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.

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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

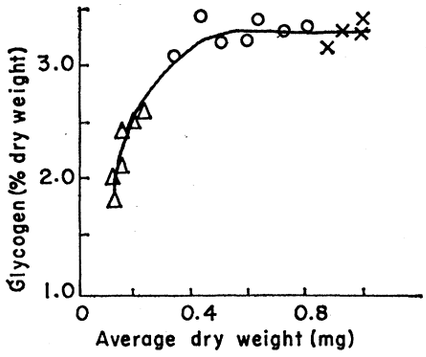

Fig. 1. The glycogen content of *Limnoria lignorum* in relation to growth. Young, △; adult, ○; gravid female, ×.

were of the same order as those determined in the Warburg apparatus.

The glycogen content of *L. lignorum* increases as the animal grows; after it matures, its glycogen content does not vary significantly (Fig. 1). Reproductive activity does not significantly change the amount of glycogen in *Limnoria*, nor does it have a pronounced effect on the amount in *Teredo* (*4*). Gravid sea-urchins, *Strongylocentrotus purpuratus*, have a higher glycogen content than nongravid ones do; the glycogen content of mature ovaries is 4.96 percent (*11*). The average glycogen content of mature ovaries of the wood-boring pholad, *Matesia fragilis*, is 6.1 percent (*7*). However, the mature reproductive systems of several marine molluscs are reported to lack glycogen (*12*).

The mean glycogen content of newly hatched juveniles (2.01 percent dry weight) is low compared to the extremely high concentrations of glycogen in the *Teredo* larvae (*4, 5, 13*), in which it acts as an energy reserve during the nonfeeding, free-swimming stage of the larva. Young *Limnoria* do not have a glycogen reserve, but one is not essential, since the juveniles can bore into wood soon after liberation from the brood pouch and can obtain nutrients from the wood to power the boring.

The glycogen content of *Limnoria* is depleted when the animals are kept in wood-free troughs of filtered seawater. The glycogen content based on dry weight of adults starved for 1 day decreases from 3.44 percent to 2.08 percent; the content of adults starved for 1 week, is 68 percent less than the original amount (Table 2). The glycogen content of *Teredo pedicellata* starved 7 days is similarly 72 percent less than that of the fed animals (*4*). However, *Limnoria* withstands starvation for periods up to 2 months in length, which results in a total depletion of their meager supply of body glycogen and in a decrease in their metabolic rate.

In *Limnoria* there is little or no effective reserve of glycogen, compared with that of a shipworm or of other boring molluscs. The glycogen content of *Teredo* is reported to range from 14 to 58.2 percent of its dry weight, with an average of 30 percent; that of *Martesia fragilis* ranges from 4.45 to 52.1 percent of its dry weight, with an average of 21.96 percent (*4, 7*). The difference between the narrow range (2.01 to 3.44 percent) in the glycogen content of *L. lignorum* and the fairly wide range in the molluscan borers is probably attributable to differences in the availability of the sources of carbohydrates in their diets. *Martesia* bores into wood for protection and does not ingest wood, but consumes plankton filtered from the ambient water. *Teredo* feeds on wood and plankton. The quantity of plankton available to these filter feeders is seldom uniform, but fluctuates from none to vast excess; this fluctuation may account for the wide range of glycogen content in the molluscan borers. Apart from this wide range, the average glycogen content is rather high in the boring molluscs and in lamellibranchs in general (*1*). Adequate glycogen reserves enable these bivalves to survive partial or complete anaerobiosis for long periods (*8, 14*). Von Brand (*15*) has pointed out the utility of glycogen as a metabolic substrate during anaerobiosis.

Because it has a meager glycogen reserve, *Limnoria* abandons its burrow under partially anaerobic conditions brought about by pollution and organic decomposition. Eltringham (*16*) has shown that *Limnoria* leaves the wood when there is a progressive reduction in the oxygen content of the water (*16*). He (*17*) has also shown that a population of three species of *Limnoria* from Southampton harbor require a constant supply of relatively large quantities of oxygen (1853 μl/gm per hour at 25°C). The oxygen consumption of *Limnoria lignorum* at 15°C (which approximates the temperature at which they live during summer) averages 1368 μl/gm per hour. The quantitative relationship between the amount of oxygen consumed and the amount of material metabolized permits calculation of the food requirements based on oxy-

Table 1. The glycogen content of *Limnoria lignorum*. Each figure represents an average of ten separate estimations.

Condition of animal	Animals (No.)	Length (mm)	Total wet weight (mg)	Total glycogen (μg)	Glycogen (% wet wt)	Glycogen (% dry wt)
Newly hatched	34	1.0	11.88	88.8	0.75	2.01
Juvenile with reduced 7th leg	20	1.3	9.00	79.2	0.88	2.44
Young	13	1.5	6.10	72.0	1.18	3.21
Adult	10	2.5	11.57	144.6	1.25	3.44
Adult	7	3.0	11.13	132.6	1.19	3.20
Adult	8	3.5	13.50	153.6	1.14	3.11
Gravid female	7	3.5	17.88	205.2	1.15	3.12
Starved 1 day	8	2.5	10.50	80.0	0.76	2.08
Starved 1 week	7	3.0	11.50	49.2	0.43	1.13

Table 2. Glycogen content, and oxygen consumption (μl per animal per day) and its carbohydrate equivalent in *Limnoria lignorum*. The salinity (3.5 percent), temperature (15°C), and oxygen tension (130 mm-Hg) of the media were kept constant. The figures for the mean carbohydrate equivalent represent approximate values (μg) of glucose oxidized as computed from oxygen consumed per day, per animal.

