values at 0 μg/ml were: female, 1.340 ± 0.008; male, 1.231 ± 0.024. At 5 μg/ml the values were: female, 1.359 ± 0.010; male, 1.234 ± 0.046. At 10 μg/ml, they were: female, 1.301 ± 0.015; male, 1.135 ± 0.019 (N = 15 for each sex and concentration). The single individual of *D. melanogaster* P2 that emerged at 5 μg/ml was not measured. For each species and sex, a one-way analysis of variance was used to assess the significance of size variation of flies reared at different amanitin concentrations. One-way analysis of variance was also used to detect significant variation in development time as a function of amanitin concentration.

10. The difference between treatments in the sex ratios was highly significant (χ^2 = 13.8; d.f. = 1; $P < .001$). Based on 133 flies raised at 0 μg per milliliter of amanitin and 37 flies at 5 μg/ml.
11. The survival of *D. putrida* was actually greater when amanitin was added at 50 μg/ml than when the lower concentrations were used. But since this difference is significant at only the $P = .05$ level (Student-Newman-Keuls test), we refrain from speculating on the possible causes of this finding.
12. $P < .01$ by a Student-Newman-Keuls test.
13. A. H. Sturtevant, *The North American Species of* Drosophila (Carnegie Institution of Washington, Washington, D.C., 1921).
14. J. P. Phillips, J. Willms, A. Pitt, *Can. J. Genet. Cytol.* **24**, 151 (1982). The LD_{50}'s for these strains were 10, 30, and 35 μg/ml of α-amanitin in the larval medium.
15. α-Amanitin has been detected in other, nonpoisonous species of mushrooms but at extremely low, presumably innocuous, levels [H. Faulstich and M. Cochet-Meilhac, *FEBS Lett.* **64**, 73 (1976)].
16. We thank A. Ben-Yishay for help with the rearing experiments and P. Feeny, F. J. Kull, R. C. Lewontin, R. K. Selander, and D. M. Simons for helpful comments on the manuscript. Supported by NSF grant DEB80-08574 (to J.J.) and NIH grant GM 28078 (to A.L.G.).

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Combined Lipase Deficiency (*cld*): A Lethal Mutation on Chromosome 17 of the Mouse

Abstract. *Two triglyceride lipases, lipoprotein lipase and hepatic triglyceride lipase, participate in the metabolism of plasma lipoproteins. A single recessive mutation,* cld, *on mouse chromosome 17 causes an apparent deficiency of both lipoprotein lipase and hepatic triglyceride lipase activities. Mice homozygous for this defect develop lethal hyperchylomicronemia within 2 days postpartum as a consequence of nursing. Plasma triglyceride values in affected mice often reach 20,000 milligrams per deciliter (100 times higher than that in normal littermates), and total lipase activity in plasma or tissues is 5 to 20 percent of that in controls.*

The clearance of chylomicrons and very low density lipoproteins (VLDL) is dependent on an initial interaction with the enzyme lipoprotein lipase (LPL). This triglyceride hydrolase is present at the capillary endothelium of most extrahepatic tissues (for example, heart, muscle, adipose tissue) (*1*). A second lipase, hepatic triglyceride lipase (HTGL), is probably confined to the capillary endothelium of the liver, and recent studies suggest its involvement in the final conversion of VLDL to low-density lipoproteins (LDL) (*2*). The metabolism of high-density lipoproteins (HDL) may also be affected by this enzyme (*3*). Both LPL and HTGL are released into plasma by the intravenous injection of heparin (postheparin plasma).

