A question arises as to whether some of the inhibitory action of manganese is due to its blocking effect on the action potential so that it cannot reach the terminal (*2*). If this is the case, then the number of trials with no response to stimulation should be larger than the number of these failures predicted by the Poisson theorem (*9, 10*). This point was checked in ten experiments and an agreement was found between the expected and the observed number of failures. For example, when a preparation was bathed in a Ringer solution containing 50 $\mu M$ $Mn^{2+}$, 0.7 m$M$ $Ca^{2+}$, and 1.0 m$M$ $Mg^{2+}$, the mean amplitude of the end-plate potentials, on 195 trials, was 0.76 mv, and the mean amplitude of the miniature end-plate potentials was 0.35 mv, giving a quantal content of 2.17. The expected number of failures was 22.3 ($195e^{-2.17}$), while the observed number was 23.

The magnitude of the inhibiting action of $Mn^{2+}$ ions depends on the concentration of $Ca^{2+}$ in the extracellular medium; at higher $Ca^{2+}$ concentrations, more manganese is needed to achieve the same fractional inhibition. This observation suggested that $Mn^{2+}$ and $Ca^{2+}$ may be competing for a common site on the presynaptic membrane. This point was further investigated by obtaining relations between $Ca^{2+}$ concentrations and quantal content of transmitter at two different concentrations of $Mn^{2+}$. The modified Lineweaver-Burk plots [see (5)] had a common intercept, indicating that the inhibition is competitive in nature. Thus, the action of $Mn^{2+}$ on transmitter liberation resembles that of magnesium (*11*). The main difference between these two ions is their potency, manganese being at least 20 times more potent on a molar basis. The qualitative similarity between $Mn^{2+}$ and $Mg^{2+}$ at the frog neuromuscular junction is not surprising in view of their resemblance in some physiochemical properties and their mutual replacement in a number of processes (*12, 13*).

The concentration of manganese in human extracellular fluid is estimated to be on the order of 1.0 $\mu M$ (*13*), and, if the sensitivity of the various human synapses is similar to that of the frog neuromuscular junction, it is unlikely that under normal circumstances a significant fraction of transmitter release is inhibited by manganese. Recently it has been suggested that changes in manganese concentration in body fluids are associated with some neurological disorders (*15*). It would be of interest to see whether synapses exist, with high sensitivity to manganese ions, in the central nervous system.

