References and Notes

1. S. C. Litzenberger, *Phytopathology* **39**, 300 (1949); F. Meehan and H. C. Murphy, *Science* **106**, 270 (1947).
2. H. H. Luke and H. E. Wheeler, *Phytopathology* **45**, 453 (1955).
3. L. Krupka, *Science* **128**, 477 (1958); R. R. Romanko, *Phytopathology* **49**, 32 (1959).
4. F. S. Thatcher, *Can. J. Botany*, **20**, 283 (1942).
5. I. Uritani and T. Akazawa, in *Plant Pathology*, J. G. Horsfall and A. E. Dimond, Eds. (Academic Press, New York, 1959), vol. 1, pp. 349–390; A. Millerd and K. J. Scott, *Ann. Rev. Plant Physiol.* **13**, 559 (1962).
6. H. Beevers, *Respiratory Metabolism in Plants* (Row-Peterson, Evanston, Ill., 1961), pp. 161–175.

7. This investigation was supported by U.S. Public Health Service grant E-4033.

16 August 1962

Blood Groups and Splenomegaly in Chick Embryos

Abstract. The B blood group locus in chickens is shown to be associated with the graft-against-host reaction in chick embryos.

In a recent paper Schierman and Nordskog (*1*) have shown that when chicken donors and hosts have the same B blood group allele, survival of skin grafts is prolonged. A similar relationship exists in the mouse, where strains differing in alleles of the H-2 locus not only manifest accelerated rejection of tissue grafts but also differ in respect to red cell antigens (*2*). In addition Billingham (*3*) has reported more severe "runting" symptoms in baby mice made tolerant with cells from strains differing at the H-2 locus. It appears, then, that genes of the H-2 locus are involved in histocompatibility and blood group difference and that they also play a role in the graft-against-host reaction.

Information about the physiological function of cell antigens is of profound biological significance and the purpose of this investigation is to add to this knowledge by exploring the relationship between red cell antigens and the induction of splenomegaly in non-inbred lines of chickens.

Sterile blood was obtained from adult hens of three B locus genotypes, designated B 19/19, B 21/21 and B 19/21. Fifteen-day-old embryos of all three genotypes each received 0.1 ml of blood intravenously from a single donor. The weights of the spleens removed 4 days later, which were used as a quantitative indication of the graft-against-host reaction (*4*), are shown in Table 1.

Table 1. Average spleen weights and standard errors for chick embryos injected with blood from blood-grouped adult female donors.

Donor hens		Av. wt. (mg)	Embryos (No.)
Blood type	No. used		
Blood type of hosts 19/19			
19/19	1	16.9 ± 1.2	24
19/21	2	15.5 ± 0.9	24
21/21	3	98.8 ± 6.8	37
Controls		11.8 ± 0.7	12
Blood type of hosts 19/21			
19/19	1	81.3 ± 6.7	9
19/21	2	19.3 ± 1.5	3
21/21	3	118.9 ± 12.7	10
Controls		14.8 ± 0.9	13
Blood type of hosts 21/21			
19/19	1	96.1 ± 6.9	28
19/21	2	16.4 ± 0.9	22
21/21	3	18.7 ± 1.0	27
Controls		12.2 ± 0.6	11
Ungrouped			
19/19	1	76.3 ± 5.7	27
19/21	2	65.4 ± 9.0	20
21/21	3	84.1 ± 8.7	19
Controls		13.8 ± 0.7	11

From these figures it is clear that where host and donor are of the same B locus genotype, little or no splenomegaly is produced, whereas considerable enlargement of the spleen occurs where they differ. The figures from the B 19/21 donors are particularly relevant to the graft-against-host nature of this phenomenon, in that although the donors' blood type differs from that of two of the host groups, no enlargement occurs in any of them, since the hosts do not possess any B group antigens foreign to the donors. The slight enlargement seen in the groups where none would be expected if only the B locus were involved, presumably indicates that other antigens play an additional, if minor, role.

Whether the underlying genetic situation here resembles that of the H-2 region in the mouse or that of other antigen complexes in man and cattle must remain in doubt until time has afforded further opportunities for the detection of recombinations. In any event, the variety of effects associated with the B locus (see also *5* for the relationship with reproductive fitness) suggests that this region of the fowl chromosome exerts a profound effect on cell functions.

