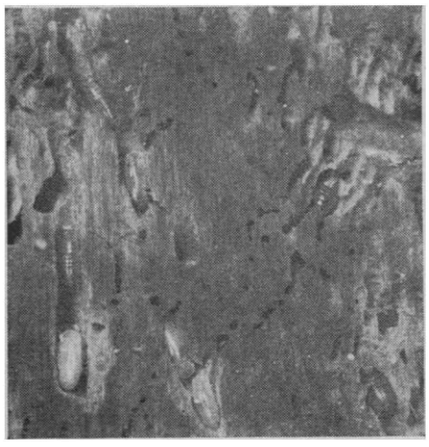

Fig. 2. The surface of a portion of a pile riddled by *Limnoria* attack. The burrow is parallel to the surface of the submerged wood and often exhibits a row of respiratory pits along the course of the tunnel.

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 $\mu$g 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 $\mu$g 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 $\mu$l) 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 $\mu$l, and the volume of feces produced ranges from 2 to 6 $\mu$l 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-

phocyte suspensions with a minimum number of contaminating erythrocytes. Lymph nodes were cleaned of perinodal fat and adventia and minced with a coarse scissors. Node fragments were then passed through a stainless steel wire mesh (60 gauge) into sterile 0.15*M* NaCl (*4*). The resulting suspension was washed three times in saline. Utilizing 7 to 10 large mesenteric nodes, we usually obtained a total of 3 to 6000 $\times$ $10^6$ lymph node cells (excluding erythrocytes). Smears of the white cell suspensions (Wright's stain) invariably showed 90 percent small lymphocytes and 4 to 8 percent large lymphocytes. The ratio of lymphocytes to contaminating erythrocytes usually varied from 10 : 1 to 3 : 1.

A saline suspension (10 ml) of canine lymphocytes (500 $\times$ $10^6$ lymph node cells per milliliter) was emulsified in an equal volume of complete Freund's adjuvant (Difco). A horse (*5*) was primarily immunized by injection of this emulsion into numerous subcutaneous sites. After 3 weeks, the horse received thrice-weekly intravenous booster injections of similar lymphocyte suspensions (10 ml per injection, 500 $\times$ $10^6$ lymphocytes/ml) without Freund's adjuvant for 7 consecutive weeks. Two weeks after the last booster, the horse was given a trial bleeding, and subsequently it was exsanguinated. The blood was allowed to clot at room temperature and to stand in the cold (4°C) for several days. The serum was separated, heated to 56°C (30 minutes) to remove the complement, filtered on a Seitz pad, treated with Merthiolate (1 : 10,000), and stored (−20°C) in small portions until used.

The capacity of horse antiserum to dog lymphocytes to agglutinate canine lymph node lymphocytes was determined by Amos and Peacocke's method (*6*). Its lymphoagglutinin titer in these experiments was 1 : 1024, and it agglutinated lymphocytes from numerous dogs to the same degree. Normal horse serum did not agglutinate canine lymphocytes or erythrocytes. The equine antiserum showed a definite titer of antibodies to canine red cells detectable as saline hemagglutinins (titer, 1:64) or hemolysins, presumably due mainly to erythrocytes contaminating the lymphocyte suspensions used for immunization. Absorption of the equine antiserum with saline-washed, packed canine red cells removed the antibody to erythrocytes, without any significant reduction in the lymphoagglutinin titer.

Studies subsequently showed that administration of large doses (500 to 1500 ml) of the unabsorbed equine antiserum either intravenously or intraperitoneally (or both) to dogs over short periods (7 to 14 days) was poorly tolerated, producing profound toxicity characterized by massive hemolysis, anemia, and bloody diarrhea (*7*). In contrast, injection of small doses of this serum subcutaneously was devoid of toxicity and was extremely well tolerated, even if continued for many weeks. For this reason, daily subcutaneous administration was employed in attempts to prolong the survival of canine renal allografts.

