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7. A train of 0.3 second of square negative pulses at a frequency of 100 hertz and 0.1-second duration was delivered with each lever press. The mean self-stimulation rate was 150 presses per 10 minutes.
8. The [$^{14}$C]DA had a specific activity of 60 mc/mmole and the concentration in the stock solution was 3.1 $\mu$g per microliter per 0.5 $\mu$c expressed as the free base. [$^{14}$C]Dopamine was obtained from Amersham/Searle Corporation, Arlington Heights, Ill.
9. R. D. Myers, in *Methods in Psychobiology*, R. D. Myers, Ed. (Academic Press, London, 1972), vol. 2, p. 169. The push-pull cannulas were connected by polyethylene tubing to calibrated 1.0-ml B-D tuberculin syringes mounted on a Harvard apparatus infusion-withdrawal pump. The artificial cerebrospinal fluid was always freshly prepared in the following concentrations: $Na^+$, 127.65 m$M$; $K^+$, 2.55 m$M$; $Ca^{2+}$, 1.26 m$M$; $Mg^{2+}$, 0.93 m$M$; and $Cl^-$, 134.58 m$M$. Ascorbic acid in a concentration of 0.2 mg/ml was added to bring the $p$H of the solution to 3.8 in order to retard the degradation of the amine. In every experiment, the artificial cerebrospinal fluid was passed through a Swinnex Millipore filter, pore size 0.22 $\mu$m.
10. After the effluent was placed immediately on crushed ice, a 50 $\mu$l portion of the chilled sample was placed in a scintillation vial containing 3 ml of PCS (Amersham/Searle); it was counted for 10 minutes and the activity was converted to disintegrations per minute.
11. Using the two-way procedure of R. M. Fleming and W. G. Clark [*J. Chromatog.* **52**, 305 (1970)], which is described in detail by G. Martin and R. D. Myers [*Am. J. Physiol.* **229**, 1547 (1975)], the following amines and metabolites were analyzed: (i) 3,4-dihydroxyphenylacetic acid, (ii) 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), (iii) 3-methoxytyramine, (iv) normetanephrine, (v) 3-hydroxytyramine, and (vi) norepinephrine (see Table 1). Prior to the TLC analysis of the experimental samples, a control washout curve consisting of six samples was analyzed to quantitate the relation between dopamine, norepinephrine, and their respective metabolites.
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13. The data in Fig. 1B illustrate the average percentage of change in the total amount of [$^{14}$C]DA recovered from thin-layer chromatograms between the third and fourth perfusion ($t$ = 2.03, $P$ < .05) and the third and fifth perfusion ($t$ = 1.67, $P$ < .10). Elevated DA activity in the sample collected after electrical stimulation was terminated is not readily explained, but could be due either to the distance between the cannula tips and the dopaminergic terminals involved in the behavior or to a recurrence or persistence of functional activity of these neurons beyond the interval of local excitation.
14. Those sites that did not support self-stimulation yet were in close proximity to active sites were used as control sites for the nonspecific activity of electrical stimulation. The dimensions of the regions of stimulated-induced release of DA were demarcated stereotaxically within anterior-posterior, 9.5 to 10.5; lateral, 0.25 to 1.25; and horizontal, +3.0 to +4.5. Inactive sites at which the pattern of DA release followed a washout were, for example, as little as within 0.75 mm lateral to this region.
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17. In one experiment, we did find increases in newly synthesized norepinephrine paralleling the release of dopamine [R. D. Myers and F. Mora, *Brain Res. Bull.* **2**, 105 (1977)].
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## Immune Response in the Sea Urchin *Lytechinus pictus*

Abstract. *The sea urchin shows an immune response to grafted tissue similar to that found in vertebrates. Unrelated animals rejected allografts in about 30 days. Acceptance of allografts was observed for tissue exchanged between some $F_2$ and $F_3$ inbred animals. The percentage of acceptances reflected the degree of inbreeding. Accelerated second set rejection was also found. These grafts were rejected in one-third of the time compared to first sets.*

