Table 1. Effect of 2,3-DPG on oxygen affinity of cat hemoglobins.

2,3-DPG (mM)	Hemoglobin A (P_{50}, mm-Hg)	Hemoglobin B (P_{50}, mm-Hg)
0	10.6	15.0
0.2	11.4	15.0
2.0	14.6	15.8

the maintenance of a high concentration of 2,3-DPG is energetically expensive. Its synthesis involves bypassing the phosphoglycerate kinase reaction, a step resulting in the synthesis of adenosine triphosphate.

The marked differences in 2,3-DPG reactivity among these animal hemoglobins should be considered in light of the known structural differences among these proteins. Such a structural-functional approach may provide additional information as to the site of 2,3-DPG binding. Earlier data of Benesch *et al.* (*2*) indicated that 2,3-DPG binds in a one-to-one molar ratio with the β chains of deoxyhemoglobin tetramer, probably somewhere in the central cavity along the diad axis of symmetry. From a comparison of 2,3-DPG reactivity among different major and minor components of human hemoglobin, Bunn and Briehl concluded that the α amino groups of the β chains were involved in 2,3-DPG binding (*9*). Benesch *et al.* also came to this conclusion from "affinity labeling" studies on hemoglobin in which the same amino group was covalently linked to pyridoxal phosphate (*10*). Recently Perutz has fitted 2,3-DPG to an atomic model of human deoxyhemoglobin built from a Fourier synthesis at 3.5-Å resolution (*11*). One molecule of 2,3-DPG was placed into the internal cavity in such a way that the phosphates could form salt bonds with the two α amino groups of the β chains and the imidazoles of the β-H21 histidines, while the carboxyl was within bonding distance of the ε amino of one of the β-EF6 lysines. Among the hemoglobins used in this study, the primary sequences of man, horse, sheep A and B, goat A, cow A, and rabbit have been reported (*12*). These hemoglobins all contain β-H21 histidine and β-EF6 lysine. Perutz has recently pointed out that the ruminant hemoglobins contain a deletion at the NH_2-terminal end of the β chains (*11*). Thus the distance between α amino groups would be about 6 Å greater than in the hemoglobins having the usual 146 residues per chain. Because of this, one molecule of 2,3-DPG would not be expected to bind to both α amino groups, and thus the strength of binding at this proposed site would be considerably attenuated.

The structure of cat A and B hemoglobins has been partially worked out by Lessard (*13*). Among differences in their β chains, the NH_2-terminus of hemoglobin B is acetylated. In 0.2 mM 2,3-DPG neither of these hemoglobins interacts appreciably with 2,3-DPG (Table 1). However, in the presence of a high concentration of 2,3-DPG (2 mM), the oxygen affinity of cat A hemoglobin decreased considerably while that of cat B, hardly at all. Cat B resembles human F_1 and A_{IC}. All three hemoglobins have a blocked α amino group on the β chain and decreased reactivity to 2,3-DPG (*9*). Recently Taketa, Lessard, and Mauk have found a similar difference in the oxygenation of the two cat hemoglobins in the presence of a high concentration of 2,3-DPG (*14*).