Adult mongrel female dogs received daily subcutaneous injections of small doses of the equine antiserum to dog lymphocytes (approximately 1 ml/kg) for 1 to 3 weeks before operation and were then subjected to renal allotransplantation. The right or left donor kidney was placed in the opposite iliac fossa of the recipient, the donor renal artery being anastomosed end-to-end to the recipient's divided common iliac artery, and the donor renal vein being sutured end-to-side to the recipient's common iliac vein. Ureteral continuity was reestablished by implantation of the donor ureter into the recipient bladder (uretero-neocystostomy). Recipients were bilaterally nephrectomized at the time of transplantation. All operative procedures were performed under general (Pentothal) anesthesia. From the time the injections of the antiserum to lymphocytes were begun, all dogs were bled thrice weekly for determination of the total number of white blood cells (standard Neubauer chamber), differential cells (Wright's stain), and blood urea nitrogen. Daily injections of the antiserum were continued after the operation. The dose of serum used was calculated on a basis of 1 ml per kilogram of body weight plus 1 ml/kg for each percent, greater than 1, of lymphocytes on the peripheral-blood smear. The usual total daily dose was 10 to 20 ml to each dog. The serum injections were continued postoperatively until the graft was rejected or until the injection schedule was changed as described below. Control dogs were paired with individual experimental animals and underwent similar operations, but received normal horse serum according to the same dose schedule until kidney grafts were rejected.

The total white cell counts and the percentage of large and small lymphocytes on smears varied markedly in normal dogs. Of 20 animals studied, total white blood cell counts ranged between 7600 and 25,000 cells (mean, 14,000), and the total percentage of lymphocytes varied from 14 to 60 percent (mean, 28 percent), with total lymphocyte counts of 1800 to 9000 cells/mm$^3$ (mean, 4000). The antiserum, given daily as described, produced rapid and profound peripheral lymphopenia in all of ten dogs treated with antiserum to lymphocytes, prior to and after renal allotransplantation. The mean percentage of lymphocytes of all types on peripheral smear was 0 to 2 percent 72 hours after the initial injection. The total granulocyte counts remained the same or frequently rose significantly, while the mean total lymphocyte count fell to 0 to 300/mm$^3$.

Daily injections of the equine antiserum maintained the initial lympho-

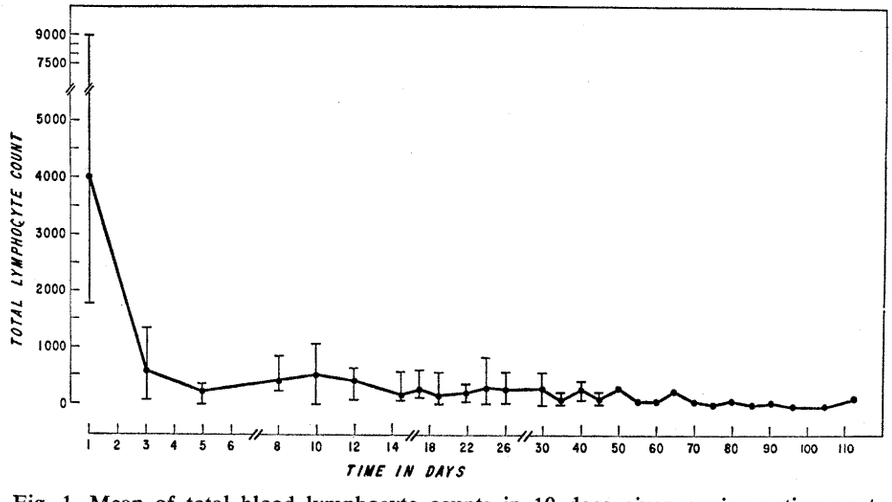


Fig. 1. Mean of total blood lymphocyte counts in 10 dogs given equine antiserum to lymphocytes daily subcutaneously for varying periods of 21 to 116 days. After day 50, the average total lymphocyte counts (only 4 dogs) are charted.

