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Long-term Pilot Trial of D-Penicillamine, Minerals and Vitamins in Patients with Advanced Laennec's Cirrhosis

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ABSTRACT

A long-term intensive study of the effects of D-penicillamine and selected nutrients in patients with severe Laennec's cirrhosis showed striking improvement without the usually reported incidence of side effects of the drug. We have, however, encountered a possible new adverse effect, influencing glucose and lipid metabolism and the heart, that might result from trace mineral imbalance and that should be preventable. Early signs of benefit were correction of iron-refractory anemia and (in patients with a hepatitis-component) diminution of hepatic pain and enlargement. Hyperammonemia, pre-coma, abnormal encephalogram and confusion have diminished at doses of 1 g/day (1 g/d). With sustained and increased dosage (in most to 2 g/d), pre-existing thrombocytopenia, inverted albumin/globulin ratios and hepatic fibrosis all showed improvement. The nutrients alone did not achieve these effects.

INTRODUCTION

Laennec's cirrhosis that progresses despite prolonged hospitalization and conventional therapy is an unresolved problem. In Wilson's disease, D-penicillamine has improved hepatic dysfunction (12,20,48,65,78) and decreased parenchymal cell degeneration and fibrosis (18,20,48,74). Early and controlled studies of up to one year suggest that it has potential value in primary biliary cirrhosis (13,21,38,46). Benefit has been reported in chronic active liver disease (1,2,45,72,74) and controlled studies have shown penicillamine to suppress chronic hepatitis as effectively as does prednisone (71). Patients with acute alcoholic hepatitis given penicillamine had less histologic damage than did controls (63). However, liver disease patients given even low dosage (1 g/d) of D-penicillamine have had almost as high an incidence of side effects (13,21,38) as do rheumatoid patients (5,10,27,28, 33,34,60). In contrast, our long-term trial of its use in doses twice as high but in combination with nutrients with which it interferes or which have complementary activities (68) has shown few adverse reactions. A possible new serious (cardiovascular) complication that should prove preventable has been encountered. Among 12 patients with advanced Laennec's cirrhosis so treated for 8-60 months were 3 whose condition regressed when D-penicillamine was decreased or stopped, even though nutrients were continued. Two more were given only nutrients without notable change.

MATERIALS AND METHODS

Chronic Laennec's cirrhosis was verified by needle biopsy in 10 of the 14 patients in this study. Pre-treatment evaluation included hemograms, prothrombin times, serum bilirubin, ceruloplasmin, minerals, transaminases, lactic dehydrogenase, alkaline phosphatase, blood glucose, ammonia, creatinine,

urea nitrogen, urinalysis, 24-hr urinary outputs of Zn, Cu and Mg. Electrocardiograms (ECG) and electroencephalograms (EEG) also were taken. Blood counts and urinalyses were done at least monthly to detect early adverse reactions. Lipoprotein profiles and hair Zn/Cu ratios were recently added to the test schedule, particularly for patients late in the course of therapy.

At least a month before starting D-penicillamine and during its administration, oral nutrients were given with each meal as follows: vitamins B_6 (50 mg), B_1 (50 mg), B_{12} (25 µg), C (100-500 mg, depending on need to acidify urine), E (400 I.U.), Zn (originally 15 mg as the gluconate with each meal, reduced to 0 or 10 mg/d) and Mg (100 mg as the hydroxide or amino acid chelate, depending on bowel habits). Copper supplements (3 mg/d or more as the sulfate) were added if ceruloplasmin levels fell to subnormal from high levels or if the hair Cu/Zn ratio was lower than normal. A high potency multivitamin capsule was given with one meal. Intramuscular injections of vitamin B_{12} (2000 µg) and Mg (2 ml 50% $MgSO_4$) were given weekly. D-penicillamine was administered 3 times daily, at least 1-2 hours apart from meals, beginning with 250 mg/day and increasing at no less than one month intervals by 250 mg daily. Patients then received the lowest amount of the drug at which improvement was maintained. The highest total daily dosage was 2.75 g, the usual upper limit was 2 g/d. Three patients received uninterrupted treatment with 2-2.75 g/d of D-penicillamine and the nutrients for 1.3-5 years. One (case 1) is still in hospital on lower dosage; one (case 2) and 2 others given 1-2.25 g/d for 8-12 mo were discharged much improved on medication but have been lost to follow-up after 1-3 re-hospitalizations for evaluation. A fifth (case 3) was his own control, having received the combination regimen, the nutrients alone and the combination again. Another's nutrients were continued when his D-penicillamine was permanently stopped. Of the remainder, 2 were taken off the program because of lack of cooperation, 4 signed themselves out as soon as they felt better and 2 received only nutrients.