Deficiency of LPL activity is a rare familial trait in humans (*4*). This recessive genetic disorder produces a hypertriglyceridemia that is expressed primarily as a buildup of chylomicrons after consumption of a fatty meal. Although, when uncontrolled, severe hyperchylomicronemia can produce pancreatitis in humans, this complication is effectively managed by restricting dietary fat. The same clinical syndrome results from genetic deficiency of the activator of LPL, apoprotein C II (*5*). In both disorders, HTGL activity is present in plasma at normal or reduced levels after heparin injection. Deficiency of HTGL activity was described in a single family with an associated accumulation of intermediate-density lipoproteins (IDL) and lighter HDL (HDL_2) (*6*). No disorder resulting in deficiency of both enzyme activities has been described in humans. There are no reports of a primary deficiency of either LPL or HTGL activity in laboratory animals.

We now report on a mouse autosomal recessive mutation, named combined lipase deficiency (*cld*), which causes a deficiency of both LPL and HTGL activities. The *cld* mutation was extracted from a chromosome bearing mutations at the *T/t* complex of mouse chromosome 17 (*7*). The original *t* haplotype was characterized as carrying t^{w73}, a recessive embryonic lethal acting in homozygotes at 5 days of gestation (*8*). Any chromosome carrying one recessive lethal could carry undetected numerous other later-acting lethals with no further genetic detriment. Genetic dissection of *t* chromosomes by recombination analysis has separated the different component genes carried by complete *t* haplotypes (*9*). In the chromosome bearing t^{w73}, a parasitic lethal gene, *cld*, acting postnatally, was uncovered (Fig. 1). The two relevant *cld* phenotypes can be distinguished immediately by inspection of the tail phenotype because of the close linkage of the tail mutation and *cld* (94 percent co-inheritance of the two traits through the female and 98 percent through the male). Such a distinction can be made with confidence even on animals delivered by cesarean section.

Although apparently normal at birth, tailless (*cld/cld*) animals show decreasing mobility, poor weight gain, paleness, and progressive cyanosis leading to death between 36 and 48 hours postpartum. Gross examination of tissue reveals small hemorrhages in the heart, lungs, and liver, suggesting diffuse microinfarction. Plasma glucose levels taken during the first 48 hours postpartum are within normal limits (40 to 80 mg/dl) in both normal and affected mice, indicating that the mice are not diabetic. Hypertriglyceridemia is not present in the affected mice examined in utero, and cholesterol levels are comparable to those in normals (Fig. 2). During the first 2 days, a progressive rise in plasma triglycerides occurs. The serum becomes opaque, and triglyceride concentration is often more than 20,000 mg/dl by the second day postpartum. Cholesterol values also rise from approximately 60 mg/dl in utero to more than 450 mg/dl after 48 hours (Fig. 2). Sudan black staining of plasma lipoproteins separated by electrophoresis in 1 percent agarose (*10*) shows chylomicron accumulation in plasma of affected mice. A pre–β-lipoprotein (VLDL) band is less discrete, and the α-lipoprotein (HDL) band shows reduced staining in comparison with that of normal animals. β-Lipoprotein (LDL) is virtually undetectable in affected and unaffected mice. It seems highly probable that the extreme increase in chylomicrons results in coalescence of particles and obstruction of capillary fields in vital organs.

We assayed endothelial bound lipase activity released into the plasma by heparin, as well as liver and heart tissue triglyceride lipases. The lipolytic activity of postheparin plasma and tissue homogenates in the affected animal was 5 to 20 percent of control activity for both LPL and HTGL. The liver homogenate of normal neonatal mice showed an increase in activity with the addition of serum, a property normally ascribed to LPL. Total liver lipolytic activity was reduced by 1.5M sodium chloride or protamine sulfate (data not shown). This is compatible with previous reports of an activity similar to LPL in addition to HTGL activity in the neonatal rat liver (*11*). The decreased lipase activity observed in *cld/cld* mice is not the result of an inhibitor present either in the plasma or in tissue homogenates, because addition of an equal volume of heart or liver homogenate from an affected mouse did not reduce the activity of homogenates from normal mice. Some inhibition of lipolytic activity (20 to 25 percent) was seen when an equal volume of post-