penia for as long as injections were continued (Fig. 1). In none of the dogs was there any suggestion of loss of ability of the serum to produce lymphopenia. After 116 days two dogs which had undergone renal allotransplantation survived, and these dogs subsequently received the antiserum (1 ml/kg) only once per week. Whereas daily injections of antiserum had maintained lymphopenia in the 0- to 2-percent range, weekly injections thereafter maintained lymphopenia at 3 to 7 percent (average total lymphocyte count, 300 to 900 cells/mm$^3$) for 250 days more, without evidence of significant escape from the effectiveness of the antiserum treatment. All dogs given daily subcutaneous injections showed only a 5- to 10-percent fall in hematocrit, even if injected for over 100 days. Absorption of the antiserum to dog lymphocytes with washed, packed canine erythrocytes to remove antibody to erythrocytes prior to administration was accordingly not done in these experiments. No evidence of wasting, as frequently seen in mice after prolonged and continuous treatment with heterologous antiserum to lymphocytes, was seen in these dogs (*1*).

Eight dogs treated with normal horse serum for 1 to 3 weeks preoperatively rejected renal allografts in 6 to 11 days (mean, 8.5 days). No significant lymphopenia secondary to therapy was noted with normal horse serum. Of ten dogs given the antiserum to lymphocytes, three succumbed to surgical complications (arterial thrombosis, intussusception, retroperitoneal hemorrhage) within 5 days of transplantation and were excluded from further consideration. Only one of the remaining seven dogs treated with antiserum underwent normal rejection (Table 1). Four dogs survived 20, 26, 52, and 76 days while two others were alive beyond 350 days, and their blood urea nitrogen (10 to 20 mg per 100 ml) is normal. The duration of survival was not clearly related to the length of time of prior treatment with antiserum, nor was the degree of lymphopenia correlated closely with the survival of the transplants.

Kidney grafts of dogs Nos. 1 and 2, examined at day 11 and day 20 respectively, showed typical advanced rejection in spite of persistent lymphopenia, that is, smears showing only 0 to 1 percent lymphocytes, even when 400 to 500 cells were counted. Dog No. 3 survived 26 days and succumbed to uremia with hydronephrosis secondary to stricture at the site of uretero-neocystostomy. The histologic picture indicated infiltration with interstitial, mononuclear cells, fibrosis, and an increase in the glomerular cells, a picture consistent with chronic infection rather than rejection. Dogs Nos. 4 and 5 showed increased survival over Nos. 1 to 3, although the degree of lymphopenia obtained was generally somewhat less striking and less consistent, that is, 2 to 4 percent lymphocytes on peripheral smear. Since all dogs were significantly lymphopenic, however, the differences in transplant survival may reflect differences in degree of histocompatibility between the donors and recipients. Dogs Nos. 6 and 7 are surviving at present beyond 350 days with normal renal function. Except for a brief period of slight elevation in blood urea nitrogen during the first 12 days after transplantation, consistent with recovery from ischemia associated with the surgical procedure, the dogs never showed an increase in the blood urea nitrogen and had no episodes of acute rejection. These two dogs had lymphopenia maintained at 0 to 2 percent (0 to 300 lymphocytes/mm$^3$) for the first 100 days by daily injections of the antiserum to dog lymphocytes, after which weekly doses of antiserum resulted in less pronounced lymphopenia (3 to 7 percent, 300 to 900 lymphocytes/mm$^3$). In spite of this, no deterioration of function has occurred, and the blood urea nitrogen has now been normal for another 250 days, with only weekly injections being given. Biopsy of kidneys of dogs Nos. 6 and 7 on day 107 and 116, respectively, showed normal renal parenchyma in both kidneys with no cell infiltration in one, and only a few cells apparent in the other (Fig. 2).

