Since, from studies of nuclear magnetic resonance, it now appears firmly established (*6*), that most, if not all, of the added Mn^{++} and Co^{++} becomes attached to the nucleic acid phosphates, the possibility of a quenching mechanism that operates by way of several saturated bonds must be seriously considered.

The quenching data presented here should aid in establishing mechanisms of radiation damage in DNA since such states may occur by way of the excited triplet.

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References and Notes

1. R. Bersohn and I. Isenberg, *J. Chem. Phys.* **40**, 3175 (1964).
2. I. Isenberg, S. L. Baird, R. Rosenbluth, *Anal. Biochem.*, in press.
3. H. Linschitz and L. Pekkarinen, *J. Amer. Chem. Soc.* **82**, 2411 (1960); G. Porter and M. R. Wright, *Discussions Faraday Soc.* **27**, 18 (1959).
4. G. W. Robinson and R. P. Frosch, *J. Chem. Phys.* **37**, 1962 (1962); **38**, 1187 (1963).
5. R. O. Rahn, R. G. Shulman, J. W. Longworth, *Proc. Nat. Acad. Sci. U.S.* **53**, 893 (1965).
6. R. G. Shulman, H. Sternlicht, B. J. Wyluda, *J. Chem. Phys.*, in press.
7. We thank Professor R. Bershon for discussions. Supported by NIH grant GM-10383.
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Allograft Survival: Effect of Antiserums to Thymus Glands and Lymphocytes

Abstract. *Although antiserums to lymphocytes and thymus cells have similar effects on lymphocytes and thymocytes in vitro, the antiserum to thymus has more persistent lymphopenic effect in vivo and prolongs allograft survival time more markedly. Since only thymus glands of animals treated with antiserum to thymus showed depletion of small lymphocytes, antibody to thymic humoral factor may be operational.*

Antiserums to lymphocytes and thymocytes produce lymphopenia and suppress delayed hypersensitivity reaction (*1, 2*). Antiserum to lymphocytes, however, has lymphopenic effect of relatively short duration. On the other hand, antiserum to thymocytes, given daily from the day of birth, has long-lasting lymphopenic effect in mice (*2*). We now report a significant difference between antiserum to rat lymphocytes and antiserum to rat thymus in the effect of each on prolongation of allograft survival in young adult rats.

Osborne-Mendel strain (not inbred) rats kept in a closed colony for 35 to 36 generations were used. Antiserums were produced in albino rabbits (3 to 4 kg) by immunization with a mixture of 20-percent saline suspension of homogenized thymus or mesenteric lymph nodes (from rats, aged 6 to 8 weeks) and Freund's complete adjuvant, in equal volume. The mixture (5 ml) was injected into the rabbits intramuscularly and subcutaneously in multiple locations once a week for 5 weeks, and the animals were bled 1 week after the last injection for the subsequent 4 weeks. Some animals were given two booster injections at weekly intervals and bled weekly for another 4 weeks. All the serums were heated at 56°C for 30 minutes and pooled.

The effect of the antiserums was studied by agglutination tests with rat red blood cells, lymph node cells, and thymocytes. Agglutinin titers expressed by reciprocals of the maximum dilutions of serum giving positive agglutination are shown in Table 1. Thymocyte agglutinin titers were equal in both antiserums and lymphocyte agglutinin titers differed only by one dilution.

Two groups of seven animals each were injected daily intraperitoneally with 1 ml of either antiserum for 4 weeks or more, and the lymphocyte count was made daily on each animal. The mean lymphocyte count, prior to injection, for the group receiving antiserum to lymphocytes was 4774 ± 458, and it was 5225 ± 480 for the group receiving antiserum to thymus. The injections of antiserum to lymphocytes produced lymphopenia lasting up to 3 to 4 weeks, but the white counts gradually returned toward normal thereafter. The mean value of the lowest lymphocyte counts observed in the seven animals after treatment with antiserum to lymphocyte for at least 3 weeks was 2645 ± 520. In contrast, injection with antiserum to thymus resulted in more persistent lymphopenia of intense degree, and the mean of the lowest lymphocyte counts in this group after the animals had been injected with this antiserum for at least 3 weeks was 1388 ± 510. There was no significant change in the number of polymorphonuclear leukocytes, and the hematocrit remained essentially unchanged in most of the animals receiving either antiserum.