Table 1. Triglyceride lipase values in normal and affected mice. Organs were removed and homogenized at 0°C in 5 volumes of 10 m*M* sodium phosphate, 0.15*M* sodium chloride, *p*H 7.4, containing sodium heparin (10 U/ml; Lipohepin, Riker). Plasma was collected 5 minutes after intravenous injection of heparin (0.1 U per gram of body weight). The radiochemical triglyceride emulsion system of Baginsky and Brown (*19*) was used to assay homogenate supernatants and postheparin plasma were assayed for LPL activity, HTGL activity, or total postheparin lipolytic activity (PHLA), with normal mouse serum or pooled human serum as the source of apoprotein C II. Sample means were compared with a two-tailed *t*-test for which a normal distribution was assumed. Abbreviations: LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; PHLA, postheparin lipolytic activity; and N.D., not determined.

Source	Prenatal				At 48 hours			
	N	Normal	Affected	*P*	*N*	Normal	Affected	*P*
Heart (LPL)*	4	0.96 ± 0.17	0.22 ± 0.19	< .01	5	2.4 ± 0.72	0.45 ± 0.14	< .01
Liver (HTGL)*	4	0.40 ± 0.10	0.14 ± 0.10	< .01	6	0.49 ± 0.08	0.10 ± 0.10	< .01
Carcass (PHLA)*		N.D.	N.D.		4	21.7 ± 4.2	1.4 ± 0.2	< .01
Plasma (PHLA)†		N.D.	N.D.		6	6.4	0.55	< .01

*Measured in micromoles of free fatty acid released per hour per milligram of protein. †Measured as micromoles of free fatty acid released per hour per milliliter of plasma.

heparin plasma from affected mice was added. However, this is no greater than that expected to result from dilution of the substrate by the large amounts of triglyceride-rich lipoproteins present in affected plasma. The enzyme activity detected in small amounts in the tissues of the affected mice represents either residual LPL and HTGL activity or the presence of other intracellular lipases in the liver and the heart. This activity was not increased by the addition of serum (not shown).

No evidence of a gene dosage effect was observed in the activity levels of LPL or HTGL (data not shown). The plasma triglyceride and cholesterol levels of wild-type and +/*cld* heterozygote weanling littermates were virtually identical (mean ± standard deviation for triglyceride, 80 ± 18; mg/dl and for cholesterol 59 ± 10 mg/dl; $N = 13$). Affected animals survived the gestational period in good health, as evidenced by the fact that litter size was comparable to that seen in control litters (+/*cld* × +/+). As discussed above, prenatal levels of triglyceride and cholesterol were not increased in affected mice, even though their LPL and HTGL activities were deficient in utero (Table 1). In addition, tissue LPL levels were depressed prenatally in the unaffected mice when compared with values at 48 hours postpartum. These data indicate that, in the mouse, this lipolytic system is probably not required to provide substrates for cholesterol and triglyceride metabolism during the late prenatal period.

The severe deficiency of both LPL and HTGL activity appears to result from a single autosomal recessive mutation, although a deletion of two closely linked genes cannot be excluded. Various defects can explain the simultaneous deficiency in activity of these two triglyceride lipases. The synthesis of each enzyme is thought to occur in parenchymal cells; hepatocytes for HTGL and adipocytes, myocytes, and macrophages for LPL (*12, 13*). Both enzymes are glycosylated before being secreted from the cell, and evidence has been presented that an energy-requiring activation precedes secretion of LPL (*14*). After leaving the parenchymal cell, the enzymes bind to the capillary endothelium at sites containing glycosaminoglycans, probably heparan sulfate (*15*). Although this binding site is not thought to be specific for the enzyme, a defect at this site could explain marked reduction in enzyme activity. Alternatively, it seems probable that the deficiency in these animals relates to a defect occurring before secretion from the cell, such as a primary sequence abnormality or some essential posttranslational modification common to both enzymes. Previous work on these lipases purified from human postheparin plasma has shown marked similarities in their amino acid compositions, molecular weights, and peptide maps (*16*). However, other investigators have reported differences in the amino acid content of the purified proteins, and antibodies to the two enzymes do not cross-react (*17*). Thus the relationship between the two enzymes is unclear. A defect may exist in a common posttranslational modification, such as glycosylation. Other mutations within