Table 1. Survival of dogs given daily subcutaneous injections of horse antiserum to lymphocytes, before and after transplantation of renewal allografts.

| Dog No. | Days treatment prior to graft | Days surviving after graft |
|---|---|---|
| 1 | 23 | 11 |
| 2 | 7 | 20 |
| 3 | 14 | 26 |
| 4 | 7 | 52 |
| 5 | 7 | 76 |
| 6 | 7 | 350* |
| 7 | 21 | 350* |

* Still surviving with normal blood urea nitrogen.

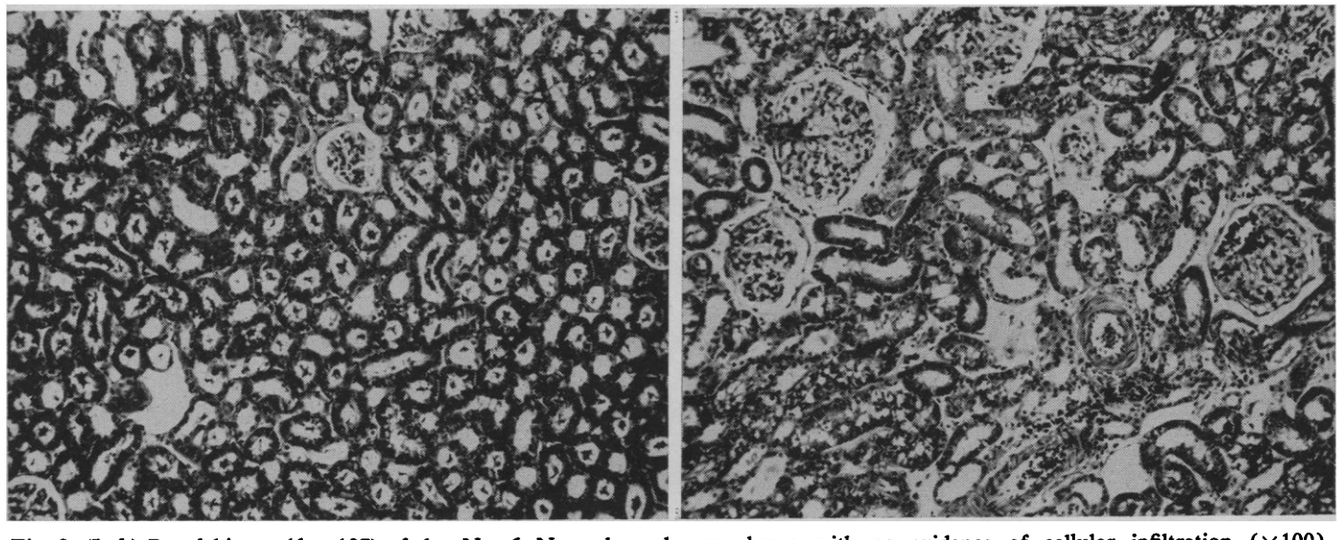

Fig. 2. (Left) Renal biopsy (day 107) of dog No. 6. Normal renal parenchyma with no evidence of cellular infiltration (×100). (Right) Renal biopsy (day 116) of dog No. 7. Normal renal parenchyma with exception of small area of minimal mononuclear cell infiltration (×140).

Limited examination of the lymphoid tissues of two of the dogs (Nos. 4 and 5) given the antiserum revealed striking alterations in the lymph nodes and spleen. Lymph nodes were definitely reduced in size. Marked depletion of both large and small lymphocytes was evident and germinal centers were totally absent. Few plasma cells were seen. Although lymphocyte depletion was striking, there was little evidence of tissue necrosis. Gross weights of the spleens of these dogs were markedly reduced, with lymphocyte depletion and reduction in white pulp apparent histologically. Depletion of Peyer's patches was only slight. The thymus glands were not examined. In spite of long-maintained lymphopenia and associated tissue lymphocyte depletion, susceptibility to infection was not apparently significantly increased by daily administration of the antiserum. Except for (i) a terminal pneumonia in dog No. 5, after a prolonged period of uremia secondary to chronic attenuated rejection, and (ii) the chronic interstitial nephritis secondary to ureteral obstruction in dog No. 3, no infections were encountered in dogs treated with antiserum.

