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 γ-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

Table 1. Excretion of C^{14} in 24-hour period following oral administration of C^{14}-labeled *N*-cyclohexyl linoleamide and linoleic acid-1-C^{14} in rats. Test material was diluted with a suitable amount of the nonlabeled compound and given as a peanut oil solution, 2 ml (10^7 count/min) per kilogram of body weight. Results are expressed as the percentage recovery of administered C^{14} activity, means ± standard errors. Numbers in parentheses indicate number of animals used.

Dose (mg/kg)	Recovered from: Feces (%)	Urine (%)
	N-cyclohexyl linoleamide	
500 (3)	72.3 ± 1.2	10.5 ± 0.7
100 (4)	58.8 ± 1.7	18.5 ± 0.8
50 (4)	53.9 ± 7.7	25.2 ± 4.1
	Linoleic acid	
100 (4)	6.85 ± 0.03	9.4 ± 2.7

been fed cholesterol; and (iii) absence of other pharmacological effects. Comparison of these effects with those of linoleic acid suggested that the amide possesses some characteristics that are different from those of the acid (*2*). We now report that the fate of orally administered *N*-cyclohexyl linoleamide is different from that of linoleic acid and that its cholesterol-lowering action probably results from interference with the absorption of cholesterol in the intestine.

In preliminary studies, the amide linkage of *N*-cyclohexyl linoleamide was found to be quite stable to hydrolysis, in the ordinary chemical procedure. It was not hydrolyzed when it was suspended in simulated gastric or intestinal fluid (*3*) for several hours at 37°C, nor when incubated with intestinal contents of the rat. To examine the metabolism of this compound in animals, male Wistar rats, weighing 120 to 150 g, were administered, by a stomach tube, C^{14}-labeled *N*-cyclohexyl linoleamide prepared from linoleic acid-1-C^{14} and cyclohexylamine. Feces, urine, and respiratory CO_2 were collected for 24 hours, and the radioactivities were measured by a liquid scintillation counter. As shown in Table 1, more than 50 percent of the activity of administered C^{14} was recovered from the feces, by thin-layer chromatography, as unchanged *N*-cyclohexyl linoleamide (*4*), which indicated that about 30 to 50 percent of the administered compound was absorbed from the gastrointestinal tract at these dose levels. This amount of absorption was markedly less than the absorption of linoleic acid. Urinary excretion of C^{14} was about 10 to 25 percent of that administered, which corresponded to 30 to 50 percent of the absorbed amount, and it was more than that of linoleic acid. The C^{14}-labeled product in urine was a water-soluble, slightly acidic substance, not identified so far. Respiratory C^{14}-labeled CO_2 was determined by the method of Jeffay and Alvarez (*5*). At a dose level of 100 mg/kg, 4.5 percent of administered C^{14} was recovered from respiratory CO_2, and most of the C^{14} activity was expired within 12 hours after feeding.

Table 2. The effect of *N*-cyclohexyl linoleamide on cholesterol absorption from the thoracic duct lymph. Rats were given oral dose of C^{14}-labeled cholesterol [12.5 mg (4.5×10^5 count/min) per rat] suspended in 0.25 ml of peanut oil. *N*-Cyclohexyl linoleamide was dissolved in the same solution. C^{14} activity of 24-hour lymph lipids was analyzed. Values are means ± standard errors. Numbers in parentheses indicate number of animals used.

Dose (mg/rat)	C^{14} recovered (%)	Cholesterol esterified (%)
0 (5)	13.6 ± 3.4	69.8 ± 4.3
25 (4)	11.3 ± 2.4	53.3 ± 4.9
50 (5)	9.5 ± 1.9	43.6 ± 4.7