Table 1. Agglutinin titers of antiserums to rat thymus (ARTS) and lymphocytes (ARLS) for red blood cells (RBC), lymphocytes, and thymocytes.

Rabbit serum	Agglutinin titers (maximum dilution)		
	RBC	Lympho-cyte	Thymo-cyte
ARTS	1:40	1:320	1:160
ARLS	1:80	1:160	1:160
Normal	< 1:5	< 1:5	< 1:5

A full-thickness graft of circular skin (18 mm in diameter) was exchanged between 6- to 8-weeks-old male rats (120 g). The graft was sutured with silk to a bed prepared on the lateral thoracic wall. No dressing was applied, and the graft was observed daily. Complete disappearance of macroscopically intact grafted skin, also confirmed microscopically, was scored as the time of rejection. Mean survival time in nontreated animals was 10.9 days (Table 2). In animals receiving daily intraperitoneal injection of 1 ml of antiserum to lymphocytes beginning 1 day before grafting, the mean survival time was prolonged to 18.8 days. In the group of animals treated with daily injection of 1 ml or less of antiserum to thymus intraperitoneally, however, survival was more than three times longer than that of the nontreated group. Daily injection of 1 ml of antiserum to thymus not infrequently resulted in death of animals with intact graft. Reduction of the dose to as low as 0.5 ml every 5 days has been tolerated well without ill effect on the graft.

Table 2. Survival time of allografts, in groups treated with antiserum either to lymphocyte or to thymus.

Dose (ml/day)	Animals (No.)	Graft survival (days)	Mean
		No treatment	
0	15	10, 10, 10, 10, 10, 10, 11, 11, 11, 11, 11, 12, 12, 12, 12	10.9
		Antiserum to lymphocytes	
1	12	11, 13, 14, 14, 14*, 15, 15*, 17, 18, 25, 34, 36	18.8
		Antiserum to thymus	
1 or < 1	12	19*, 20*, 20*, 27*, 27*, 35†, 36*, 37†, 56*, 56†, 71†, 71†	39.8

* Animals died with intact graft. † Animals still living with intact graft.

Since effects of either antiserum on thymocytes and lymphocytes in vitro as determined by agglutination test showed no significant difference, the observed difference in effect on lymphopenia and prolongation of allograft survival time between these serums must depend on factors other than the agglutinins. Thymectomy in adult animals has relatively little effect on immunological competence unless peripheral lymphoid tissues are destroyed simultaneously, for instance, by lethal irradiation (*3*). Histological examination of the animals receiving antiserum to thymus showed depletion of small lymphocytes in the thymus glands, whereas thymus glands in animals receiving antiserum to lymphocytes remained intact. It seems, therefore, that treatment with antiserum to thymus results in an equivalent of thymectomy plus irradiation.

Antiserum to thymus, however, has several advantages: (i) no surgical intervention is required; (ii) lymphopenia and desired effect can be regulated by adjusting the dose of the serum; (iii) anemia or neutropenia inherent to irradiation are usually not present. Since thymic humoral factor is the only stimulus known for the production of thymocytes (*4*), depletion of thymocytes and the resulting interference with allograft rejection may be mediated by antibody to the thymic humoral factor.

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References and Notes

1. B. H. Waksman, S. Arbouys, B. G. Arnason, *J. Exptl. Med.* **114**, 997 (1961); M. F. A. Woodruff and N. A. Anderson, *Nature* **200**, 702 (1963); J. H. Sachs, D. R. Filippone, D. M. Hume, *Transplantation* **2**, 60 (1964); H. Nagaya and H. O. Sieker, in preparation.
2. H. P. Russe and A. J. Crowle, *J. Immunol.* **94**, 74 (1965).
3. J. F. A. P. Miller, S. M. A. Doak, A. M. Cross, *Proc. Soc. Exptl. Biol. Med.* **112**, 785 (1963).
4. D. Metcalf, in *The Thymus*, V. Defendi and D. Metcalf, Eds. (Wistar Institute Press, Philadelphia, 1964), p. 53.
5. Supported by NIH training grant 2T 1 AI 31-06 and by a grant from North Carolina Tuberculosis Association.