H. Franklin Bunn
Thorndike Memorial Laboratory,
Boston City Hospital,
Boston, Massachusetts 02118

References and Notes

1. S. Rapoport and G. M. Guest, *J. Biol. Chem.* **138**, 269 (1941); D. R. Harkness, J. Ponce, V. Grayson, *Comp. Biochem. Physiol.* **28**, 129 (1969); G. Bartlett, in *Red Cell Metabolism and Function*, G. Brewer, Ed. (Plenum, New York, 1970), p. 255.
2. R. Benesch and R. E. Benesch, *Biochem. Biophys. Res. Commun.* **26**, 162 (1967); A. Chanutin and R. R. Curnish, *Arch. Biochem. Biophys.* **121**, 96 (1967); R. Benesch, R. E. Benesch, C. I. Yu, *Proc. Nat. Acad. Sci. U.S.* **59**, 526 (1968).
3. R. E. Benesch, R. Benesch, C. I. Yu, *Biochemistry* **8**, 2567 (1969).
4. J. W. Drysdale, P. Righetti, H. F. Bunn, *Biochim. Biophys. Acta* **229**, 42 (1971).
5. T. H. J. Huisman and A. M. Dozy, *J. Chromatogr.* **19**, 160 (1965).
6. T. H. J. Huisman and C. A. Meyering, *Clin. Chim. Acta* **5**, 103 (1960).
7. A. S. Keitt, *J. Lab. Clin. Med.* **77**, 470 (1971).
8. D. W. Allen, K. F. Guthe, J. Wyman, Jr., *J. Biol. Chem.* **187**, 393 (1950); A. Riggs, *J. Gen. Physiol.* **35**, 23 (1951).
9. H. F. Bunn and R. W. Briehl, *J. Clin. Invest.* **49**, 1088 (1970).
10. R. E. Benesch, R. Benesch, R. D. Renthal, *8th Int. Congr. Biochem.* (abstracts) (1970), p. 6.
11. M. F. Perutz, *Nature* **228**, 726 (1970).
12. M. O. Dayhoff, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Md., 1969), pp. 50–61.
13. J. L. Lessard, thesis, Marquette University (1970).
14. F. Taketa, J. L. Lessard, G. Mauk, *J. Biol. Chem.*, in press.
15. Supported in part by NIH grants FR76, HE08251, and HE/AM05391 and by contract No. 70-C-0042 from the U.S. Army Medical Research and Development Command. I thank Miss Allison Chao for expert technical help and Drs. Hyram Kitchen and T. H. J. Huisman for blood specimens.

28 January 1971

Lymph Node Cells: Their Differential Capacity to Induce Tolerance of Heart and Skin Homografts in Rats

Abstract. *Rats of the BN strain, inoculated at birth with (Lewis × BN)F_1 hybrid lymph node cells are not tolerant of Lewis skin grafts but do display high degrees of tolerance of Lewis hearts.*

In rats high degrees of tolerance of Ag-B incompatible skin grafts, that is, skin grafts that differ from their host at the major histocompatibility locus of the species (*1*), can be induced effectively by inoculating neonatal recipients only with bone marrow cells (*2*). By comparison, splenic and lymph node cells are poor in inducing tolerance in respect to skin grafts, and thymocytes are ineffective (*3*). Nevertheless, when injected into neonatal hosts Ag-B incompatible lymphocytes are highly effective in inducing tolerance in respect to themselves (*4*). Thus blood lymphocytes from BN rats injected at birth with BN/Lewis F_1 hybrid lymph node cells display a significantly diminished reactivity when exposed to BN/Lewis lymphocytes in vitro in the mixed lymphocyte interaction, regardless of whether the inoculated animals are tolerant of Lewis skin grafts. On the other hand, BN rats treated in similar fashion with BN/Lewis F_1 hybrid thymocytes are neither tolerant of Lewis skin grafts nor do their lymphocytes have a diminished level of responsiveness when confronted with BN/Lewis lymphocytes in vitro (*4*). Inasmuch as tolerance of skin homografts is easily obtained with either lymphoid cells or thymocytes in Ag-B *compatible* strain combinations (*2, 5*), one explanation for these findings is that in rats skin and bone marrow cells may possess some Ag-B specificities that are absent or poorly expressed on lymphoid cells and thymocytes, and that the latter, in turn, are deficient in some Ag-B determinant or determinants present on lymph node and splenic cells.

To establish whether this inferior ability of lymphoid cells to induce tolerance of skin homografts applies to

homografts of other organs as well, we have determined the capacity of these cells to induce tolerance of Ag-B incompatible auxiliary heart transplants.