W. P. JAFFE

School of Veterinary Science, University of Bristol, England

E. M. MCDERMID

Thornber Brothers Ltd., Mytholmroyd, Halifax, England

References

1. L. W. Schierman and A. W. Nordskog, *Science* **134**, 1008 (1961).
2. P. A. Gorer, *Advan. Cancer Res.* **4**, 149 (1956).
3. R. E. Billingham, *Science* **130**, 947 (1959).
4. W. P. Jaffe and L. N. Payne, *Brit. Poultry Sci.* **2**, 133 (1961); *Immunology*, in press.
5. W. E. Briles, *Poultry Sci.* **35**, 1134 (1956); R. C. Fanguy, T. M. Ferguson, J. M. Quisenberry, *ibid.* **40**, 1400 (1961).

20 April 1962

Calcium and Other Ions in Blood and Skeleton of Nicaraguan Fresh-Water Shark

Abstract. The bull shark, *Carcharhinus leucas*, employing archaic but effective means of regulating the physical-chemical composition of its body fluids, thrives in tropical fresh-water rivers and lakes. The ionic strength of the serum and the concentrations of total solutes, calcium, urea, and other ions are below the levels found in marine elasmobranchs but higher than the levels in teleosts. The patterns of the calcium deposits of the vertebrae are identical in marine and fresh-water subspecies.

Migrations into fresh water of the bull sharks of Lake Nicaragua in Central America, Lake Sentani of western Dutch New Guinea, and the Ganges and other Asiatic rivers have been reported by Herre (*1*) and Boeseman (*2*). The physiology of these fishes presents extraordinary mechanisms of ion and osmotic regulation. Smith (*3*) reported a decrease in the serum concentration of urea with relatively little change in phosphate or chloride in small sharks and sawfish found in rivers of Malaya and Siam; no data were recorded about the other solutes of the blood. My previous research (*4*) has included work on the chemistry of calcium and the major components of the blood and skeleton of the bull shark of the Atlantic, *Carcharhinus leucas*, and 13 other species of marine elasmobranchs, but none were fresh-water migrants.

On 27 March 1962, one tarpon (*Megalops atlanticus*) and four bull sharks, three females and one male, ranging in length from 5 feet 11 inches to 7 feet 1 inch, were taken in southeastern Nicaragua from Rio San Juan (*5*) near El Castillo. Small fishes, alligators, and viscera of swine were used as bait, but only freshly caught tarpon brought results. The fish were photographed after exsanguination by cardiac puncture and section of the caudal artery. Blood was collected in thermos

containers. Serum was separated immediately after the blood had clotted, with a hand-operated centrifuge and then was frozen in liquid nitrogen for transportation to Los Angeles. Vertebrae were excised and fixed in solutions of 10-percent formalin. Specimens of water were collected from Lake Nicaragua and the region of Rio San Juan in which the fish were caught. Chemical methods were the same as in previous reports (*4*); values given are the mean of three determinations on each specimen.

Photographs of two specimens were examined by Eugenie Clark and Stewart Springer but could not be positively identified as *C. leucas*. Possibly a subspecies can be recognized in which (i) the first dorsal fin is behind the pectoral fins, (ii) the third gill slit is short, and (iii) the dental formula is identical. There is now considerable uncertainty (*6*) about whether the data collected to date can justify two subspecies, *C. leucas leucas* and *C. leucas nicaraguensis*; only a statistically significant number of specimens can resolve this situation. The limits of the range of *C. leucas nicaraguensis* is not known. The general assumption is that it originally inhabited oceanic lakes extending from the sea, adjusted to fresh water when the salt water turned fresh by a slow process of dilution, and became landlocked by historic earthquakes that raised the bedrock and produced the rapids of Rio San Juan. At El Castillo, El Toro rapids have channels through them that I found were easily passable for large tarpon, sharks, and even fishermen traveling in dugout canoes during the dry season.