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Iodine: Accumulation by Balanoglossus gigas

Abstract. *The whole body of the giant Brazilian enteropneust,* Balanoglossus gigas, *shows an accumulation of iodine, but the concentration in the hepatic region is 854.7 percent higher than it is in the proboscis, where the amount of iodine is the lowest. Such an iodine concentration has not yet been detected in any other enteropneust or protochordate.*

Balanoglossus gigas is the largest enteropneust known; it was rediscovered by Sawaya (*1*) on certain shores in Brazil (Araçá, in the state of São Paulo, 23° 48′7″S and 45° 23′29″W; Gambôa, in the state of Santa Catharina, 27° 22″S and 48° 32″W).

Enteropneusts are animals which characteristically produce a copious secretion of mucus, which has a pervading odor reminiscent of iodoform. These organisms live in the intertidal zone, and they burrow long galleries, ingesting sand; this material is expelled by the intestine, and it accumulates at the posterior opening of the gallery in the form of spiral casts.

The whole body of the animal, the mucus, and the casts have the strong smell mentioned above; yet, in spite of several analyses, iodoform has not yet been detected.

The animals are of considerable size (up to 2 m in length). Hence, in order to detect any possible differences in iodine content in regions of the body, some regions were subdivided artificially into two or three subregions, excluding the proboscis and collar. From the branchial region we made two subregions (I and II), and from the genital, hepatic, and intestinal regions we made three (I, II, and III in each case). Only the hepatic and intestinal subregions can be differentiated easily by color: I is dark brown; II is light brown; and III is rosy.

The total iodine content of the mucus (fresh material) and the several regions and subregions of the body (dry weight) of four adult animals was determined (*2*), and the results are listed in Table 1.

The whole body shows an accumulation of iodine, but the concentration in the hepatic region is 854.7 percent higher than it is in the proboscis, where the amount of iodine is the lowest (116.7 μg/100 g).

The total iodine content in the fresh mucus from the four animals was 8.3, 7.5, 8.0, and 7.8 μg/ml (mean, 7.9 μg/ml; SD = ±0.41).

These results lend support to the hypothesis that the hepatic region has a thyroid-like function. Such an iodine concentration in that region is noteworthy and, as far as we know, such amounts have not yet been detected in any other enteropneust or protochordate.

The protochordates, to which the enteropneusts belong, are very close to the chordates, and despite the contentions of Van der Horst (*3*) and Hyman (*4*), some authors retain the enteropneusts in the phylum Chordata (*5*). Some protochordates—for example *Ciona* in the urochordates and *Branchiostoma* in the cephalochordates—are provided with the endostyle, an organ in which the concentration of iodine is high (*6, 7*). Several authors (*5, 6, 9*) state that the endostyle is the precursor

Table 1. Total iodine content in different regions of the body of *Balanoglossus gigas.*

Body region	Iodine (μg/100 g, dry wt.)*				
	No. 1	No. 2	No. 3	No. 4	Mean ± S.D.
Proboscis	163	101	97	107	116.75±30.94
Collar	217	226	218		220.00± 4.12
Branchial I	274	282	276	266	274.50± 6.60
Branchial II	479	480	473	477	477.50± 3.10
Genital I	385	368	366	337	364.00±19.86
Genital II	377	393	377	391	384.50± 8.83
Genital III	457	503	450	502	478.00±28.40
Hepatic I	855	844	876	880	876.25±13.24
Hepatic II	1039	971	984	996	997.5 ±23.09
Hepatic III	896	966	936	935	933.25±28.62
Intestinal I	601	597	596	623	604.25±12.68
Intestinal II	360	358	340	338	349.00±10.50
Intestinal III	342	350	356	392	360.00±22.09

* Four animals were used.