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### References and Notes

1. A. W. Cuthbert, Ed., *Calcium and Cellular Function* (Macmillan, London, 1970), p. 450.
2. P. Fatt and B. D. Ginsborg, *J. Physiol.* **142**, 516 (1958); R. K. Orkand, *ibid.* **164**, 103 (1962); S. Hagiwara and S. Nakajima, *Science* **149**, 1254 (1965); *J. Gen. Physiol.* **49**, 793 (1966); Y. Hashimoto and M. E. Holman, *Aust. J. Exp. Biol. Med. Sci.* **45**, 533 (1967); K. Takeda, *J. Gen. Physiol.* **50**, 1049 (1967); M. Kleinfeld and E. Stein, *Amer. J. Physiol.* **215**, 593 (1968); E. Büibring and T. Tomita, *Proc. Roy. Soc. Ser. B.* **172**, 121 (1969).
3. P. F. Baker, A. L. Hodgkin, E. B. Ridgeway, *J. Physiol.* **208**, 80P (1970); *ibid.* **218**, 709 (1971).
4. B. Katz and R. Miledi, *Proc. Roy. Soc. Ser. B* **167**, 8 (1967); *J. Physiol.* **192**, 407 (1967); *ibid.* **203**, 459 (1969).
5. P. A. Dodge, Jr., and R. Rahamimoff, *J. Physiol.* **193**, 419 (1967).
6. J. S. Kelly, *Nature* **205**, 296 (1965); R. I. Birks and M. W. Cohen, in *Muscle*, W. M. Paul, E. D. Daniel, C. M. Kay, G. Monckton, Eds. (Pergamon, Oxford, 1965), p. 403; R. Rahamimoff and F. Colomo, *Nature* **215**, 1174 (1967).
7. P. W. Gage and R. S. Eisenberg, *J. Gen. Physiol.* **53**, 298 (1969).
8. J. del Castillo and L. Stark, *J. Physiol.* **116**, 507 (1952); B. Katz and R. Miledi, *Proc. Roy. Soc. Ser. B* **161**, 496 (1965).
9. J. del Castillo and B. Katz, *J. Physiol.* **124**, 560 (1954).
10. K. Krnjevic and R. Miledi, *ibid.* **149**, 1 (1969).
11. J. del Castillo and L. Engbaek, *ibid.* **124**, 370 (1954); J. del Castillo and B. Katz, *ibid.*, p. 553; D. H. Jenkinson, *ibid.* **138**, 434 (1957).
12. P. D. Boyer, H. Lardy, K. Myrebäck, Eds., *The Enzymes* (Academic Press, New York, ed. 2, 1959).
13. K. Kostial and Z. Juričić, *Arh. Hig. Rada* **7**, 27m (1956); S. M. Kirpekar, W. Dixon, J. C. Prat, *J. Pharmacol. Exp. Ther.* **174**, 72 (1970); S. P. Arpil and J. P. Reuben, *Fed. Proc.* **30**, 557 (1971).
14. H. J. M. Bowen, *J. Nucl. Energy* **3**, 18 (1956); H. Fore and R. A. Morton, *Biochem. J.* **51**, 600 (1952); H. A. Schroeder, *Advan. Intern. Med.* **8**, 254 (1956); E. M. Butt, R. E. Nusbaum, T. C. Gilmour, S. L. Didio, Sister Mariano, *Arch. Environ. Health* **8**, 60 (1964); D. A. Olehy, R. A. Schmitt, W. F. Bethard, *J. Nucl. Med.* **7**, 917 (1966); M. L. N. Lubol, K. Chaywatanangkur, S. Kritalugsana, *ibid.* **9**, 178 (1968).
15. I. Mena, C. Marin, S. Fuenzalida, G. Cotzias, *Neurology* **17**, 128 (1967); G. Cotzias, K. Horiuchi, S. Fuenzalida, I. Mena, *ibid.* **18**, 376 (1968); P. S. Papavazilian, S. T. Miller, G. Cotzias, *Nature* **220**, 74 (1968); I. Mena, K. Horiuchi, K. Burke, G. Cotzias, *Neurology* **19**, 1000 (1969).
16. Supported in part by the S. Lunenfeld and R. Kunin Medical Research Foundation.

23 September 1971; revised 8 December 1971 ■

## Deficient Activity of Hepatic Acid Lipase in Cholesterol Ester Storage Disease

Abstract. *Absence of lysosomal acid lipase activity in the liver is described in cholesterol ester storage disease and Wolman's disease. This enzyme deficiency may result in the excess hepatic cholesterol ester found in both conditions. However, clinical, genetic, and histopathologic differences suggest that the two conditions are separate diseases not completely explained by deficient enzyme activity.*

Cholesterol ester storage disease is a rare familial disorder of lipid metabolism characterized by asymptomatic hepatomegaly, normal liver function studies, and hypercholesteremia (*1–3*). The liver contains cholesterol esters at 100 to 500 times the normal concentration (*1, 4*). Neutral cholesterol esterase activity is normal (*3*). The ultrastructural appearance of hepatocytes indicates excessive lipid in membrane bound vacuoles (presumably abnormal lysosomes) (*2, 3*). We now describe deficiency of hepatic acid lipase (a lysosomal enzyme) activity in a previously reported patient (*3*). Liver biopsy for the determination of acid lipase activity was performed when the patient was 20 years of age. Total serum cholesterol was 275 mg per 100 ml of serum with 210 mg/100 ml as cholesterol ester.

Mahadevan and Tappel described a lysosomal acid lipase in rat liver and kidney with optimum activity at *p*H 4.2 (*5*). Using similar assay methods, Patrick and Lake reported lipolytic activity in human liver and spleen with optimum activity at *p*H 4.6 (*6*). More recent histochemical and ultrastructural studies provide evidence that human hepatic acid lipase is located in the lysosome (*7*).