CASE REPORTS: RESULTS

Case 1. A 60-year-old white man had had a major myocardial infarct and deep thrombophlebitis several years before his most recent admission to Bellevue Hospital with ascites, purpura, icterus and congestive heart failure. He was transferred to this chronic care hospital in June, 1971 when his bilirubin had dropped to 0.1/1.8 and his transaminases were almost normal. He was anemic (rbc=3.8; hemoglobin/hematocrit [Hgl/Hct] = 11.7/47) and his albumin/globulin ratio was inverted (2.4/4.7). In 3 months he was stable except for psychotic episodes and worse iron-refractory anemia (Fig. 1-A). A year later his liver became tender, enlarged, nodular and stony hard. Needle liver biopsy showed fibrosis and parenchymal degeneration (Fig. 2A). A very tender subxiphoid bulge (diameter: 10 cm) appeared, and spider nevi and purpuric showers were noted April, 1973. He then had high globulin levels (IgG =1900; IgM=160; IgA=500), ceruloplasmin (1265 U [normal: 28-57]) and 24-hr urinary Cu (300 mg [normal: 70-140]). Liver scan, normal alkaline phosphatase, lack of fetoproteins and Australia antigens suggested chronic active liver disease superimposed on Laennec's cirrhosis, with a large regenerative nodule. Preparatory to a trial of D-penicillamine, the patient was supplemented with vitamins B_6, E and B_{12} and with Mg, additional to maintenance multivitamins and B_1. He exhibited increased Hgl/Hct, improved strength and a sense of well-being on this program, so it was continued when D-penicillamine was started. Within 2 mo his subxiphoid nodule had decreased in size and pain. The dosage was gradually increased at 1-3 mo intervals to 1.5 g/d and maintained at that level for 6 mo. Although zincuresis and cupriuresis developed, only Zn was repleted. Despite vitamin E supplementation, its blood levels remained subnormal. The D-penicillamine dosage was gradually increased, first to 2 and then to 2.75 g/d when hepatic pain recurred. Hepatic needle biopsy (September 1975) showed normal parenchyma and minimal fibrosis (Fig. 2B). Ecchymoses increased after several months on the high

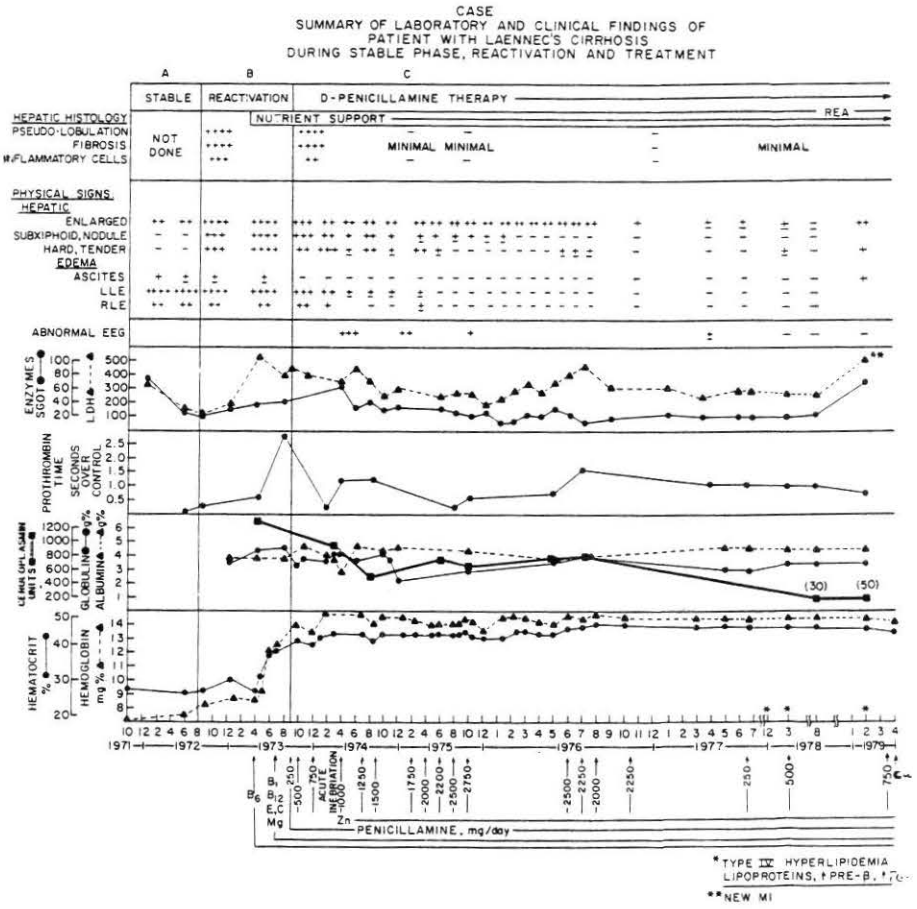

FIGURE 1.

dosage and gradually subsided when dosage was decreased to .25-.5 g/d. This patient improved markedly, even his EEG being normal, and developed none of the other usual D-penicillamine reactions. However, his previously normal glucose tolerance curve became abnormal and his urinary output of Cr was very low after 4 years of high dose therapy. Multiple xanthomata appeared in the fifth year of treatment; then Type IV hyperlipidemia was identified. He also developed ECG changes indicative of a fresh myocardial infarct. His ceruloplasmin dropped markedly to 30 I.U. His diabetes improved on feeding Cr-rich brewers yeast. His Zn supplements were stopped and 3 mg Cu added to the regimen.

