Alpha Globulin Injections and Decreased Gamma Globulin Production in Chickens

Abstract. *Electrophoretic analysis shows a strong similarity in the blood serums of chickens subjected to egg parabiosis and of chickens repeatedly injected with fraction IV (alpha globulins). In both instances there is an evident rise in the albumin-plus α_1 titer and an evident decrease in the gamma globulin levels. These findings suggest that the injection of alpha globulins has the same serum effect on the animal as parabiotically induced blood chimerism, namely, a reduction in circulating gamma globulin (immunoglobulins?).*

In a recent report, Davis and Boxer (*1*) found that chromatographically prepared bovine α-globulins (*2*) injected into reciprocally skin-grafted DBA/2 and BALB/c mice on days 1 and 3 did not produce immune tolerance nor prolonged homograft survival. These authors also did hemagglutinin titers and found that their animals had been sensitized and produced hemagglutinins in a high titer. They found no evidence of immunosuppression by the injection of preparations containing α_2-globulins. In contrast to this, in 1958 I had demonstrated (*3*) that repeated injections (five or more) of α_2-globulin serum fractions resulted in significant increases in successful parabiotic union of pen-bred albino rats. Using a similar regimen, I reported (*4*) that rat serum fraction VI (17 percent α_1 and 83 percent α_2) and human fraction IV-4 (59.5 percent α_1-, 33.9 percent α_2-, and 6.6 percent β-globulins) significantly increased the prolonged retention of skin allografts. In an attempt to obtain more purified α_2-globulin preparations, Mowbray (*2*) used chromatographic methods of separation. His preparation C gave results confirming mine. In an attempt to explain Davis and Boxer's failure to obtain immunosuppression, that is, indications of prolonged homograft survival, Mowbray (*5*) pointed to the necessity of proper timing and repeated injections of the material.

There is a need to investigate this subject further and to develop insight into the probable mechanisms involved in the prolonged retention of skin allografts. Comparison with a well-researched phenomenon such as blood chimerism and concomitant suppression of the immune rejection phenomenon would aid in clarifying the probable mechanism. I have now made electrophoretic examinations of the blood serums of (i) normal hybrid chickens, (ii) normal White Leghorn chickens, (iii) normal chickens (hybrids) subjected to skin allografting, (iv) normal chickens (White Leghorns) subjected to skin allografting, (v) hybrid chickens derived from egg parabiosis and subjected to skin allografting, (vi) White Leghorn chickens derived from egg parabiosis and subjected to skin allografting, (vii) hybrid chicks subjected to injections of serum containing 5 mg of fraction IV (chicken) at varying ages and then homografted, and (viii) White Leghorn chicks subjected to injections of serum containing 5 mg of fraction IV (chicken) at varying ages and then homografted.

All serums were analyzed by the Antweiler microelectrophoresis apparatus, with Veronal buffer, *p*H 8.6, ionic strength 0.10. A constant amperage reading of 1.5 ma was maintained for all serums. The voltage fluctuated between 75 and 95 volts and appeared to depend on the temperature of the circulating ice water which surrounded the mantle and cell. The temperature of the bath varied from 8° to 13°C.

All the chickens used in the experiment were hatched from eggs maintained at a constant temperature, humidity, and rotation in the forced draft incubator. The hatching eggs were placed in the hatching tray on the 20th day of incubation and were then removed to a heated brooder after hatching.

Fertile hybrid (Barred Rock × Rhode Island Reds) eggs and White Leghorn eggs were joined in parabiosis on the 11th, 12th, or 13th day of incubation. Vascular union of the two developing embryos was encouraged by the gravitational collapse following air-cell suction or by placing fragments from 11-day-old embryos on the chorioallantoic membrane prior to joining the eggs, or by placing fresh chicken red-blood-cell clot on the joined chorioallantoic membrane. Out of 486 unions, 12 coparabionts and 16 single surviving parabionts were obtained. Several of the chicks died by the 5th day so that only 7 pairs were available for reciprocal skin allografts.