The vertebrae of the shark of Nicaragua were sectioned for radiographic examination of the calcium deposits and for comparison with typical specimens of *C. leucas* which had been collected in the Atlantic Ocean and supplied by Clark. The configurations of the calcium deposits are exactly the same, and somewhat similar to those of other Carcharhinidae. The vertebrae of tarpon consist of true bone rather than calcified cartilage (Fig. 1).

Analyses of waters of Nicaragua revealed typical medium soft fresh water. In Lago de Nicaragua, and Rio San Juan, respectively, such analyses showed the following ions (in millimoles per liter): total ions, 8.5 and 5.1; Na, 1.3 and 0.7; K, 0.1 and 0.1; Ca, 0.9 and 0.8; Mg, 0.6 and 0.2; Cl, 1.8 and 0.8; HCO_3, 3.0 and 1.7; SO_4, 0.7 and 0.7; and PO_4, 0.001 and 0.001.

In *C. leucas nicaraguensis* from Rio San Juan, the total concentration of the serum is approximately 83 percent, the calcium 66 percent, and the uremia 30 percent of the levels which were found in *C. leucas* from a marine habitat (Table 1). To appreciate the remarkable adjustments that these elasmobranchs can make in fresh water, the chemical composition of the serum in marine habitat (*4*) must be noted. In the sea, the level of serum urea fluctuates between 300 and 350 mmole/liter, and the total ion concentration rises and falls only slightly in order to maintain osmotic equilibrium and homeostasis. The ionic strength, μ, is as high as 0.3 (*7*). Elasmobranchs do not drink sea water but absorb calcium and other ions from the diet through the gut. The active concentration of calcium ions is sustained by the kidneys and gill membranes at levels required for normal neuromuscular tone and for calcification of cartilage. For these functions, the optimum concentration of total serum calcium (3.0 mmole/liter is ionized) for the shark in marine habitat is 4.5 mmole/liter. Further efforts to investigate ion regulation in fishes requires experiments designed to study thermodynamic mechanisms. In sea water, the gills expend energy to: (i) hold back ions from moving too fast toward concentration and electropotential gradients; (ii) maintain unidirectional flow; and (iii) regulate each ion separately. The concentration of calcium in sea water is 10.10 mmole/

Table 1. Chemical composition (millimoles per liter) of serums of sharks and tarpon.

Item	*C. leucas leucas*	*C. leucas nicaraguensis*	*M. atlanticus*
Ions:			
Na	223.4	200.12	101.0
K	9.0	8.2*	6.2
Ca	4.5	3.0	2.5
Mg	2.9	2.0	1.4
Cl	236.0	180.5	140.0
HCO_3	5.1	6.0	10.1
SO_4	0.6	0.5	0.4
PO_4	2.0	4.0*	5.0*
Total	483.5	404.3	266.6
Urea nitrogen	333.0	132.0	6.0
Ultrafilterable Ca	3.7	1.8	1.1
Protein (g %)	2.4	3.4	6.0
Alkaline phosphatase	0.9	3.0	2.0
Acid phosphatase	1.0	1.1	12.0

*Hemolyzed blood.