Case 2. A 54-year-old white female, who had undergone a portocaval shunt 4 years earlier, was admitted to this hospital from a mental institution with organic brain syndrome, massive ascites, hyperammonemia and anemia (Fig. 3). She had hypomagnesemia and hypokalemia but her other electrolytes and bilirubin and albumin/globulin levels were almost normal. Needle liver biopsy confirmed severe fibrosis (Fig. 4A). Her entry into the study was therapy of last resort. She had riboflavin deficiency, refractory to oral B_2; malabsorption was proved by a d-xylose absorption test. D-penicillamine dosage was increased very slowly because of pre-treatment thrombocytopenia and fluctuating platelet counts during the early months. She attained a

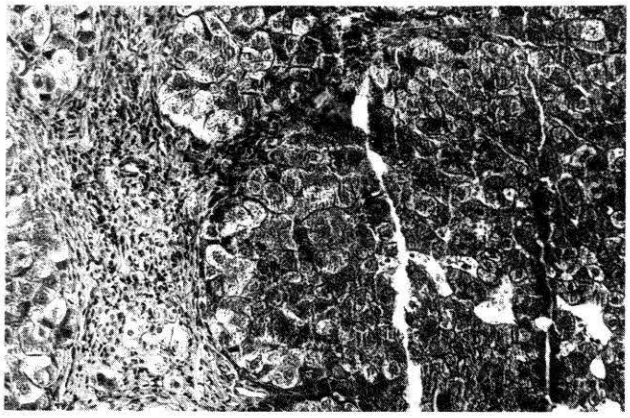

FIGURE 2A.

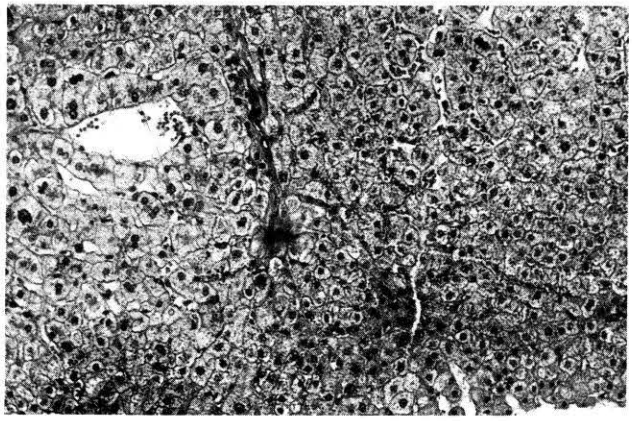

FIGURE 2B.

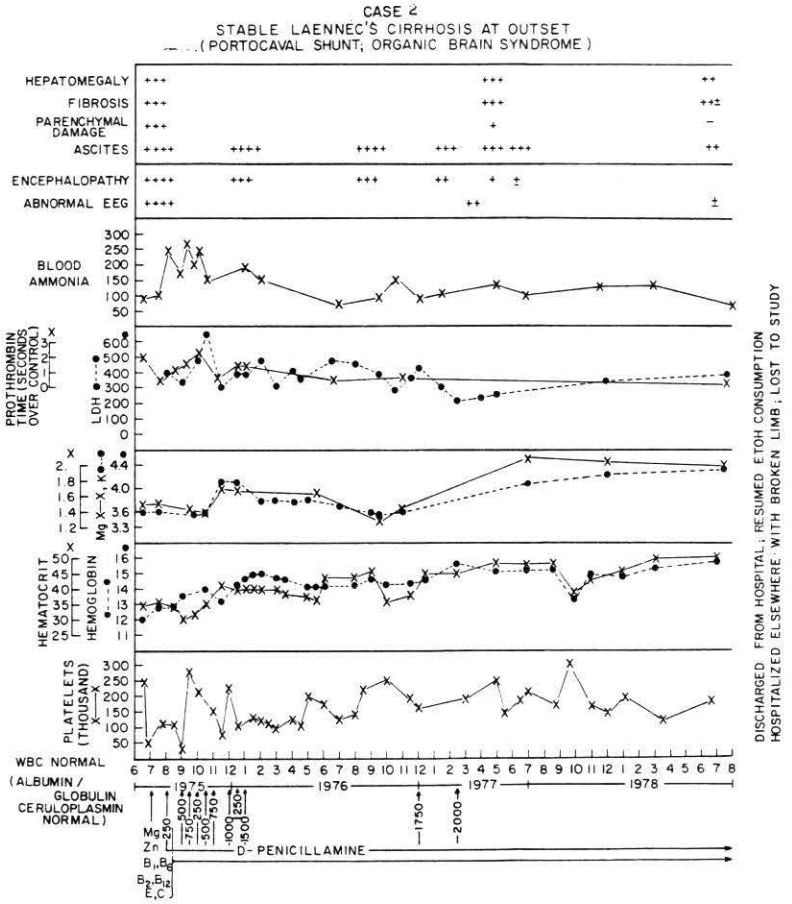

FIGURE 3.

normal platelet count about a year after therapy was begun, by which time Malabsorption, hypomagnesemia, anemia, byperammonemia and to a lesser degree ascites had begun to improve. Her blood urea nitrogen rose to normal from subnormal levels, she became alert, her EEG improved and the liver biopsy taken after 22 mo of treatment showed less fibrosis (Fig. 4B). She left the hospital on therapy, but returned to drink and has been lost to follow-up.