Chicks of both strains were randomly selected for injection of fraction IV (chicken-Pentex) initially at age 1 day, 5 days, 16 days, 18 days, and 21 days. The 5 mg of dissolved fraction IV in serum was injected intraperitoneally at least five times on alternate days. On the 21st to the 25th days, reciprocal leg skin allografts were orthotopically exchanged between the strains as well as within the strains. In most of the cases, prolonged survival of viable grafts was obtained (*6*). The data in this report showed that 5 out of 14 grafts (hybrids) were retained two or more times longer than the control mean survival time.

Table 1 shows the mean percentage

Table 1. Comparison of electrophoretic analyses of control and experimental animals. Composite data for ages 1 through 75 days.

Subjects	No.	Albumin + α_1 (%)	α_2 (%)	β (%)	$\gamma \pm$ S.D. (%)	p*
		Hybrids (Barred Rocks × Rhode Island Reds)				
Controls						
Unoperated	12	54.0	9.5	12.0	24.5 ± 3.8	
Grafted	12	46.3	13.8	13.5	26.4 ± 3.6	
Experimental						
Grafted parabionts	15†	60.5	7.3	14.5	17.7 ± 3.3	.01
Fraction IV injected (grafted)	11‡	57.2	8.4	22.6	11.8 ± 3.7	.01
		White Leghorns				
Controls						
Unoperated	10	54.1	10.1	11.4	21.4 ± 3.5	
Grafted	10	49.9	10.5	16.6	23.0 ± 4.3	
Experimental						
Grafted parabionts	8§	56.7	8.4	19.4	15.5 ± 3.2	.01
Fraction IV injected (grafted)	13¶	55.8	10.5	18.5	15.2 ± 4.8	.01

* The probabilities, determined by the *t*-test, apply to the differences in γ-globulin between hybrid grafted controls and the two experimental hybrid groups, respectively, and the differences in γ-globulin between White Leghorn grafted controls and two experimental White Leghorn groups, respectively. † Only seven animals survived for the entire 75-day period. ‡ Only six animals survived for the entire 75-day period. § Only four animals survived for the entire 75-day period. ¶ Only seven animals survived for the entire 75-day period.

of each of the electrophoretically separated components of each group of animals. Since the time span (1 to 75 days) is the same for all the groups, it is interesting to note that both groups of grafted controls show a lower albumin-plus-α_1 percentage than do the normal unoperated controls. This drop probably reflects the utilization of albumin during the healing process, which is initiated from the day of surgery and continues for a week or more during the post-slough period. The increase in the γ-globulin percentage titers probably discloses that the animals were sensitized during the period that the graft was retained, and probably indicates increased antibody formation.

The electrophoretic analyses of the immune tolerant animals and those of the animals injected with α-globulin are markedly similar. Contrary to the finding in the controls which were grafted, the albumin-plus-α_1 percentage is high, suggesting that no stress is involved in the healing process. In each strain, the γ-globulin percentage is lower, clearly providing evidence that the production of this globulin is partially inhibited or impaired. Since there is widespread acceptance that antibodies are analogous to or even identical with moieties of γ-globulin, decrease in the percentage of γ-globulin may represent a degree of suppression of the rejection apparatus. In addition, the prolonged retention of allografts by both parabiotic animals and animals injected with α-globulin varied widely, from a period of 13 days to over 100 days, suggesting that there are degrees of suppression; probably other mechanisms are involved in the phenomenon.

It is speculated that either α_2-globulins or some protein intimately associated with it has a suppressive effect, by covering or hindering the production of antibodies by specific lymphocytes or by the incorporation of a particular fragment of the α_2-globulin into the cytoplasm of specific lymphocytes. In either case, there appears to be a lack of recognition of the foreignness of the grafted tissue. Because of the long duration of the effects of α-globulin injection (γ-globulin depression is seen 50 days after the last injection) and the theoretical short half-life of the protein, it is assumed that incorporation of the protein in the constantly replicating immunological cell is the hypothesis of choice.