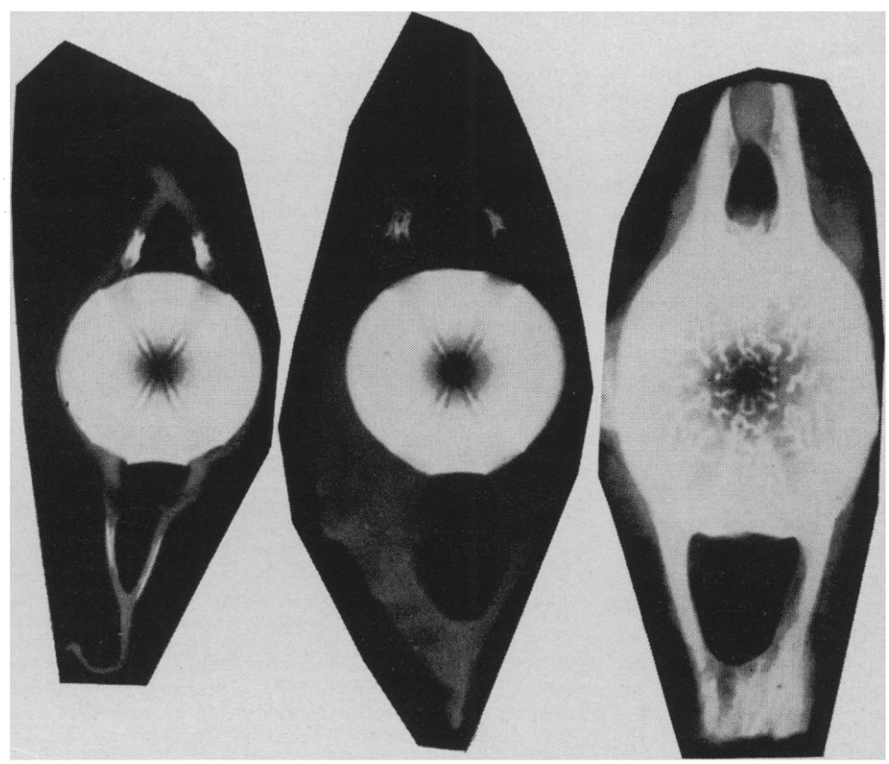

Fig. 1. Radiographs in the frontal view, showing the anatomy of the lumbar vertebrae of *C. leucas leucas* (left), *C. leucas nicaraguensis* (middle), and the tarpon, *M. atlanticus* (right). These deposits consist of calcified cartilage in the elasmobranchs, but true bone exists in the teleosts. The identical pattern in the two sharks suggests that they are of very closely related, if not the same, species.

liter, but only a fraction is chemically active when this value is corrected to infinite dilution and ion association is taken into consideration.

In the fresh water, the serum concentration of urea is sustained at approximately 132 mmole/liter. Elasmobranchs require at least this concentration of urea for normal cardiac function (*7, 8*). The concentration of total ions is 404.3 mmole/liter, 83 percent of the ion concentration of the serum of marine elasmobranchs; the ionic strength, μ, is estimated to be .2790. Under these conditions, the active concentrations of calcium ions increase to the extent that precipitation of calcium phosphate and depression of neuromuscular irritability can be avoided only if the gill membranes and the kidneys lower the calcium concentration in the body fluids. This level appears in the fresh-water shark to be approximately 3.0 mmole/liter of total and 1.8 mmole/liter of ultrafilterable calcium. The most abundant ions in Rio San Juan and other bodies of fresh water are Ca^{++} and HCO_3^{--}, but the total ion concentration is very low (5.1 mmole/liter), compared with 404.3 mmole/liter in serum. Here, the gills expend energy obtained from intracellular glycolysis to pump ions against concentration and electropotential gradients.

The teleost fish that is found in the same geographic area as the Nicaraguan shark has an entirely different composition of the serum; μ equals 0.15, total ions constitute 266.6 mmole/liter, and the total calcium is only 2.5 mmole/liter. Uremia is absent (Table 1). The blood is less concentrated, more viscous, better aerated, and homeostasis is achieved presumably by dynamic mechanisms using the functions of bone cells and bone tissue as a buffer (*7*). Teleosts are ionically independent and highly advanced, compared with the elasmobranchs; they evolved bone for finer regulation of ionic composition of the blood. The apatite mineral in bone is metabolized by means of a two-way process of resorption and formation, and constitutes the storage depot of a closed cycle or servosystem for utilizing hydronium, sodium, calcium, phosphate, and other ions. In addition to gills, kidneys, and bone, integument functions as an organ of ionic homeostasis. It serves as a semipermeable membrane in lamprey (which have gills but no calcium deposits) and in certain adult amphibians (which possess true bone but no gills and yet retain urea). In elasmobranchs and teleosts, the body covering is waterproof, and facilitates osmoregulation. Thus, the interactions and combinations of organs used by vertebrates for water balance and mineral homeostasis are numerous and highly complex. Physiologic experiments are necessary to measure the capacity of the bull sharks for adaptation. A tagging project would be of great interest to determine the limits of their range, reproductive cycle, species specificity, and ascertain whether they readapt to marine habitat.