Case 3. A 64-year-old black man was transferred to this hospital with biopsy-confirmed Laennec's cirrhosis and hepatic decompensation with hyperammonemia. Nutrients were given for 3 mo before starting D-penicillamine, which was increased very slowly because of pre-treatment and persistent thrombocytopenia (Fig. 5). He required Neomycin to control his hyperammonemia and more frequent than weekly I.M. Mg for his hypomagnesemia. His blood ammonia started to drop and pre-coma occurred less frequently toward the end of his first course of D-penicillamine (at 1 g/d), which was stopped when he developed a resistant urinary infection requiring a new aminoglycoside. His nutrient therapy was continued, but he was not restarted on D-penicillamine for 8 mo, during which time his frequent episodes of pre-coma and hyperammonemia recurred. His liver became shrunken and an attempt to obtain a needle biopsy was unsuccessful. A second course of D-penicillamine

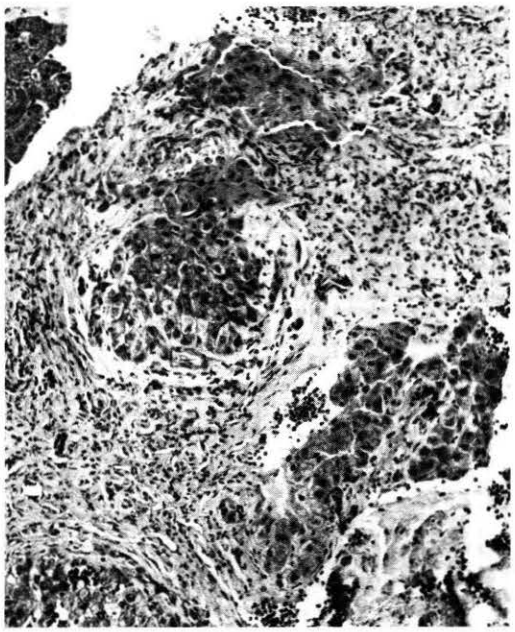

FIGURE 4A.

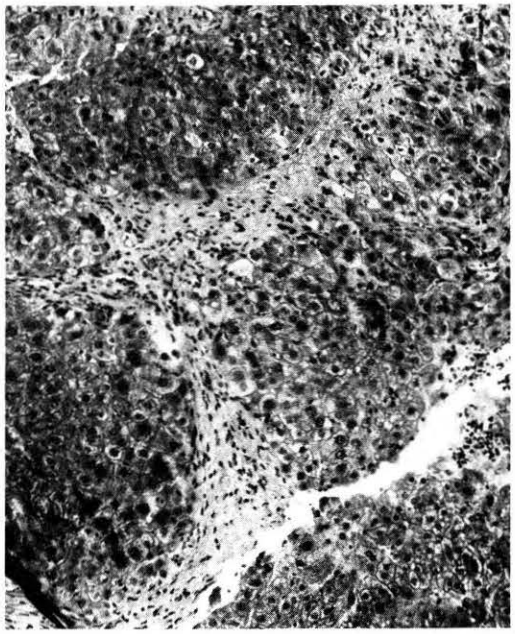

FIGURE 4B.

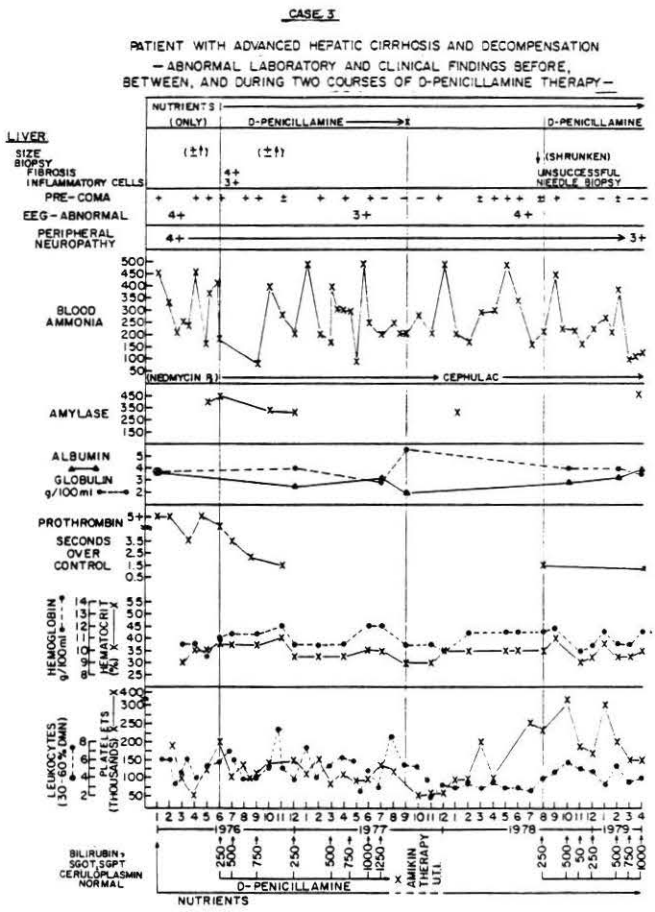

FIGURE 5.

was started cautiously, and when dosage reached .75-1.25 g/d his condition improved (Fig. 5).