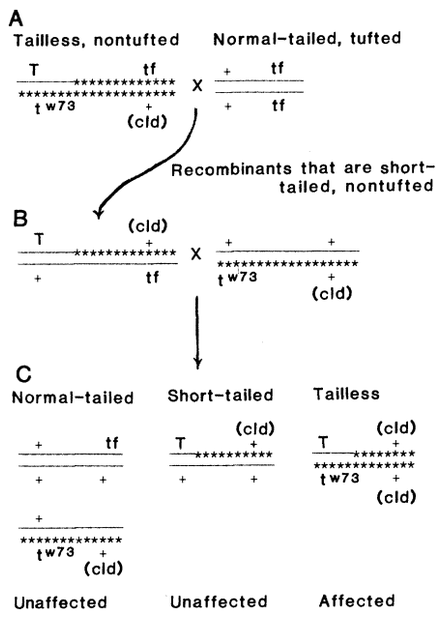

Fig. 1. Genetic extraction and detection of *cld*. The t^{w73} chromosome contains t^T (not indicated), the tail interaction factor, formally allelic to *T* and causing taillessness in the *T*/t^{w73} heterozygote; t^{w73}, the early embryonic lethal gene close to t^T; and *cld*, either very close to tufted on the proximal side or distal. *T* causes a short tail when heterozygous with wild type (+); tufted (*tf*) is a recessive marker causing a distinctive hair growth pattern in homozygotes. Stars designate *t*-derived chromatin [see (*9*) for explanation]. (A) The cross to detect recombinants. (B) Sixteen different short-tailed, nontufted recombinants between *T* and *tf* were selected and mated to mice with the original t^{w73} chromosome. The progeny, indicated in (C), include two genotypes of normal-tailed mice, short-tailed normal mice, and tailless *cld* homozygous mice affected with hyperchylomicronemia. The mice used in experiments were the littermates indicated in (C).

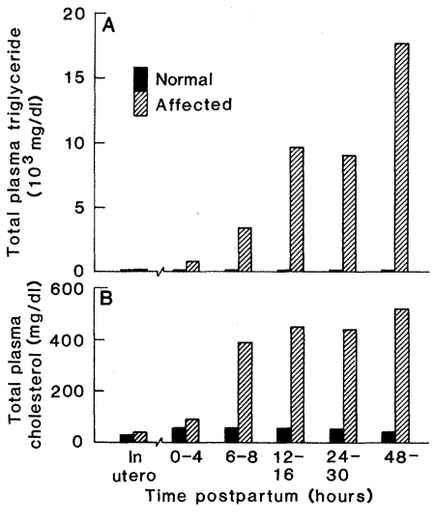

Fig. 2. Plasma triglyceride and cholesterol values during the perinatal period. Mouse blood is anticoagulated with EDTA (1 mg/ml) before separation of plasma and determination of triglyceride and cholesterol values (Biochromic Analyzer ABA-100; Abbott). All data are the means of at least six samples. The differences between the average triglyceride levels of normal and affected mice are significant ($P < .01$) at all times postpartum.

the *t* complex, such as t^{12}, affect the glycosylation of cell surface glycoproteins (*18*). Elucidation of the molecular defect in the *cld* mouse should contribute greatly to our understanding of the synthesis and relationship between these two enzymes.

The *cld* mutation may prove to be an important model for studying the clearance of plasma triglycerides since both enzymes are involved in their metabolism. The effect of the absence of either activity, as in the human syndromes, is probably modulated by the presence of the alternate pathway. In the mouse, when both enzymes are deficient, dietary fat is a lethal insult.