Our experiments show that heterologous antiserum to lymphocytes is as potent a biological lymphopenic and immunosuppressive agent in dogs as it is in smaller animals. Previous workers have noted loss of serum effectiveness (as measured by lymphopenia) after short treatment with antiserum, presumably secondary to development in serum-treated animals of antibody to heterologous $\gamma$-globulin, leading to inactivation of the antibody to lymphocytes (*3*). We have found that chronic administration of heterologous antiserum to lymphocytes for up to 28 days in mice (*1*) is not necessarily associated with loss of serum effectiveness. Similar results in rats have been reported (*8*). The fact that antiserum to dog lymphocytes was administered for more than 350 days without loss of lymphopenic and immunosuppressive effects in our experiments is noteworthy. A likely reason for persistence of serum effectiveness is the fact that immunologically competent cells, which might otherwise have made antibody against the heterologous serum, are destroyed by contact with it. The heterologous antiserum to lymphocytes thus appears to potentiate its own biological effectiveness by preventing formation of neutralizing antibody, a finding previously suggested by us in studies of a similar serum in mice (*1*). Another explanation may be that repeated injections of antiserum to lymphocytes produce a state of immunological paralysis to the heterologous proteins, with subsequent failure to form neutralizing antibody (*9*). Serum from dogs chronically injected with the antiserum to lymphocytes is currently being examined by immunoelectrophoresis for the presence of excess circulating antigen (horse serum proteins), antibody, and antigen-antibody complexes. In this regard, no dogs showed any evidence of serum sickness from chronic administration of the antiserum.

Although long-term administration of the antiserum was well tolerated, experiments in small animals suggest that continued serum administration may not be necessary for continued survival of allogeneic transplants. Mice (*10*) and rats (*11*), thymectomized as adults prior to administration of heterologous antiserum to lymphocytes, recover much more slowly from the lymphopenic and immunosuppressive effects of antiserum to lymphocytes than unthymectomized, serum-treated animals. The effect of adult thymectomy in dogs prior to serum administration is as yet unknown, particularly in regard to survival of whole organ grafts. The effectiveness of chemotherapeutic agents and steroids in prolonging canine renal grafts is established, as is the increased effectiveness of such agents when employed in states of lymphocyte depletion with other allograft systems (*12*). A brief period of intensive treatment with the antiserum to lymphocytes may magnify the effectiveness of these drugs and decrease the doses required for maintenance of immune suppression. We have recently produced a stable state of specific tolerance and chimerism in mice by infusion of appropriate donor allogeneic cells during the period of profound lymphopenia induced by antiserum to lymphocytes and adult thymectomy (*13*). Thus, heterologous antiserum to lymphocytes may be useful in the production of specific tolerance and chimerism in large animals.

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## N-Cyclohexyl Linoleamide: Metabolism and Cholesterol-Lowering Effect in Rats

Abstract. *More than half of orally administered* N*-cyclohexyl linoleamide-carboxyl-*$C^{14}$ *was recovered from feces of rats, and 30 to 50 percent of the absorbed carbon-14 activity was excreted in urine.* N*-Cyclohexyl linoleamide had an inhibitory effect on the absorption of cholesterol from the thoracic duct and caused a decrease in the deposition of cholesterol in the livers of rats that had been fed cholesterol.*

In studying the effects of polyunsaturated fatty acid derivatives on cholesterol metabolism, we found that some amide derivatives of linoleic acid showed a remarkable cholesterol-lowering effect in experimental animals. Among those, *N*-cyclohexyl linoleamide [Linolexamide (*1*)] is one of the most effective, and its action is as follows (*2*): (i) Suppression of experimental atherosclerosis in rabbits fed 80 mg of *N*-cyclohexyl linoleamide per kilogram per day; (ii) decrease of serum cholesterol in mice, rats, and rabbits that had