Condition of animal	Length (mm)	Average wet weight (mg)	Glycogen (μg/mg wet wt)	Average glycogen per animal (μg)	Mean of O_2 consumed (μl)	Mean of carbohydrate equivalent (μg)
Juvenile	1.0	0.35	7.5	2.65		
Juvenile	1.3	0.45	8.8	3.96		
Juvenile	1.5	0.47	11.8	5.54	7.85	11.06
Adult	2.5	1.16	12.5	14.46	12.8	17.15
Adult	3.0	1.59	11.9	18.94	20.8	28.45
Adult	3.5	1.66	11.4	17.06	21.6	28.68
Gravid	3.5	2.22	9.5	21.12	25.12	33.83
Starved 1 week	3.0	1.64	4.3	7.00	11.8	15.81

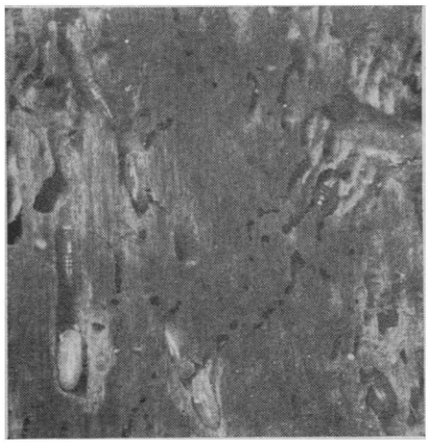

Fig. 2. The surface of a portion of a pile riddled by *Limnoria* attack. The burrow is parallel to the surface of the submerged wood and often exhibits a row of respiratory pits along the course of the tunnel.

gen consumption. The total amount of oxygen consumed each day by *Limnoria* of different sizes at 15°C is presented in Table 2, as are the glycogen contents of *Limnoria*.

The mean amount of substrate catabolized ranges from 11.06 to 33.83 μg of glycogen, depending on the size of the animal (Table 2). Assuming that the respiratory quotient is one, the amount of carbohydrate oxidized daily exceeds the total carbohydrate content of the body. In all probability a single *Limnoria* catabolizes 6 to 40.87 μg of glycogen daily. To meet the metabolic demands and still maintain the small glycogen reserve, this quantity of glycogen must be synthesized. Wood forms the major, or perhaps the sole, dietary source for *Limnoria* in which cellulase is present (*18*). Among the crustaceans, *Limnoria* may be unique in its ability to subsist totally on wood. This ability may account for the fact that their glycogen content (0.75 to 1.25 percent of their wet weight) is distinctly higher than that of other crustaceans such as the copepod, *Calanus fimnarchicus* (0.19 to 0.4 percent of its wet weight); the mysid, *Neomysis americana* (0.19 to 0.23 percent); the euphausid, *Thysanoessa* sp. (0.09 to 0.11 percent); the amphipod, *Euthemisto compressabispinosa* (0.25 to 0.62 percent); and the ostracod, *Conchoecia* sp. [0.31 percent (*3*)].

At regular intervals, I measured the volume of the burrow with a microsyringe (50 μl) to assess the exact volume of wood excavated and ingested by a burrowing, adult *L. lignorum*. On an average, the amount of wood consumed by an individual in one week ranges from 3 to 8 μl, and the volume of feces produced ranges from 2 to 6 μl The maximum rate of ingestion and fecal production always occurred during initial attack until the animal became completely embedded in the wood. *Limnoria* constructs a tunnel that is parallel to the surface and is well ventilated by a row of circular perforations through the thin film of wood bridging the lumen of the tunnel (Fig. 2). These holes are about 0.5 mm in diameter and are spaced at 1 to 2 mm along the course of the tunnel. In order to obtain sufficient oxygen to oxidize the carbohydrates synthesized from ingested wood, it seems essential that freshly oxygenated sea water be flooded over the constantly beating respiratory pleopods. The glycogen synthesized from the daily intake of food is oxidized to meet the metabolic requirements while the meager glycogen level remains little changed. It is now clear that in the absence of an adequate glycogen reserve, such as occurs in molluscan borers, *Limnoria* lacks the capacity to survive temporary or accidental anoxia which is likely to occur in deeper burrows and, thus, its zone of infestation is limited to the surface of submerged wood.

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Antiserum to Lymphocytes: Prolonged Survival of Canine Renal Allografts

Abstract. *A horse immunized with dog lymphocytes produced an antiserum which agglutinated canine lymphocytes in vitro and caused prolonged lymphopenia in dogs in vivo. Renal transplants in dogs treated with this antiserum survived for long periods, two of the grafts surviving beyond 350 days with normal function and histologic appearance.*

There has been intensive interest in the use of heterologous antiserum to lymphocytes to modify immune responses. Mice given such antiserum show profound lymphopenia, tissue lymphocyte depletion, and associated immunological incompetence demonstrable as reduced ability to produce humoral antibody and to reject skin allografts and xenografts. After a period of time, as the number of lymphocytes returns to normal, immune competence is restored (*1*). Allograft rejection in rats (*2*) and delayed sensitivity reactions in guinea pigs (*3*) are likewise suppressed by antiserum to lymphocytes. All reported studies with heterologous antiserums to lymphocytes have been performed in small animals. The effects of such antiserum on the survival of whole organ, immediately vascularized allografts in larger species, have not been reported. The experiments below show that heterologous (equine) antiserum to dog lymphocytes is a potent lymphopenic and immunosuppressive agent when given to dogs, and results in prolonged survival of canine renal allografts.

Suspensions of canine lymph-node lymphocytes were prepared from mesenteric nodes of dogs previously exsanguinated as blood donors for cardiovascular experiments. Use of exsanguinated donors markedly reduced the amount of blood in the nodes, thus producing lym-