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References and Notes

1. J. Augustin and H. Greten, in *Atherosclerosis Reviews*, R. Paoletti and A. M. Gotto, Jr., Eds. (Raven, New York, 1979), vol. 5, p. 91.
2. I. J. Goldberg, N. A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, W. V. Brown, *J. Clin. Invest.* **70**, 1184 (1982).
3. T. Kussi, P. K. J. Kinnunen, E. A. Nikkila, *FEBS Lett.* **104**, 384 (1979); H. Jansen, A. Van Tol, W. C. Hulsmann, *Biochem. Biophys. Res. Commun.* **92**, 53 (1980); J. O. Grossen, O. Schrecker, H. Greten, *J. Lipid Res.* **22**, 437 (1981).
4. D. S. Fredrickson, J. L. Goldstein, M. S. Brown, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, *et al.*, Eds. (McGraw-Hill, New York, ed. 4, 1978), p. 604.
5. W. C. Breckenridge, J. A. Little, G. Steiner, A. Chow, M. Poapst, *N. Engl. J. Med.* **298**, 1265 (1978).
6. W. C. Breckenridge *et al.*, *Atherosclerosis*, in press.
7. D. Bennett, *Cell* **6**, 441 (1975).
8. M. Spiegelman, K. Artzt, D. Bennett, *J. Embryol. Exp. Morphol.* **36**, 373 (1976); B. Babiarz, J. Garrisi, D. Bennett, *Genet. Res.* **39**, 111 (1982).
9. K. Artzt, P. McCormick, D. Bennett, *Cell* **28**, 463 (1982).
10. R. P. Noble, *J. Lipid Res.* **9**, 693 (1968).
11. M. Llobera, A. Montes, E. Herrera, *Biochem. Biophys. Res. Commun.* **91**, 272 (1979).
12. G. S. Sundaram, K. M. M. Shakir, G. Barnes, S. Margolis, *J. Biol. Chem.* **253**, 7703 (1978).
13. M. Rodbell, *ibid.* **239**, 753 (1964); T. Chajek, O. Stein, Y. Stein, *Biochim. Biophys. Acta* **528**, 456 (1978); P. Wang-Iverson, A. Ungar, P. Bukberg, J. C. Gibson, W. V. Brown, *Biochem. Biophys. Res. Commun.* **104**, 923 (1982).
14. J. E. Stewart and M. C. Schotz, *J. Biol. Chem.* **249**, 904 (1974).
15. G. Bengtsson, T. Olivecrona, M. Hook, J. Riesenfeld, U. Lindahl, *Biochem. J.* **189**, 625 (1980); C. F. Cheng, G. M. Oosta, A. Bensadoun, R. Rosenberg, *J. Biol. Chem.* **256**, 12893 (1981).
16. J. Augustin, H. Freeze, P. Tejada, W. V. Brown, *J. Biol. Chem.* **253**, 2912 (1978).
17. A-M. Ostlund-Linqvist, *Biochem. J.* **179**, 555 (1979).
18. B. D. Shur, *Genet. Res.* **38**, 225 (1981); C. C. Cheng and D. Bennett, *Cell* **19**, 537 (1980).
19. M. L. Baginsky and W. V. Brown, *J. Lipid Res.* **20**, 548 (1979).
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Direct in vivo Monitoring of Dopamine Released from Two Striatal Compartments in the Rat

Abstract. *Microvoltammetric electrodes were used to monitor dopamine released in the caudate nucleus of the rat after electrical stimulation of the medial forebrain bundle. The time resolution of the technique is sufficient to determine in vivo concentration changes on a time scale of seconds. Direct evidence identifying the substance released as dopamine was obtained both voltammetrically and pharmacologically. Administration of α-methyl-*p*-tyrosine terminates the release of dopamine, although tissue stores of dopamine are still present. Thus there appears to be a compartment for dopamine storage that is not available for immediate release. This compartment appears to be mobilized by amfonelic acid, since administration of this agent after α-methyl-*p*-tyrosine returns the concentration of dopamine released by electrical stimulation to 75 percent of the original amount.*