Hepatic tissue was weighed, frozen on solid $CO_2$, and stored at −20°C or −75°C for less than 12 months before assay. Post-mortem specimens were obtained within 6 hours of death. Nitrogen was determined by the microkjeldahl method (*8*). The assay method for acid lipase was similar to that used by Patrick and Lake (*6*) (Table 1).

These findings indicate deficient activity of hepatic acid lipase in cholesterol ester storage disease. Patrick and Lake demonstrated a similar enzyme deficiency in Wolman's disease (*6*), an observation confirmed by our data. Activities of other lysosomal enzymes (that is, of β-galactosidase and of total hexo-

Table 1. Acid lipase activity of liver. The assay system contained McIlvaine phosphate-citrate buffer, *p*H 4.4 (0.25 ml); 15 m*M* substrate dispersed in 16 percent Triton X-100 (0.25 ml); and tissue homogenate in 0.45*M* sucrose, frozen, and thawed three times (0.25 ml). The concentration of homogenate was 5 percent with glyceryl tripalmitate or cholesteryl oleate as substrate, and 2 percent with glyceryl tridecanoate as substrate. After incubation at 37°C for 1 hour, fatty acid liberation was measured colorimetrically (*16*). T.H. and F.D. were the patients studied.

| Subject | Diagnosis | Fatty acid liberated per milligram of nitrogen ($\mu$mole/hour) | | |
|---|---|---|---|---|
| | | Glyceryl tridecanoate | Glyceryl tripalmitate | Cholesteryl oleate |
| | *Liver (biopsy)* | | | |
| T.H. | Cholesterol ester storage disease | 0 | 0 | 0 |
| Control 1 | Mucosulfatidosis | 7.30 | 1.83 | 0.489 |
| Control 2 | Metachromatic leukodystrophy | 6.28 | | 0.260 |
| Control 3 | Gaucher's disease | 16.35 | 4.30 | 0.364 |
| Control 4 | Abetalipoproteinemia | 4.33 | | 0.626 |
| Control 5 | Neonatal hepatitis | 9.39 | 2.14 | 0.474 |
| Control 6 | Hodgkin's disease | 6.28 | | 0.260 |
| | *Liver (autopsy)* | | | |
| Control 7 | Reye's syndrome | 5.17 | 2.49 | 0.780 |
| Control 8 | Reye's syndrome | 3.13 | 1.74 | 1.060 |
| Control 9 | Erythroblastosis | 7.70 | 2.69 | 0.203 |
| F.D. | Wolman's disease | 0 | 0 | 0 |
| | Mean of controls ± S.D. | 7.33 ± 3.65 | 2.53 ± 0.85 | 0.50 ± 0.27 |

saminidase) were normal in our liver homogenates from Wolman's disease and cholesterol ester storage disease.

Hepatic lipid composition was determined after extraction by the method of Folch *et al.* (*9*). Total lipid was determined gravimetrically. Free cholesterol, esterified cholesterol, and triglyceride were separated and identified by silica-gel thin-layer chromatography (*10*). Each fraction was quantitated by gas liquid chromatography. Total liver lipid (mg/g, wet weight) was: in cholesterol ester storage disease, 177; in Wolman's disease, 228; and in five normal controls 45.4 (S.D. ± 5.7). In cholesterol ester storage disease, total cholesterol was 148.3 mg/g, wet weight; esterified cholesterol was 146.9 mg/g, wet weight; and triglyceride was 16.6 mg/g, wet weight. In Wolman's disease, total cholesterol was 92.9 mg/g, wet weight; esterified cholesterol was 87.4 mg/g, wet weight; and triglyceride was 127 mg/g, wet weight. In the five control specimens, the total cholesterol was 4.8 mg/g, wet weight (S.D. ± 2.5); esterified cholesterol was 1.1 mg/g, wet weight (S.D. ± 0.27); and triglyceride was 10.1 mg/g, wet weight (S.D. ± 4.4). Thus in both diseases, there was a great excess of cholesterol ester, but in Wolman's disease the triglyceride concentration increased tenfold while in cholesterol ester storage disease, hepatic triglyceride concentration was within the normal range. Similar values have been reported by others (*4, 11, 12*).