In the absorption studies we used male rats, weighing 200 to 250 g, with the thoracic duct lymph fistula (*6*). They were kept restrained in cages and supplied a diet of commercial pellets and 0.9-percent solution of sodium chloride for drinking. Lymph was collected for 24 hours following administration of test materials, and the lipids were isolated and separated by thin-layer chromatography (*4*). When 100 mg of C^{14}-labeled *N*-cyclohexyl linoleamide was administered per kilogram of body weight, the percentage recovery of C^{14} activity was as follows (mean of four rats ± standard error): lymph, 9.58 ± 0.86; feces, 41.2 ± 4.6; and urine, 8.6 ± 1.4. The excretion of the C^{14}-labeled product in urine indicated that a large part of this compound was absorbed through pathways other than the thoracic duct, because the lymph had been collected separately. In view of the fact that long-chain fatty acids are absorbed almost completely by way of the lymph (*7*), the partition of the amide between lymph and portal vein blood seems to be different from that of long-chain fatty acids.

When cholesterol-4-C^{14} was administered with 50 mg of *N*-cyclohexyl linoleamide to the rat, recovery of C^{14}-labeled cholesterol from the lymph was decreased to about 70 percent of the control, and the percentage of C^{14}-labeled cholesterol in esterified form was markedly lowered (Table 2). This effect is similar to that of plant sterols reported by Hernandez *et al.* (*8*).

Table 3. The effect of *N*-cyclohexyl linoleamide on the levels of liver lipids in rats. At the end of the 12th and 18th weeks of the feeding period, four rats of each group were killed. Group 1, cholesterol diet; group 2, cholesterol diet + 0.2 percent *N*-cyclohexyl linoleamide; group 3, cholesterol-free diet; and group 4, cholesterol-free diet + 0.2 percent *N*-cyclohexyl linoleamide. Results are given as percentage of fresh tissue, means ± standard errors.

Week	Total cholesterol (%)	Total lipids (%)
	Group 1	
12th	9.45 ± 1.31	21.23 ± 2.42
18th	9.74 ± 0.98	18.44 ± 1.53
	Group 2	
12th	4.55 ± 1.27*	11.52 ± 1.79*
18th	4.76 ± 1.25*	11.31 ± 0.98*
	Group 3	
12th	0.28 ± 0.02	4.99 ± 0.33
18th	0.27 ± 0.01	4.58 ± 0.17
	Group 4	
12th	0.28 ± 0.05	5.10 ± 0.18
18th	0.22 ± 0.03	4.44 ± 0.18

* Significantly different from group 1 ($P < 0.05$).

Table 3 shows that addition of 0.2 percent of *N*-cyclohexyl linoleamide to the cholesterol diet significantly lowered levels of cholesterol as well as those of total lipids of the liver. But the addition of the compound to the cholesterol-free diet did not show any significant effect. In this experiment, 5-week-old male Wistar rats were housed in mesh-bottomed individual cages and received weighed amounts of the diet (*9*) so that the daily intake of each rat in groups 1 and 2 was the same and that of groups 3 and 4 was the same. Throughout the experimental period, both groups treated with *N*-cyclohexyl linoleamide showed almost the same gain in body weight as the pair-fed controls. At the end of the 12th and 18th weeks the animals were decapitated and the liver lipids were extracted. Total cholesterol was determined by the method of Herrmann (*10*), and total lipids were estimated gravimetrically. Since in the rat the deposition of excess cholesterol in the liver is considered a sensitive indicator of cholesterol absorption (*11*), the decrease of cholesterol deposition can be regarded as being due to the action on cholesterol absorption.

These findings suggest that the effect of *N*-cyclohexyl linoleamide is very similar to that of plant sterols and the brain extracts or phrenosine (*12*). Since the latter substance has a fatty acid amide linkage in the molecule, structural analogy can also be considered.

In comparing these actions, however, the effective dose of *N*-cyclohexyl linoleamide seems to be considerably smaller than that of the other substances.

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Chloroplast DNA from Tobacco Leaves

Abstract. *DNA from tobacco leaf chloroplasts was isolated as a single component with a buoyant density in CsCl of 1.702 compared to 1.697 for nuclear DNA. 5-Methylcytosine is present in nuclear DNA but absent in chloroplast DNA. Chloroplast DNA, with a guanine-cytosine content of 43 percent, has a melting temperature of 86°C and renatures completely on slow cooling, whereas nuclear DNA (melting temperature, 84°C; guanine-cytosine content, 40 percent) does not renature. About 9 percent of the total DNA in tobacco leaves is chloroplast DNA representing about* 4.7×10^{-15} *gram of DNA per chloroplast with a molecular weight of approximately* 4×10^{7}.