Rats of the isogenic BN (Ag-B^3) strain were inoculated within 24 hours of birth with 30×10^6 lymph node cells obtained from young adult Lewis/BN (Ag-B^1/Ag-B^3) F_1 hybrids (*6*). Some of these animals were challenged with Lewis skin grafts when 8 to 10 weeks of age (*7*), whereas others received Lewis hearts. The survival of these homografts was compared with the survival of similar transplants made to normal (that is, untreated) BN animals of the same age. The hearts were transplanted by the technique of Ono *et al.* (*8*). The donor pulmonary artery was anastomosed to the recipient's inferior vena cava, and the donor aorta to recipient's abdominal aorta, and the donor pulmonary veins and venae cavae were ligated. The beating hearts could be palpated easily through the anterior abdominal wall, and this proved to be the most convenient means of assessing function daily. Heart homograft survival was defined as the last day on which the heart was beating. When a beat could not be detected by palpation, surgical exploration usually confirmed that no contractile activity was present, though in a few instances the heart continued to quiver slightly.

It is evident from the results of these experiments (Table 1) that there was a pronounced discrepancy in the ability of Lewis lymph node cells to induce tolerance of skin and heart grafts in BN recipients. While only one of eight (12.5 percent) recipients of lymph node cells displayed tolerance of a skin homograft, 10 of 11 (91 percent) were rendered tolerant of Lewis hearts (that is, accepted such grafts for more than 14 days). Moreover, seven (64 percent) of these inoculated animals accepted their hearts for more than 50 days—our criterion that they had been rendered highly tolerant.

Because recipients of BN lymph node cells were frequently made unresponsive to Lewis heart homografts but not to Lewis skin, we decided to determine whether inoculated animals that rejected skin homografts with the promptitude of normal (that is, untreated) rats would nevertheless accept a subsequent heart transplant. Accordingly, five rats inoculated with lymph node cells, which had displayed no evidence of tolerance when challenged with a Lewis skin graft, were subsequently challenged with a Lewis heart. Four of these organ grafts were accepted permanently (Table 2, experiment 2). As expected, four BN rats not inoculated with lymph node cells at birth, but which had previously rejected Lewis skin grafts, rejected their heart homografts in an accelerated fashion (Table 2, experiment 4).

To determine whether rechallenge with a skin graft could prejudice the survival of a tolerated cardiac homograft, four BN rats neonatally inoculated with Lewis lymph node cells, and bearing Lewis hearts, were grafted with Lewis skin after the heart transplants had been in residence for 60 days. Two of these rats had already rejected one Lewis skin graft prior to the implantation of the auxiliary Lewis heart. All four animals rejected the skin grafts within 9 days but in no case did this prejudice the survival of the established heart graft, which was still beating when the experiments were terminated 35 to 105 days later.

Finally, to evaluate the possibility that humoral "blocking" antibodies directed specifically against graft antigens, that is, immunological enhancement (*9*), was responsible for the prolonged survival of these heart homografts, three normal BN rats which received Lewis heart transplants were injected with one donor-equivalent of serum taken from BN animals which had maintained a Lewis heart graft for 102, 160, and 169 days, respectively. The schedule of injections used was the same as that described by French and Batchelor to prolong the survival of rat kidney allografts (*10*): 1 ml intravenously immediately after reestablishing the blood supply to the organ, 1 ml intravenously at 24 hours, and the remainder in three intraperitoneal injections at 48, 72, and 96 hours. This treatment failed to prolong the survival of the Lewis hearts (Table 2, experiment 5).

These experiments show that Lewis/BN lymph node cells can induce tolerance of Lewis heart homografts but not of Lewis skin grafts, when inoculated into newborn, Ag-B incompatible, BN recipients. The reason for this could be: (i) heart, like lymphoid tissue, is deficient in at least one antigen represented on skin; (ii) in contrast to the situation with skin homografts, the rejection of heart homografts, like kidney, could result primarily from sensitization to "passenger" leukocytes (*11*); (iii) lymph node cells confer a degree of tolerance sufficient to greatly extend the survival of a whole organ homograft, but insufficient to prolong the survival of skin grafts; or (iv) in spite of the fact that with our protocol we were unable to enhance the survival of heart homografts with serum from rats that had accepted their heart grafts, some humoral enhancing mechanism may be responsible for the better survival of these grafts than those of skin.

Situations in which lymphoid or hematopoietic cells, or both, are unable

Table 1. Survival of Lewis skin and heart grafts in adult BN rats injected at birth with 30 million L/BN F_1 hybrid lymph node cells.