BENJAMIN B. KAMRIN
Department of Animal Behavior, American Museum of Natural History, New York 10024

References and Notes

1. W. C. Davis and L. A. Boxer, *Transplantation* **3**, 673 (1965).
2. J. F. Mowbray, *ibid.* **1**, 15 (1963).
3. B. B. Kamrin, *Ann. N.Y. Acad. Sci.* **73**, 848 (1958).
4. ———, *Proc. Soc. Exptl. Biol. Med.* **100**, 58 (1959).
5. J. F. Mowbray, *Transplantation* **3**, 676 (1965); *Nature* **197**, 1019 (1963).
6. B. B. Kamrin, in preparation.
7. Supported in part by NIH grant MH-11057-01.

11 July 1966

Glycogen Content in the Wood-Boring Isopod, Limnoria lignorum

Abstract. *The glycogen content of the isopod,* Limnoria lignorum, *ranges from 2.01 to 3.44 percent of its dry weight. Starvation of the animal for 1 week results in a decrease of the glycogen content by about 68 percent. Measurements of its oxygen consumption indicate that sufficient carbohydrate, probably its sole source of energy, is oxidized daily to meet the carbon requirements. The infestation zone of* Limnoria *is restricted to the surface of submerged wood, since this isopod borer, unlike the molluscan borers, does not have a glycogen reserve sufficient to meet prolonged periods of anaerobiosis.*

Glycogen serves animals as a primary food reserve and a principal source of energy. Most of the studies of glycogen in marine invertebrates have been of lamellibranchs and zooplankton (*1–3*). The distribution and amount of glycogen in the larvae and adults of a shipworm, *Teredo* (*4, 5*), and in a pholad, *Martesia*, have also been studied (*6, 7*). These wood-boring mollusks have a rich glycogen reserve (30 to 50 percent of their dry weight) which is catabolized during unusually long periods of anaerobiosis [up to 7 weeks in *Teredo navalis* (*8*)]. This study establishes the glycogen content of the crustacean borer, *Limnoria lignorum*, in relation to its growth and sex and to the availability of food, and it shows the relationship between the total glycogen content and the amount of glycogen oxidized daily. In addition, I have tried to determine why *Limnoria*, unlike the molluscan borers, is restricted to the surface of submerged woods.

A continuous supply of *Limnoria lignorum* was obtained from the old pier at Roche Harbor, Washington (48° 33′N, 123° 00′W). Preliminary experiments showed that the glycogen content of specimens analysed a day after dislodgement from the infested wood decreased slightly. Therefore, the animals emerging from their burrows were pipetted and then weighed on an H-16 Mettler semi-micro balance, and without delay, glycogen was determined by the methods of Mendel *et al.* (*9*) and Kemp and Kits van Heijningen (*10*). The animals were homogenized in 5 ml of a deproteinizing solution of 5 percent trichloroacetic acid containing 0.1 percent Ag_2So_4, and the homogenates were then heated for 15 minutes in a bath of boiling water, cooled, and centrifuged at 3000 rev/min. One milliliter of the resulting supernatant was boiled for 6½ minutes with 3 ml of concentrated H_2SO_4 (specific gravity, 1.84). By this procedure, glycogen was hydrolyzed to glucose and a pink color developed, the intensity of which depended upon the concentration of glucose. The intensity was measured with a Spectronic-20 colorimeter at a maximum absorption of 520mμ. The results obtained with the Spectronic-20 agreed reasonably well with those obtained at the same wave length with a Beckman DU-model spectrophotometer. The glycogen content of *L. lignorum* is given in Table 1.

The oxygen consumption of *Limnoria lignorum* was measured at 15°C in Braun's Warburg apparatus (model V "85"). Not less than 20 specimens were put in a flask containing 2.5 ml of filtered sea water (3.5 percent salinity). The constant beat of pleopods and occasional swimming movements helped to mix the medium. Mechanical agitation was avoided since it stimulated activity and consequently raised the metabolic rate. Care was taken to choose specimens free of the macroscopic, heterotrichid ciliates which are usually found attached to the telsons of 50 to 90 percent of the population. Debris clinging to the dense fringe of setae was removed by repeated rinsings before the animals were transferred to the respiratory flasks. The respiratory rates determined in preliminary experiments by means of a polarographic oxygen-electrode connected to a model 160 Beckman physiological gas analyzer