Typical Wolman's disease has a fulminant course (*13, 14*). Affected children usually die in the first year of life after a prolonged illness characterized by intestinal malabsorption, vomiting, punctate adrenal calcification, and hepatic failure, which is in marked contrast to the clinical presentation and benign course of patients with cholesterol ester storage disease who reach adult life with no symptoms but hepatomegaly (*1–3*). Recessive inheritance has been demonstrated in Wolman's disease (*13*). In this family with cholesterol ester storage disease, we tend to believe that inheritance may be dominant with variable expressivity because one female sibling has the same histologic lesion, four of five other siblings have hepatomegaly and minimal but definite histologic and ultrastructural abnormalities resembling the index case, and all six siblings with hepatomegaly as well as the father and the paternal grandfather have an abnormal serum bile acid pattern although clinical or biochemical evidence of liver disease is absent (*3*).

A common feature of both diseases is the hepatic excess of cholesterol ester (*4, 11, 12*). However, in Wolman's disease excessive amounts of cholesterol ester in the liver are present mainly in the macrophages of the reticuloendothelial system (*15*), whereas in cholesterol ester storage disease cholesterol ester is primarily found in the hepatocytes (*2, 3*). The liver in Wolman's disease contains triglyceride at 10 to 20 times the normal concentration, most of which is present in hepatocytes (*12, 15*), but in cholesterol ester storage disease the hepatic triglyceride concentration is normal (*4*). We propose that these differences in the pattern of hepatic lipid storage suggest that there may be different acid lipases in hepatocytes and reticuloendothelial cells. If so, the acid lipase determination as performed may be specific for only one such lipase that is shared by both cell types.

The differences between cholesterol ester storage disease and Wolman's disease in clinical course, histopathology, lipid analysis of the liver, and possible mode of inheritance suggest that these are different diseases not completely explained by the observed identical deficiency of hepatic lysosomal acid lipase activity.

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### References and Notes

1. D. S. Fredrickson, *Ann. Intern. Med.* **58**, 718 (1963).
2. A. Lageron, J. Caroli, H. Stralin, P. Barbier, *Presse Med.* **75**, 2785 (1967).
3. L. Schiff, W. K. Schubert, A. J. McAdams, E. L. Spiegel, J. F. O'Donnell, *Amer. J. Med.* **44**, 538 (1968).
4. R. Infante, J. Polonovski, J. Caroli, *Presse Med.* **75**, 2829 (1967).
5. S. Mahadevan and A. L. Tappel, *J. Biol. Chem.* **243**, 2849 (1968).
6. A. D. Patrick and B. D. Lake, *Nature* **222**, 1067 (1969).
7. B. D. Lake and A. D. Patrick, *J. Pediat.* **76**, 262 (1970).
8. A. Steyermark, *Quantitative Organic Microanalysis* (Academic Press, New York, 1961), p. 188.
9. J. Folch, M. Lees, G. H. Sloane Stanley, *J. Biol. Chem.* **226**, 497 (1957).
10. V. P. Skipski and M. Barclay, *Methods Enzymol.* **14**, 530 (1969).
11. G. C. Guazzi, J. J. Martin, M. Philippart, H. Roels, H. Van der Eecken, L. Vrints, M. J. Delbeke, C. Hooft, *Eur. Neurol.* **1**, 334 (1968).
12. E. C. Kyriakides, N. Filippone, B. Paul, W. Grattan, J. A. Balint, *Pediatrics* **46**, 431 (1970).
13. M. Wolman, V. V. Sterk, S. Gatt, M. Frenkel, *ibid.* **28**, 742 (1961).
14. A. C. Crocker, G. F. Vawter, E. B. D. Neuhauser, A. Rosowsky, *ibid.* **35**, 627 (1965); W. C. Marshall, B. G. Ockenden, A. S. Fosbrooke, J. N. Cumings, *Arch. Dis. Childhood* **44**, 331 (1969).
15. J. Lough, J. Fawcett, B. Wiegensberg, *Arch. Pathol.* **89**, 103 (1970).
16. W. G. Duncombe, *Biochem. J.* **88**, 7 (1963).
17. We thank Dr. George Hug for suggestions and discussion. Supported by NIH grants (RR-123 and RR-05535) from the General Clinical Research Centers Program of the Division of Research Resources.

30 September 1971; revised 11 January 1972