearly demonstrated the absence of anesthetic effect upon the host heart rate. Anesthesia and depth of the anesthetic state can be discounted as variables which might affect pulsatile activity of the implanted grafts.

Polygraph tracings obtained from the implanted hearts are characteristic of the ventricular depolarization with no apparent auricular electrical activity.

In some instances there appeared to be a double beat, but the initial beat apparently was not coupled to the second portion of the beat. Presence of the double beat was not required to initiate the contraction represented by the larger electrical pattern. The double beat pattern may represent

TABLE II. SUMMARY OF POLYGRAPH FINDINGS ON HOST HEART RATE AND GRAFT PULSATILE ACTIVITY.

	2.4 Host:15.16 Implant					15.16 Host:2.4 Implant				
No. weeks implanted	No. animals observed	Host heart rate	Range	Implant contraction rate	Range	No. animals observed	Host heart rate	Range	Implant contraction rate	Range
1	9					9				
2	7	321	260–370	14	0–50	9	257	150–330	32	10–130
3	2	380	370–390	33	30–40	5	326	260–350	14	0–60
4						6	371	240–380	59	0–132
5	3	360	350–370	27	0–60	8	310	240–360	43	10–100
6	2	360	330–390	0		8	309	270–390	26	10–70
7	2	305	260–350	20	10–30	7	294	240–360	31	0–90
8	2	350	330–370	10	0–20	7	300	210–370	41	0–100
9	1	380		70		3	297	270–320	40	20–80
10										
11										
12	1	350		110						
13	2	390		55	40–70	1	240		10	

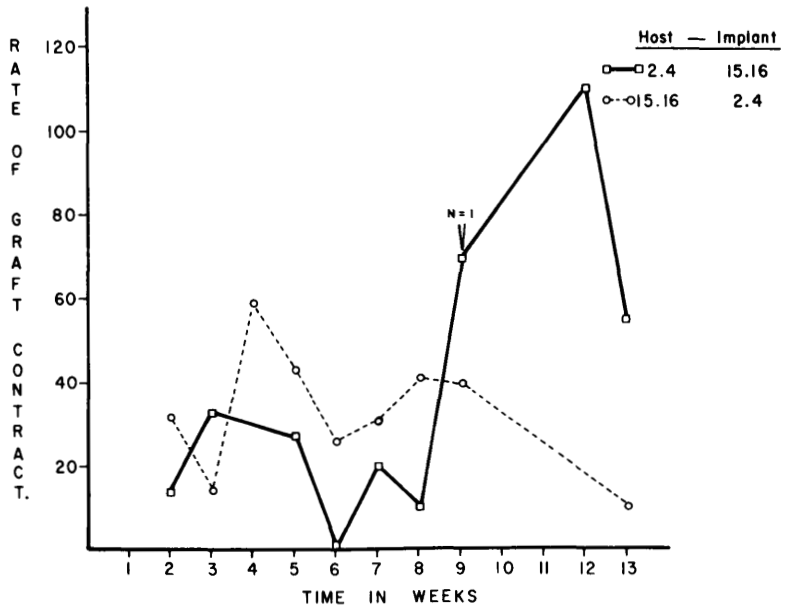

FIG. 3.

uncoupled auricular contraction or multiple foci of cardiac tissue capable of independent contraction. Evaluation of all records and subsequent histological study suggest that the double beat is the result of multiple foci of viable myocardium beating at random.

As with the maturation of adult heart rate, an apparent maturational pattern of electrical activity is exhibited by the grafted tissue. Strain differences are negligible in the early maturational patterns of electrical activity in grafts, but with "rejection" or fatty infiltration, the pattern is grossly altered. This relationship is most easily observed in graphical presentation of the maximal graft activity (Fig. 5). The marked depression of observed maximal voltages is interesting but unexplainable. This pattern has been displayed in both strains studied