Type of graft	Number of rats	Reactivity of hosts to their grafts and distribution of graft survival times		
		Not tolerant (< 14 days)	Tolerant (14 to 50 days)	Highly tolerant (> 50 days)
Skin	8	7*	0	1
Heart	11	1	3	7

* Mean survival time of Lewis skin grafts on normal BN hosts is 8.1 ± 0.4 days.

Table 2. Survival of Lewis hearts in treated and normal BN recipients.

Exp. No.	Treatment of BN recipients	Number	Survival of cardiac homografts (days)
1	30 million L/BN cells at birth	6	16, 32, 47, > 50*, > 102†, > 169†
2	30 million L/BN lymph node cells at birth, rejected Lewis skin graft	5	13, > 62*, > 72*, > 88‡, > 160†
3	None	6	6, 7, 7, 7, 7, 8
4	Lewis skin homograft, which was rejected	4	2, 3, 3, 4
5	Transfer of 5 ml of serum from animals in experiments 1 and 2	3	7, 7, 7

* Died with beating heart grafts during anesthesia for second skin graft. † Killed and exsanguinated for serum used in experiment 5. ‡ Died of intraperitoneal abscess.

to induce unresponsiveness to skin grafts of the same genotype have also been reported to occur in certain H-2 incompatible mouse strain combinations (*12*). Indeed, in this species, inoculation of both C57/A bone marrow and splenic cells is totally ineffective in inducing tolerance of A or C57/A skin grafts in lethally irradiated adult C57 recipients (*13*). Similar observations have been reported in cattle (*14*) and humans (*15*).

The occurrence of one or more antigens on skin (possibly determined by components of the major H-locus) which are not present or poorly represented in some or all of the cells of the lymphohematopoietic system (*13*) might well contribute to the now-established fact that skin is the most difficult organ to homograft successfully.

CLYDE F. BARKER
DAVID M. LUBAROFF
WILLYS K. SILVERS
Department of Surgery and *Immunobiology Research Unit, Departments of Medical Genetics* and *Pathology, University of Pennsylvania School of Medicine, Philadelphia 19104*

References and Notes

1. W. L. Elkins and J. Palm, *Ann. N.Y. Acad. Sci.* **129**, 573 (1966).
2. W. K. Silvers and R. E. Billingham, *Transplantation* **8**, 167 (1969).
3. R. E. Billingham and W. K. Silvers, in *The Thymus*, V. Defendi, Ed. (Wistar Institute Press, Philadelphia, 1964), pp. 41–48.
4. W. K. Silvers, D. M. Lubaroff, D. B. Wilson, D. Fox, *Science* **167**, 1264 (1970).
5. V. Kren, D. Krenova, C. Stark, *Folia Biol.* **16**, 305 (1970).
6. R. E. Billingham, in *Transplantation of Tissues and Cells*, R. E. Billingham and W. K. Silvers, Eds. (Wistar Institute Press, Philadelphia, 1961), pp. 87–106.
7. R. E. Billingham, in *ibid.*, pp. 1–26.
8. K. Ono, E. S. Lindsey, O. Creech, Jr., *Transplantation* **7**, 176 (1969).
9. G. Snell, *Surg. Gynecol. Obstet.* **130**, 1109 (1970).
10. M. E. French and J. R. Batchelor, *Lancet* **1969-II**, 1103 (1969).
11. R. D. Guttman and R. R. Lindquist, *Transplantation* **8**, 490 (1969); F. P. Stuart, E. Bastien, A. Holter, F. W. Fitch, W. L. Elkins, *Transplant. Proc.*, in press.
12. N. L. Warner, L. Herzenberg, L. J. Cole, W. E. Davis, Jr., *Nature* **205**, 1077 (1965).
13. E. A. Boyse, E. M. Lance, E. A. Carswell, S. Cooper, L. J. Old, *ibid.* **227**, 901 (1970).
14. W. H. Stone, R. G. Cragle, E. W. Swanson, D. G. Brown, *Science* **148**, 1355 (1965).
15. M. F. A. Woodruff and B. Lennox, *Lancet* **1959-II**, 376 (1959); J. O. W. Beilby, I. S. Cade, A. M. Jelliffe, D. W. Parkin, J. W. Stewart, *Brit. Med. J.* **1960-I**, 96 (1960).
16. Supported by PHS grants AI-09275 and AI-07001. C.F.B. is a Markle scholar in academic medicine. W.K.S. is a PHS career development awardee. The technical assistance of J. Johnson, C. Stoffel, and G. Sawchuck is acknowledged. We also are indebted to Dr. R. E. Billingham for critical review of the manuscript.