The understanding of the dynamics of neurotransmitter interactions in mammalian brain requires in vivo chemical sensors that are not perturbational (*1*). With the use of direct chemical measurements it is possible to circumvent the assumptions that are necessary with the use of labeled compounds or in vitro methods, and such direct methods should give a much more realistic measure of the important factors that regulate neurotransmission. In vivo voltammetry with microvoltammetric electrodes (~ 20 μm total diameter) is one approach to this ideal since they show chemical specificity for dopamine, with a response time to concentration changes limited primarily by diffusion through brain tissue (*2–4*). These electrodes, when used to oxidize dopamine, give rise to voltammograms that are unique for catecholamines (*3*). In contrast, voltammograms for 5-hydroxytryptamine and its metabolites and metabolites of dopamine, ascorbic acid, and uric acid, all of which are also easily oxidized in mammalian brain, are poorly defined. This difference in definition is advantageous, since direct observations of the neurotransmitter dopamine can be made without interference, as long as the concentration of this compound in extracellular fluid exceeds ~ 5 μ*M*. Dayton *et al.* have shown that these electrodes respond in a relatively rapid fashion to changes in concentration in vivo (*4*). We demonstrate here that electrically stimulated dopamine release is easily monitored, that the release is dependent on the availability of dopamine and on its storage compartments, and that these factors can be altered by pharmacological agents.

In vivo electrochemical measurements were made in the caudate nucleus of male Sprague-Dawley rats anesthetized with chloral hydrate and maintained as described by Dayton *et al.* (*4*). As we and others have noted, dopamine concentrations in extracellular fluid of the rat caudate are sufficiently low that they are obscured by the other easily oxidized substances in the brain (*2, 5*). To in-

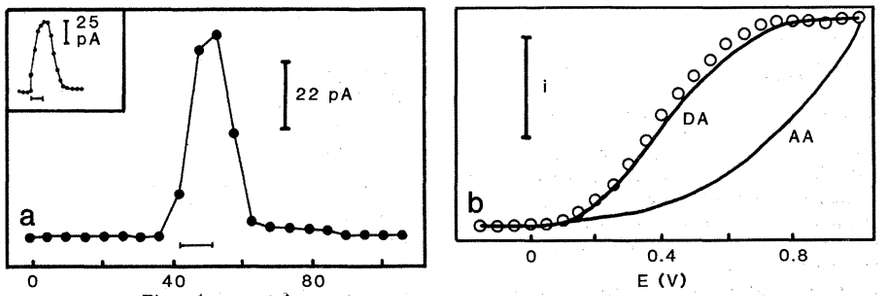

Fig. 1. Oxidation current from a microvoltammetric electrode placed in the caudate nucleus (2.4 mm anterior to bregma, 2.4 mm lateral to the midline, 4.0 mm below the dura) during a 10-second, 130-μA, 60-Hz stimulation (horizontal bar) of the ipsilateral medial forebrain bundle. (a) Chronoamperometric current versus time at 0.5 V; 6 seconds between measurements. Inset: Chronoamperometric current versus time for an identical experiment with 2-second intervals between measured points. (b) Difference normal pulse voltammogram, the voltammogram obtained by subtracting a voltammogram before stimulation from that at the peak (circles), compared to voltammograms obtained for dopamine (*DA*) and ascorbic acid (*AA*) in vitro after the experiment (solid lines). Ascorbic acid is used for comparison because it is the predominant easily oxidized substance present in extracellular fluid of the caudate. Current (*i*) scales for the voltammograms were as follows: i = 16 pA for 25 μ*M* dopamine, i = 28 pA for 200 μ*M* ascorbic acid, and i = 32 pA for stimulation. The average change in the extracellular dopamine concentration after stimulation was 35.5 ± 3.40 μ*M* (N = 35), as determined with postcalibration data. In all experiments the reference electrode was a saturated calomel electrode.