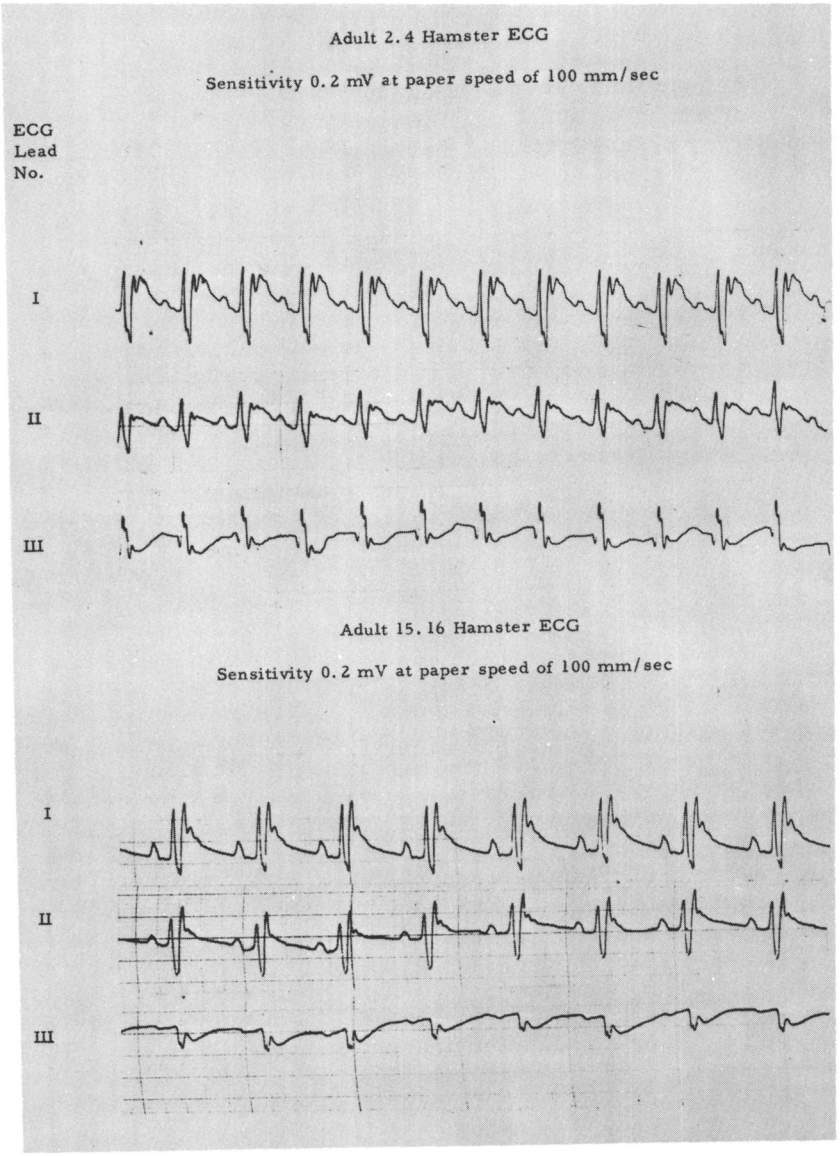

FIG. 4.

to date. Following the decline, there was a rapid elevation in voltage during weeks 3 and 4. The decline that followed the 3- to 4-wk peak seemed to persist. Figure 6 shows that after 7 wk voltage of the BIO 15.16 heart transplanted to the BIO 2.4 host began to elevate. This result was based upon observations on only one surviving implant of nine started. This behavior is unusual, since all other implants of BIO 15.16 hearts displayed no overt electrical activity after the sixth week.

Due in part to graft mobility within the cheek pouch, it was impossible to analyze the waveform generated by the grafted tissue for anything other than rate and voltage magnitudes. Measures of vector length and orientation were precluded in this sort of experiment.

Discussion. The Syrian hamster (*Mesocricetus auratus*) has long been recognized by immunologists as a successful recipient of a variety of allogeneic cell and tissue transplants (9, 10). Adams *et al.* (1) suggested

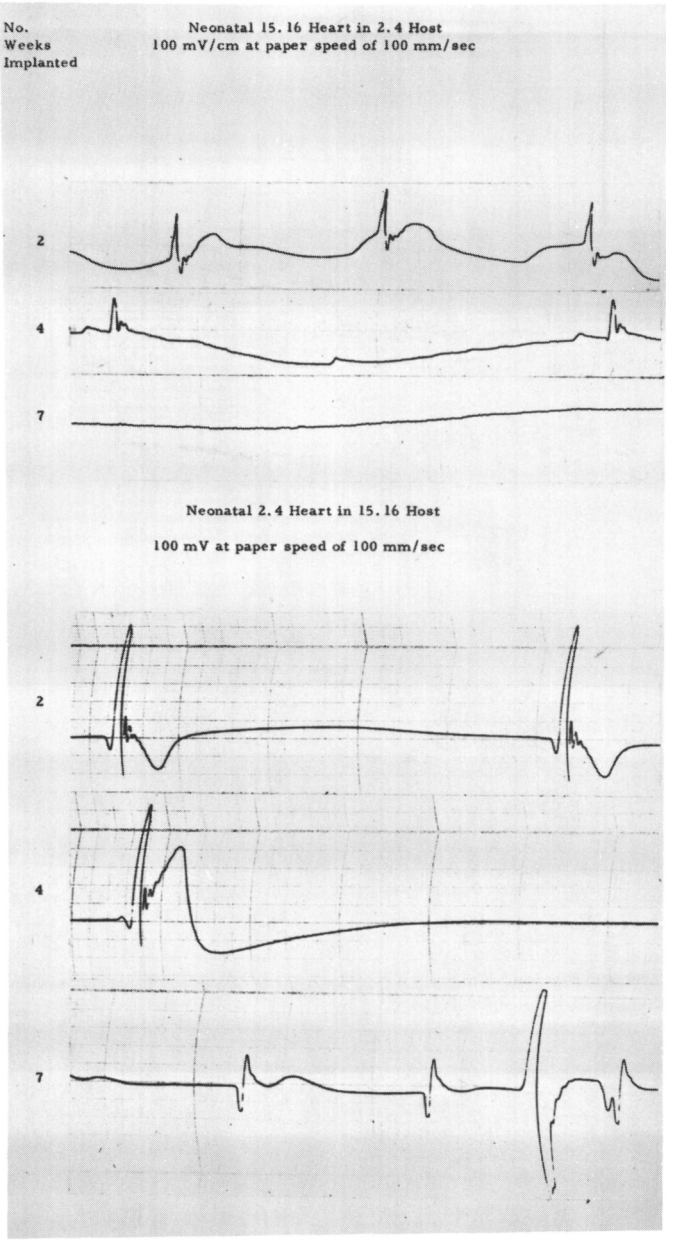

FIG. 5.