Shorter-term data. A 68-year-old man, admitted with enormous hepatomegaly, hyperammonemia and severe anemia after hemorrhage from esophageal varices, showed improvement in all parameters within 3 mo of starting the D-penicillamine + nutrient regimen. He was treated for 12 mo in the hospital and for 4 mo as an out-patient, then was lost from the study. A 66-year-old man responded similarly, but developed painless hematuria (diagnosed as cystitis). D-penicillamine was stopped when he refused a renal biopsy to rule out immunocomplex glomerulitis. His nutrients were continued, his prothrombin time rose and he left the hospital. He died of hepatic decompensation the next year following return to drink. A 48-year-old woman with a fatty, fibrotic liver and peripheral neuropathy improved after nutrients + no more than 1 g/d of D-penicillamine for 8 mo. She left the hospital on therapy but was lost to further study. The remainder on this program for 4-6 mo left as soon as they felt better or were discontinued because of lack of cooperation. Two given only nutrients are indistinguishable from other cirrhotic patients being treated conventionally.

DISCUSSION

D-penicillamine treatment of Laennec's cirrhosis was undertaken to: 1) lower the high Cu levels of cirrhosis (8,9,21,26,64) since excess Cu causes hepatic damage (46) and D-penicillamine mobilizes tissue Cu (59), and 2) increase collagen turn-over (29,32,56,57). Its efficacy in autoimmune diseases such as rheumatoid arthritis and vasculitis (11,28,34-36,60), rheumatoid lung disease (51) and scleroderma (3,6,31,32,60) led to its trial in liver diseases (1,2,13,38,45,46,63,72,79) which often have auto-immune components (14), e.g. chronic active liver disease (1,2,7,45,55,79), primary biliary cirrhosis (16,55,70) and alcoholic hepatitis (54).

The nutrient-supplement-associated clinical improvement in our first patient led us to continue the supplementation when D-penicillamine was started. He had an unusually prompt response for this drug, which is noted for a long lag-time. Prompt responses have also been seen in our other patients, once adequate doses are reached. Early improvement of iron-refractory anemia might be partially attributed to pyridoxine (24,25), which was given to compensate for B_6-inactivation by penicillamine (37,42). Correction of deficiences of Mg and vitamin E, which like the B-vitamins are low in cirrhotics (4,19,22,47,49,61,77,80) might have been contributory, since Mg and E deficiency each shortens erythrocyte survival time (17,58,72). Removal of excess Cu may also have played a role (66,67). The Mg deficiency of these patients was more often detected by urinary retention of over 40% of an I.M. load (23) than by presence of frank hypomagnesemia. Since most of the patients were being given digitalis or diuretics or both and these drugs cause Mg loss (15,44,50,69,77), all of our patients were supplemented with Mg.

Metabolic improvement (in Wilson's disease patients) having been attributed to D-penicillamine's activation of sulfhydryl (SH) enzymes (78), patients were given pharmacologic amounts of the SH-protective vitamins E, B_1, B_{12} and C (68) in which cirrhotic patients are low (4,19,47). Because pancreatitis interferes with B_{12} absorption (52), it was given I.M. weekly. Zinc was given to compensate for penicillamine's Zn chelation (43) and depletion (41) in cirrhotic patients who are already Zn deficient (62,75,76). This may have contributed to a new problem in patients kept on the Cu-depleting regimen for a long time, even though excess Cu may have been part of their original disorder. It has been suggested that high Zn/Cu dietary intakes may be a factor in hyperlipidemia and myocardial infarction (39,40). This possibility is now being investigated in view of the episode with case 1. His development of a diabetic glucose tolerance curve may have been the result of Cr depletion (53), a premise supported by subnormal urinary Cu excretion.

Except for this newly noted, serious possible side effect of long-term D-penicillamine therapy, which might be preventable by keeping Cr, Zn and Cu levels normal, our incidence of adverse effects has been very low. The high incidence of side effects which occurred in 2 double-blind studies of D-penicillamine treatment of biliary cirrhosis (38,71) and the fatablity in another (21) have encouraged investigators to employ such low doses as 0.6 g/d (38) to reduce the risk of serious reactions. None of our patients, however, has shown sustained benefit on doses less than 1 g/d.

CONCLUSIONS

D-penicillamine has shown value in several forms of liver disease, but its adverse effects have limited its application in doses that we have found necessary for reversal of Laennec's cirrhosis. We have found that selected nutrients in higher than customary doses have reduced the incidence of customary side effects (69) and permitted long-term administration to cirrhotic patients at twice the dosage tolerated in short-term double-blind studies. Use of this potent mineral chelator for prolonged periods, however, may re-

quire careful monitoring of trace minerals such as Cr, Zn and Cu to avoid production of new adverse reactions caused by deficiencies and imbalances.