MARSHALL R. URIST

Division of Orthopedics, Department of Surgery, University of California Medical Center, Los Angeles 24

References and Notes

1. A. W. C. T. Herre, *Science* **122**, 417 (1955).
2. M. Boeseman, *ibid.* **123**, 222 (1956).
3. H. Smith, *Am. J. Physiol.* **98**, 279 (1931).
4. M. R. Urist, *Endocrinology* **69**, 778 (1961).
5. I am indebted to: Dr. Thomas R. Howell, Marc Buchanan, and John Zoeger of the department of zoology at UCLA, who assisted and made the arrangements in Nicaragua; Dr. J. Antonio Mora, Ing., Escuela Nacional de Agricultura y Ganaderia, who selected the area of exploration; the Nicaraguan Air Force, which transported personnel and equipment; Major Alvaro Pallais S., Commandant at San Carlos, who allocated a jeep and boats; Captain Humberto Suarez A., of the Guardia Nacional de Nicaragua, who gave invaluable time and energy and assisted in the surgery; Marshall McLean Urist of Cate School who served as laboratory technician and processsed the specimens; Dr. Eugenie Clark, Cape Haze Marine Laboratory, Placida, Florida, who provided serum and vertebrae of *C. leucas*, collected in the Atlantic Ocean; Robert Dorion, Guatemala City, Guatemala, who generously loaned his private library on the Elasmobranchii; Drs. Stewart Springer (Duke University Marine Laboratory), T. B. Thorson (University of Nebraska), and Malcolm Gordon, who contributed valuable suggestions.
6. A. B. Bigelow, W. C. Schroder, S. Springer, *Bull. Mus. Comp. Zool.* **109**, 213 (1953).
7. M. R. Urist, *Perspectives Biol. Med.* (in press).
8. W. W. Simpson and E. Ogden, *J. Exptl. Biol.* **9**, 1 (1932).
9. These investigations were supported by contract NR 104-575 between the Office of Naval Research and the University of California Medical Center, Los Angeles, and in part by grants from the Squibb Institute for Medical Research.

10 August 1962

Continuous Culture of a Melanotic Cell Line from the Golden Hamster

Abstract. A melanotic cell line derived from a malignant melanoma of the golden hamster has retained its ability to produce melanin in vitro. This cell line provides an opportunity for studying the synthesis of melanin under various controlled conditions. The modal chromosome number in this cell line is 68.

Shortly after the advent of cell culture, Burrows reported his attempts at growing human malignant melanoma in vitro (*1*). Subsequently, short-term cultures of both animal and human melanoma cells have been described (*2*). For example, Wellings *et al.* (*3*) apparently grew human malignant melanoma cells in vitro for at least 4 weeks. Similarly, cultures of melanoma cells from fish, mice, and hamsters have been described, and have been used for histochemical studies. Rosenberg *et al.* (*4, 5*) cultured both amelanotic and melanotic malignant melanoma cells from tumors which originated in the hamster colony maintained by Fortner (*6, 7*). Rosenberg *et al.* (*4, 5*) reported that the amelanotic tumor was more invasive and grew faster in vivo, an observation which had been previously made by Fortner. Unique differences in the sites of metastases were noted (*4*). They also described the morphological characteristics of monolayer cultures, and stated that there was little growth after the first transfer (*5*).

Table 1. Chromosome numbers in malignant melanoma cells of golden hamster grown in vitro.

Chromosome numbers	Cells counted on sampling dates (1962)	
	12 April	18 April
42–49	3	
50		1
52		1
53	1	
54		1
57	3	3
58	2	1
59	2	3
60	3	
61	2	
62	3	
63	2	3
64	2	4
65	5	9
66	6	11
67	7	13
68*	12	21
69	2	17
70	1	8
71	1	4
72	1	
73	1	1
Total	60	100

* Mode.

A series of melanotic and amelanotic malignant melanomas which developed in aged golden hamsters has been described by J. G. Fortner (*6*). We have successfully cultured several of the amelanotic melanoma tumors in vitro, but we found that either the pigmented tumors did not grow as permanent cell lines or else only nonpigmented cells survived. As a result of a screening program in our laboratory in which various malignant cells are grown in a variety of media with various protein supplements, a melanotic hamster cell line which has retained its ability to