In a number of organisms, extranuclear (or satellite) DNA's differing in buoyant density from DNA obtained from nuclei have been found to be localized in two cytoplasmic particles, chloroplasts and mitochondria (*1*). Lyttleton and Peterson (*2*) concluded that in tobacco leaves, chloroplast DNA, if present, must be very similar to nuclear DNA in density, whereas Shipp *et al.* (*3*) reported chloroplast DNA of density 1.703 compared to nuclear DNA of density 1.690. However, Green and Gordon (*4*), using precautions to eliminate the possibility of a contribution of DNA from a bacterial contamination, have obtained a density of 1.696 for nuclear DNA and 1.706 for chloroplast DNA in tobacco seedlings. Satellite DNA's isolated from chloroplasts and mitochondria have been associated with variable amounts of nuclear DNA. Thus, the possibility of a second satellite component with a base composition virtually indistinguishable from that of nuclear DNA could not be ruled out. We now report the isolation of highly polymerized chloroplast DNA as a single component from purified chloroplast particles. Some of the properties which distinguish chloroplast DNA from nuclear DNA are also presented.

Lamina tissue of *Nicotiana tabacum* or *N. glutinosa* from leaves 5 to 7 cm in length was chopped by razor blades in Honda medium as described previously (*5*) in order to preserve the biphasic structure of chloroplasts in a recognizable condition. This homogenizing procedure also preserves nuclei in a condition closely resembling the in vivo state. After the homogenate was filtered through four layers of fine mesh cloth, the filtrate was centrifuged at 1000*g* for 15 minutes. The pellet of chloroplasts and nuclei corresponding to 15 g of fresh leaves was resuspended in 2 ml of homogenizing medium and then layered on a 25-ml discontinuous gradient of 60 (10 ml), 45 (10 ml), and 20 percent (5 ml) sucrose in Honda medium. The gradients were centrifuged for 2 hours at 25,000 rev/min in a Spinco SW 25-1 rotor, and the green-layer band at 45 percent sucrose was collected. Examination of this preparation with the phase microscope revealed well-preserved chloroplasts. When the preparation was stained with acridine orange and again examined by fluorescence microscopy, no intact or large fragments of nuclei could be detected. The pellet at the bottom of the tube consisted mainly of nuclei but was still contaminated with some unbroken cells and chloroplasts. This pellet served as the source of nuclear DNA without attempting further purification of the nuclei.

DNA was extracted from the original 1000*g* pellet of chloroplasts and nuclei; it was also extracted from the purified chloroplasts and from the enriched nuclear pellet. Each preparation was subjected to phenol extraction in the presence of 2 percent lauryl sulfate. After three phenol extractions, the aqueous phase was dialyzed against SSC (0.15*M* NaCl, 0.015*M* sodium citrate), treated with ribonuclease (50 μg/ml) for 2 to 3 hours, again extracted with phenol; the DNA was then precipitated with alcohol. At this stage DNA fibers were formed on a glass rod from all preparations except those of the purified chloroplasts where small amounts of DNA were collected by centrifugation. All three DNA preparations were then extensively dialyzed against SSC.

The buoyant density determined according to Schildkraut *et al.* (*6*) for nuclear DNA was 1.697 and 1.702 for chloroplast DNA, values resembling those of Green and Gordon (*4*). The density of nuclear DNA also agrees closely with the value of 1.698 reported by Lyttleton and Peterson (*2*). Density of spinach chloroplast DNA's differs from that of tobacco chloroplast DNA, the density of the former being 1.719 and 1.705 (*1*). The densitometer tracings of the ultraviolet photographs of tobacco leaf DNA's are presented in Fig. 1. The difference of