that this ability to accept grafts is due to a lack of histocompatibility differences among random-bred hamsters. Indications of immunological incompetence were observed by Billingham and Hildemann (12) in certain randombred hamsters in Great Britain. However, these investigators also observed typical graft rejections when they performed intercolony skin graft exchanges between partially inbred hamster strains.

Recently, Handler and Cosman (7) assessed histocompatibility between highly inbred hamster strains and clearly demonstrated strain-specific rejection patterns. They concluded from their studies that, while relatively few strong transplantation

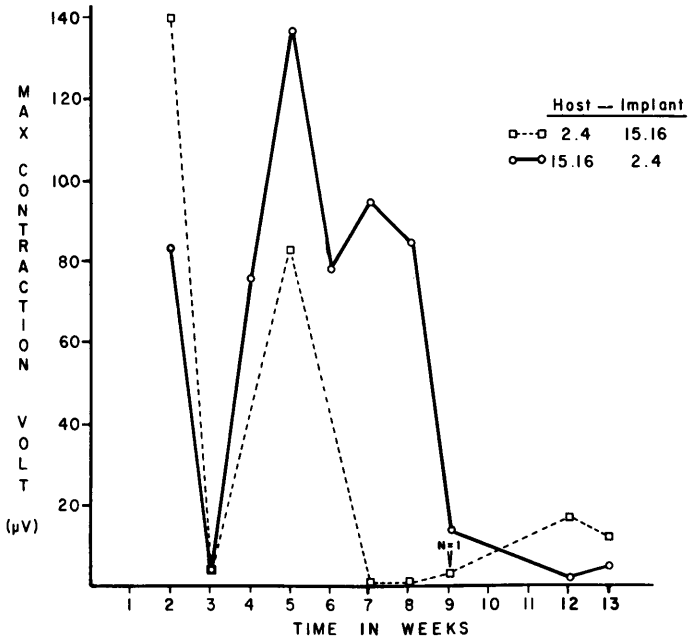

FIG. 6.

antigens were present in the genome of the Syrian hamster, lack of powerful antigenic sites did not preclude the development of a competent immunological capacity.

Billingham has maintained that the hamster cheek pouch is an immunologically privileged site (13, 15). This proposition, which is based in part upon increased survival time of allogeneic and xenogeneic normal and neoplastic tissue in the cheek pouch, may be invalid in light of recent work by Lindemann and Strauli (16), who clearly demonstrated the presence of lymphatic vessels in the cheek pouch musculature. Additional data disputing the validity of Billingham's proposition come from the work of Chadwick and Blamey (17), who showed that colloidal gold particles injected into the cheek pouch were recovered 24 hr later in the regional lymph nodes of the neck.

The degree of cheek pouch tolerance to allograft exchanges between hamster strains BIO 2.4 and 15.16 in the present study correlates well with skin allograft exchanges between these strains (7). This correlation demonstrates that allograft tolerance in inbred hamsters is truly a function of histocompatibility. These findings offer an additional suggestion that the cheek pouch lacks immunological privilege.

The ECG recording equipment originally used by Handler was only capable of detecting the electrical activity generated during the pulsatile activity of the implant, but lacked the versatility to provide sufficient amplification and display to yield meaningful patterns (8). The initial recordings also showed a low implant contraction rate. The present study confirms the earlier findings of low contraction rates which are probably due to lack of innervation of the implant or to the inherent slow neonatal heart rates in rats and hamsters (18). The pharmacological deprivation of the heart of sympathetic nervous control through reserpine administration brings about a 50% reduction in heart rate from control levels (19). There is substantial evidence to indicate that hereditary controls are exerted on the heart function in several laboratory species in addition to genetic involvement in maturation of the normal adult cardiac rate (18). The latter observation may be related to development of the sympathetic and parasympathetic nervous systems.

The factors relating to the voltages generated by implant contraction are not clearly defined. However, it is clear that the relationship of voltage to functional myocardium is maintained. Thus, use of the more sensitive recording techniques presents additional methodology for evaluation of cardiac allografts in the hamster cheek pouch, and possibly for other sites in other species.

Summary. Techniques for the recording of electrical events associated with contraction of allografted myocardium in the cheek pouch of inbred hamsters have been developed. The results of such recordings have been useful in assessing contractility and viability of the graft. The degree of tolerance to cardiac allografts in cheek pouches of inbred hamsters appears to be a function of histocompatibility and not "immunological privilege" of the cheek pouch.

The authors wish to acknowledge the technical assistance of Mr. William Bohan, Ms. Patricia Sgro and Ms. Enid Cosman.

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Received September 6, 1974. P.S.E.B.M. 1975, Vol. 148.