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LITERATURE CITED

1. Alexander, M. and M. Kludas. 1969. Collagen synthesis. Inhibition by D-penicillamine in chronic agressive hepatitis. *Munch. med. Wschr.* 111: 847-850.
2. Alexander, M. and G. Wille. 1974. D-penicillamine therapie bei leberkrankungen. Vorlaufe Ergebnisse bei 44 Fallen. *Munch. med. Wschr.* 116: 214-216.
3. Asboe-Hansen, G. 1976. Treatment of generalized scleroderma with D-penicillamine and other inhibitors of collagen synthesis. See: *Penicillamine Research in Rheumatoid Disease*, pp. 284-289.
4. Baker, H., O. Frank, H. Ziffer, S. Goldfarb and C.M. Leevy. 1964. Effect of hepatic disease on liver B-complex vitamin titers. *Am. J. Clin. Nutr.* 14:1-6.
5. Balme, H.W. and E.C. Huskisson. 1976. Side effects of penicillamine other than nephropathy and their management. See: *Penicillamine Research in Rheumatoid Disease*, pp. 250-254.
6. Bluestone, R., R. Grahame, V. Holloway and P.J. Holt. 1970. Treatment of systemic sclerosis with D-penicillamine. *Ann. Rheum. Dis.* 29:153-158.
7. Bridi, G.S., P.W. Falcon, N.C. Brackett, Jr., W.J.S. Still and I.N. Sporn. 1972. Glomerulonephritis and renal tubular acidosis in a case of chronic active hepatitis with hyperimmunoglobulinemia. *Am. J. Med.* 52:267-278.
8. Butt, E.M., R.E. Nusbaum, T.C. Gilmour and S.L. DiDio. 1956. Trace metal patterns in disease states. *Am. J. Clin. Path.* 26:225-242.
9. Butt, E.M., R.E. Nusbaum, T.C. Gilmour and S.L. DiDio. 1964. Hepatic and pancreatic cirrhosis in alcoholic patients with and without storage of iron. *Am. J. Clin. Path.* 42:437-450.
10. Day, A.T. and J.R. Golding. 1974. Hazards of penicillamine in the treatment of rheumatoid arthritis. *Postgrad. Med. J.* 50:71-73.
11. Day, A.T., J.R. Golding, P.N. Lee and A.D. Butterworth. 1974. Penicillamine in rheumatoid disease. *Brit. Med. J.* 1:180-183.
12. Deiss, A., R.E. Lynch, G.R. Lee and G.E. Cartwright. 1971. Long-term therapy of Wilson's disease. *Ann. Int. Med.* 75:57-65.
13. Deering, T.B., E.R. Dickson, C.R. Fleming, M.G. Geall, J.T. McCall and A.H. Baggenstoss. 1977. Effect of D-penicillamine on copper retention in patients with primary biliary cirrhosis. *Gastroenterology* 72:1208-1212.
14. Doniach, D. 1972. Autoimmune aspects of liver disease. *Brit. Med. Bull.* 28:145-148.
15. Duarte, C.G. 1968. Effects of ethacrynic acid and furosemide on urinary calcium, phosphate and magnesium. *Metabolism* 17:867-876.
16. Eddleston, A.L.W.F., I.G. McFarlane, C.G. Mitchell, W.D. Reed and R. Williams. 1973. Cell-mediated immune response in primary biliary cirrhosis to a protein fraction from human bile. *Brit. Med. J.* 3:274-276.
17. Elin, R.J. 1973. Erythrocyte survival in magnesium-deficient rats. *Proc. Soc. Exp. Biol. Med.* 142:1159-1161.