The rejection of tissue transplanted from one member to another member of the same species, an allogeneic transplant, is one characteristic of an animal's ability to mount an adaptive immune response to foreign tissue. Experimental allogeneic rejection has been demonstrated for all groups of vertebrates tested so far (*1*). Recent interest in the phylogenetic origin of the immune response has led to investigations of various invertebrate classes. Incompatibility responses, which have been described as nonimmunological, are found in the lower invertebrate phyla, such as the Protozoa (*2*), Coelenterata (*3*), and Porifera (*4*). Incompatibility responses in these phyla are more commonly found when xenogeneic grafts (transplants between species) are employed. Allogeneic rejection has been reported for two invertebrate phyla, the annelids (*5*) and the echinoderms (*6*).

We have tested the sea urchin *Lytechinus pictus* for the ability to reject allografts, and have found that a highly sensitive immune system is present. This work was done with the sea urchin colony that has been maintained in our laboratory for the past 7 years. The colony consists of about 4000 animals. Some of these have been kept in a mixed population and have unknown parentage, but most are kept as separate individuals with known genealogies. This allows us to begin to examine the genetics of the immune response. The methods for rearing sea urchins in the laboratory have been described (*7*). The animals were maintained in a closed seawater system at 15° to 16°C.

Allografts were exchanged between pairs of animals by removing sections of test (shell), about 3 mm square, along with the overlying epidermis and surface structures such as spines and pedicellaria. The squares were taken from between the rows of tube feet. Grafts were placed on the host graft bed on the upper third of the animal. Control tests indicate that the position of the graft on the host does not affect the immune response. No special methods were used to anchor the grafts to the host, and about 10 percent did become dislodged and were scored as technical losses. The sea urchin usually deposits calcium carbonate along the border between the graft and host, and the graft becomes firmly attached to the host test within a few days. This host-graft interface sometimes fails to form in the case of rapidly rejected allografts, such as those between unrelated animals.

Five sets of animals were used. These were grouped on the basis of genetic relationship. The sets were: lab stock, which were laboratory animals of unknown genetic relationship to one another; second-generation inbred animals; third-generation inbred animals; wild-type animals grafted to third generation; and wild type grafted to wild type. Wild-type animals were sea urchins obtained from Pacific Bio-Marine Laboratories in Venice, California. Graft survival times are summarized in Table 1.

Control autografts were lifted from the animal to ensure that all connections between the graft and underlying tissue were completely severed, and then reinserted into the original graft bed. Some controls were done by excising two sections from the animal and transposing them. In other experiments, autografts were placed on the animal when the second sets and third-party grafts were done. All autografts healed in rapidly. Grafts were positioned relative to the madriporite to facilitate future identification.

The first response to the surgical procedure took place in 2 days, with the appearance of red pigmented cells on the graft. The brightly colored cells were frequently first observed in the border between the host and graft, and appeared to migrate onto the graft from the underlying host tissue. These cells were present in slight to moderate amounts on about 75 percent of the autografts. Their number decreased between the first and third weeks after surgery until they disappeared from the autografts. These pigmented cells were present in considerably greater density on the allografts and were observable throughout the rejection period. The red pigment within

Table 1. Response of the sea urchin *Lytechinus pictus* to grafted tissue. Second sets are repeats of previous allografts, third parties are grafts from a new donor unrelated to the host or to the donor of the first graft. These were placed on a host at the same time as the second set.

| Reciprocal grafts | Number of urchins | Number of grafts rejected | Percent rejection | Days to rejection* |
|---|---|---|---|---|
| Wild type | 14 | 14 | 100 | 35 ± 5 |
| Wild to laboratory stock | 17 | 17 | 100 | 30 ± 0 |
| Laboratory stock | 13 | 13 | 100 | 53 ±21 |
| $F_2$ siblings | 16 | 10 | 62 | 56 ±10 |
| $F_3$ siblings | 16 | 5 | 31 | 70 ±38 |
| Second set | 14 | 14 | 100 | 12 ± 0 |
| Third party | 14 | 14 | 100 | 12 ± 0 |

*Average number of days ± the standard deviation.

the cells, termed enchinochrome by MacMunn in 1885 (*8*), has been found only in sea urchins. In the past we have noticed red patches of these same cells on ill animals. The physiological role of the pigment is not known, and it seems not to have been mentioned in reference to disease.