23 February 1971; revised 22 March 1971

Photoreceptors in Primitive Chordates: Fine Structure, Hyperpolarizing Receptor Potentials, and Evolution

Abstract. *Two species of primitive chordates have hyperpolarizing photoreceptor potentials, as vertebrates do. In* Salpa *the photoreceptive membrane is composed of microvilli, whereas in* Amaroucium *it is modified from cilia. There appears to be no functional correlation between fine structure of photoreceptive membrane and polarity of response to light.*

Most photoreceptors show one of two types of structural specialization at those regions of their cell membranes believed to contain photopigments—a folding of the plasma membrane into microvilli or into arrays of lamellae or disks modified from cilia (*1*). The microvilli-bearing photoreceptors which are common among invertebrates are associated with a depolarizing receptor potential, whereas vertebrate photoreceptors, whose outer segments are derived from cilia, respond to light with a hyperpolarizing potential (*2*). We have recently shown (*3*) that the ciliary-type photoreceptor of an invertebrate, *Pecten irradians*, is hyperpolarized by light. This response differs from that of vertebrate cones in two ways. It is associated with an increase, rather than a decrease, in membrane conductance, and it is linked to the generation of an "off" discharge in the axons of these cells. It is likely that a hyperpolarizing receptor potential also underlies the "off" responses of ciliary-type photoreceptors of other invertebrates (*4*). It is of some interest to determine if the relationship between the cell's fine structure and its response to light is real or simply a fortuitous correlation. To do this we have examined the membrane structure and electrical response of visual cells in the eyes of some primitive chordates, from the subphylum urochordata, whose receptors may be similar to those of higher chordates (vertebrates) (*5*). Our findings show that hyperpolarizing receptor potentials occur in both ciliary- and microvillar-type photoreceptors in chordates.

The tunicate *Salpa democratica* (class Thaliacea) has a lensless eye arising from the neural ganglion, which is directly accessible to light, being situated on the dorsal surface of the animal beneath its transparent tunic. The eye arises from a secondarily acquired portion of the nervous system and is thus not homologous with the eyes of other urochordates or those of vertebrates (*6*). Our electron microscopic observations show that the eye contains, in addition to a layer of pigment cells, several hundred elongated visual cells about 10 μm in diameter (*7*). These cells give rise to a randomly organized array of microvilli at one end (Fig. 1A), which in some sections can be seen to be in continuity with the cell's cytoplasm. No other types of neurons are present in the eye.

We made intracellular recordings from photoreceptor cells with high-resistance microelectrodes filled with KCl, using conventional recording methods and a Wheatstone bridge circuit to pass constant-current pulses across the cell membrane. The bridge was balanced after penetration to eliminate the voltage drop across the electrode, leaving the slower charging voltage drop across the cell membrane. White light from a 45-watt tungsten quartz-iodine bulb was focused to an evenly illuminated spot which covered the entire eye. For study of the response to light, the eye and the neural ganglion were removed from the animal and pinned in a small chamber under seawater.

Although the resting potentials of visual cells in the salp were low, about -10 mv, the response to a brief flash of light was a large hyperpolarization (Fig. 1B), which in some cells could be up to 70 mv in amplitude. The size of this response was graded with light intensity. The nature of the changes in membrane conductance during the receptor potential was studied by passing brief hyperpolarizing constant-current pulses across the membrane during darkness and illumination. The voltage drop caused by the test pulse across the cell membrane decreased in size during the response to light (Fig. 1C) indicating that the receptor potential is due to an increase in membrane conductance. Additional information about the response was obtained by passing steady currents across the membrane and observing the change in the amplitude