18. Falkmer, S., G. Samuelson and S. Sjolin. 1970. Penicillamine-induced normalization of clinical signs, and liver morphology and histochemistry in case of Wilson's disease. *Pediatrics* 45:260-268.
19. Fennely, J., O. Frank, H. Baker and C.M. Leevy. 1964. Peripheral neuropathy of the alcoholic. I. Aetiologic role of Aneurin and other B-complex vitamins. *Brit. Med. J.* 1290-1292.
20. Fisher, R.M. and S.C. Sherlock. 1964. Familial hepatic copper storage disease a variant of Wilson's disease. *Arch. Dis. Child.* 39:14-17.
21. Fleming, C.R., J. Ludwig and E.R. Dickson. 1978. Asymptomatic primary biliary cirrhosis. Presentation, histology and results with D-penicillamine. *Mayo Clin. Proc.* 53:587-593.
22. Flink, E.B., T.J. Konig and J.L. Brown. 1955. The association of low serum magnesium concentrations and deleterious effects of ammonia in hepatic cirrhosis. *J. Lab. Clin. Med.* 46:814-815.
23. Freeman, R. and E. Pearson. 1966. Hypomagnesemia of unknown etiology. *Am. J. Med.* 41:645-656.
24. Frimpter, G.W., R.J. Andelman and W.F. George. 1959. Vitamin B_6 dependency syndromes. *Am. J. Clin. Nutr.* 22:794-804.
25. Gehrmann, G. 1965. Pyridoxine-responsive anemias. *Brit. J. Haem.* 2:86-91.
26. Gubler, C.J., H. Brown, H. Markowitz, G.E. Costwright and M.M. Wintrobe. 1957. Portal (Laennec's) cirrhosis of the liver. *J. Clin. Invest.* 36:1208-1216.
27. Hamilton, E.B.D., F.E. Dische, D.R. Swenson and L. Fernandes. 1976. Renal disease and penicillamine. See: *Penicillamine Research in Rheumatoid Disease,* pp. 260-267.
28. Hamilton, E.B.D., A. St.J. Dixon, J. Davis, T.L. Dormandy, P.J.L. Holt, R.M. Mason, M. Thompson, J.C.P. Weber and D.W. Zutshi. 1976. Multicentre trial with synthetic D-penicillamine in rheumatoid arthritis. See: *Penicillamine Research in Rheumatoid Disease,* pp. 260-267.
29. Hansen, T.M., R. Manthorpe, B. Kofod and I. Lorenzen. 1976. Clinical improvement during penicillamine treatment of rheumatoid arthritis. See: *Penicillamine Research in Rheumatoid Disease,* pp. 93-99.
30. Harkness, R.D. 1968. *In vitro* and *in vivo* observations on the effect of penicillamine on collagen. *Postgrad. Med. J.* 44:31-34.
31. Harris, E.D., Jr. and A. Sjoerdsma. 1966. Effect of penicillamine on human collagen and its possible application to treatment of scleroderma. *Lancet* 2:996-999.
32. Herbert, C.M., K.A. Lindberg, M.I.V. Jayson and A.J. Bailey. 1974. Biosynthesis and maturation of skin collagen in scleroderma, and effect of D-penicillamine. *Lancet* 1:187-192.
33. Jaffee, I.A. 1968. Effects of penicillamine on the kidney and on taste. *Postgrad. Med. J.* 44:15-18.
34. Jaffe, I.A. 1976. Penicillamine treatment of rheumatoid arthritis. Rationale, pattern of clinical response, and clinical pharmacology and toxicology. See: *Penicillamine Research in Rheumatoid Disease,* pp. 11-24.
35. Jaffe, I.A. 1964. Rheumatoid arthritis with arteritis. *Ann. Int. Med.* 61:556-563.
36. Jaffe, I.A. 1970. The treatment of rheumatoid arthritis and necrotizing vasculitis with penicillamine. *Arth. Rheum.* 13:436-443.
37. Jaffe, I.A., K. Altman and P. Merryman. 1964. The antipyridoxine effect of penicillamine in man. *J. Clin. Invest.* 43:1869-1873.
38. Jain, S., R.J. Scheuer, S. Samourian, J.O. McGee and S. Sherlock. 1977. A controlled trial of D-penicillamine therapy in primary biliary cirrhosis. *Lancet* 1:831-834.
39. Klevay, L.M. 1975. Coronary heart disease: the zinc/copper hypothesis. *Am. J. Clin. Nutr.* 28:764-774.
40. Klevay, L.M. 1977. The role of copper and zinc in cholesterol metabolism. In: *Advances in Nutritional Research,* H.H. Draper, Ed., Plenum

Press, New York, pp. 227-252.
41. Klingberg, W.G., A.S. Prasad and D. Oberleas. 1976. Zinc deficiency following penicillamine therapy. In: *Trace Elements in Human Health and Disease*, A.S. Prasad, Ed., Academic Press, New York, pp. 51-65.
42. Kuchinskas, E.J., A. Horvath and V. Duvigneaud. 1957. An anti vitamin B-6 effect of L-penicillamine. *Arch. Biochem. Biophys.* 68:69-75.
43. Kuchinskas, E.J. and Y. Rosen. 1972. Metal chelates of DL-penicillamine. *Arch. Biochem. Biophys.* 97:370-372.
44. Kupfer, S. and J.D. Kosovsky. 1965. Effects of cardiac glycosides on renal tubular transport of calcium, magnesium, inorganic phosphate and glucose in the dog. *J. Clin. Invest.* 44:1132-1143.
45. Lange, J., K. Schumacher and H.P. Witscher. 1971. Die behandling der chronischaggressiven hepatitis mit D-penicillamine. *Dtsche. med. Wschr.* 96:139-145.
46. Leeson, P.M. and P. Fourman. 1967. A disorder of copper metabolism treated with penicillamine in a patient with primary biliary cirrhosis and renal tubular acidosis. *Am. J. Med.* 43:620-635.
47. Leevy, C.M., H. Baker, L.O. TenHove, O. Frank and G.R. Cherrick. 1965. B-complex vitamins in liver disease of the alcoholic. *Am. J. Clin. Nutr.* 16:339-346.
48. Levi, A.J., S. Sherlock, P.J. Scheuer and J.M. Cumings. 1967. Presymptomatic Wilson's disease. *Lancet* 2:575-578.
49. Lim, P. and E. Jacob. 1972. Magnesium deficiency in liver cirrhosis. *Q.J. Med.* New Ser. 41:291-300.
50. Lim, P. and E. Jacob. 1972. Magnesium deficiency in patients on long-term diuretic therapy for heart failure. *Brit. Med. J.* 3:620-622.
51. Lorber, A., R.A. Bovy and C.C. Chang. 1971. Sulfhydryl deficiency in connective tissue disorders: correlated with disease activity and protein alterations. *Metabolism* 20:446-455.
52. Matuchansky, C., J.C. Rambaud, R. Modigliani and J.J. Bernier. 1974. Vitamin B_{12} malabsorption in chronic pancreatitis. *Gastroenterology* 67:406-407.
53. Mertz, W. and E.E. Roginski. 1971. Chromium metabolism: the glucose tolerance factor. In: *Newer Trace Elements in Nutrition*, W. Mertz and W.E. Cornatzer, Eds., Dekker, New York, pp. 123-153.
54. Mihas, A.A. and D.M. Bull. 1973. Cell-mediated immunity to liver disease in acute alcoholic hepatitis. *Clin. Res.* 21:962.
55. Miller, J., M.G.M. Smith, C.C. Mitchell, W.D. Reed, A.L.W.F. Eddleston and R. Williams. 1972. Cell-mediated immunity to a human liver-specific antigen in patients with active chronic hepatitis and primary biliary cirrhosis. *Lancet* 2:296-297.
56. Nimni, M.E. 1968. A defect in intramolecular and intermolecular cross-linking of collagen caused by penicillamine. Metabolic and functional abnormalities in soft tissues. *J. Biol. Chem.* 243:1451-1466.
57. Nimni, M.E. and R.D. Harkness. 1968. Chemical and mechanical changes in the collagenous framework of skin induced by thiol compounds. *Acta Phys. Acad. Sci. Hung.* 33:325-343.
58. Oken, M.M., M.A. Lichtman, D.R. Miller and P. LeBlond. 1971. Sperocytic hemolytic disease during magnesium deprivation in the rat. *Blood* 38:468-478.
59. Osborn, S.B. and J.M. Walshe. 1958. Effects of penicillamine and dimercaprol on turnover of copper in patients with Wilson's disease. *Lancet* 1:70-74.
60. *Penicillamine Research in Rheumatoid Diseases*, Symp., Spatind, Norway, 1976, E. Munthe, Ed., Fabriteus and Sonner, Oslo.
61. Popper, H., A. Dubin, F. Steigmann and F.P. Hesser. 1949. Plasma tocopherol levels in various pathologic conditions. *J. Lab. Clin. Med.* 34:648-652.
62. Prasad, A.S., D. Oberleas and C. Rajasakaran. 1970. Essential micronutrient elements. Biochemistry and changes in liver disorders. *Am. J. Clin. Nutr.* 23:581-591.