The allografts that were accepted between related animals responded similarly to control autografts. On allografts that were rejected, the graft epidermis receded from its borders, sometimes forming large blisters. Concurrent with this event, the graft surface structures, such as spines and pedicellaria, came to lie against the graft test. In a period of about 3 to 6 weeks, the receding epidermis, including the surface structures, formed a clump in the center of the graft, which continued to deteriorate over a period of several more weeks, finally leaving essentially a cell-free piece of grafted test in the test of the host. We have defined rejection as the time of the appearance of this clump of degenerating tissues.

We have tested for memory in this system, and found that second-set grafts from original donors were rejected in less than one-third the time required for the first sets to reject. Fourteen second-set grafts were placed on animals selected from laboratory stock and $F_2$ animals which had rejected first sets with a mean of 38 ± 8 days (S.D.). Ten animals were from the laboratory stock to laboratory stock set, and four were from the $F_2$ to $F_2$ set. The second grafts from the same donor were placed on the hosts 2 months after the first grafts. Second sets were all rejected by the day 12 after surgery. Under these conditions, the rejection process was not found to be specific since third-party grafts from unrelated donors were rejected at the same rate as were second sets.

The data indicate that sea urchins respond to allogeneic differences with the synthesis or activation of products that result in the rapid destruction of foreign tissue. Although second-set specificity, of the type found in mammals, and long-term memory have yet to be demonstrated, sea urchins do have an accelerated response to second-set grafts. Our results are consistent with the genetic theory of transplantation, namely, that rejection is based on antigenic differences of graft tissue that are recognized as foreign by the host and then destroyed. Major histocompatability differences, or few common alleles, should result in the more rapid recognition and destruction of graft tissue. Thus, sea urchins that are unrelated would reject more rapidly, as was observed in the studies with wild-type animals. A somewhat slower rejection time would be expected, and was found, for a random sampling of laboratory animals. These are related to some degree since they all came from a small parental stock. In animals known to be related, the percentage of accepted allografts increases according to the extent of inbreeding between host and donor.

The rejection period of about 30 days in unrelated sea urchins suggests the presence of a sophisticated immune system and is the most rapid and consistent rejection process yet to be demonstrated among the invertebrates. This contrasts to the situation found with the earthworm, where between 5 to 15 percent of interpopulation allografts are rejected in 2 to 4 months, and intrapopulation allografts are rejected very rarely (*5*). In the sea star, some allografts between presumably unrelated animals are also accepted; rejection times are from 4 to 6 months (*6*). The rejection period found in sea urchins is also more rapid than that found in the hagfish, which has a mean rejection time of 72 days (*9*), and about the same as that seen in lampreys, stingrays, and paddlefish (*10*). Although the sea urchin and lower vertebrate rejection times seem long when compared to mammalian times of about 14 days, it must be recalled that these animals are at temperatures of 15° to 20°C, whereas the mammalian body temperature is much higher.

With the notable exceptions of the earthworm (*5*) and sea star (*6*), invertebrates have been thought to lack adaptive immunity (*11*). Invertebrate responses to foreign tissues have been termed defense mechanisms, or innate immunity, meaning that the system is not modifiable by second contact to the antigen. These generalized responses, which are usually expressed in the form of phagocytosis have been found in all animal phyla, including the sea urchin (*12*). The reaction of a sea urchin to tissue from another member of the same species suggests there is an adaptive immune system in the echinoids.

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