63. Resnick, R.H., J. Boitnott, F.L. Iber, H. Makipour and J.J. Cerda. 1974. Preliminary observations of D-penicillamine therapy in acute alcoholic liver disease. *Digestion* 11:257-265.
64. Scheinberg, I.H. and I. Sternlieb. 1960. Copper metabolism. *Pharm. Rev.* 12:355-381.
65. Scheinberg, I.H. and I. Sternlieb. 1963. Dual role of the liver in Wilson's disease. *Med. Clin. N.A.* 47:815-824.
66. Seelig, M.S. 1972. Review; relationships of copper and molybdenum to iron metabolism. *Am. J. Clin. Nutr.* 25:1022-1037.
67. Seelig, M.S. 1973. Proposed role of copper-molybdenum interaction in iron-deficiency and iron storage disease. *Am. J. Clin. Nutr.* 26:657-672.
68. Seelig, M.S. and A.R. Berger. 1977. Do trace elements, magnesium and anti-oxidants protect against D-penicillamine toxicity? In: *Trace Substances in Environmental Health-XI*, D.D. Hemphill, Ed., University of Missouri, Columbia, pp. 243-251.
69. Seller, R.H., J. Cangiano, K.E. Kim, S. Mendelsonn, A.N. Brest and C. Swartz. 1970. Digitalis toxicity and hypomagnesemia. *Am. Heart J.* 79:57-68.
70. Sherlock, S. and P.J. Scheuer. 1973. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. *New Eng. J. Med.* 289:674-678.
71. Stern, R.B., S.P. Wilkinson, P.J. Howarth and R. Williams. 1977. Controlled trial of synthetic D-penicillamine and prednisone in maintenance therapy of chronic hepatitis. *Gut* 18:19-22.
72. Sternlieb, I. 1972. (comment on Lange *et al.* (45)) D-penicillamine treatment of chronic agressive hepatitis? *Gastroenterology* 62:149-150.
73. Stormont, J.M., E.A. Hirshberg and C.S. Davidson. 1959. The peroxide-erythrocyte hemolysis test. Experiences in patients with cirrhosis, jaundice, and polyneuritis. *Am. J. Clin. Nutr.* 7:206-212.
74. Tu, J.B., W.C. Cooper, R.O. Blackwell and T-Y. Hou. 1965. Treatment of hepatolenticular degeneration (Wilson's disease) in the asymptomatic stage. *Neurology* 15:402-408.
75. Vallee, B.L., W.E.C. Wacker, A.F. Bartholomay and F.L. Hoch. 1956. Zinc metabolism in hepatic dysfunction. I. Serum zinc concentrations in Laennec's cirrhosis and their validation by sequential analysis. *New Eng. J. Med.* 255:403-408.
76. Vallee, B.L., W.E.C. Wacker, A.F. Bartholomay and F.L. Hoch. 1957. Zinc metabolism in hepatic dysfunction. II. Correlation of metabolic patterns with biochemical findings. *New Eng. J. Med.* 257:1055-1065.
77. Wacker, W.E.C. and A.F. Parisi. 1968. Magnesium metabolism. *New Eng. J. Med.* 278:772-776.
78. Walshe, J.M. 1959. Current views on the pathogenesis and treatment of Wilson's disease. *Arch. Int. Med.* 103:155-161.
79. Wildhirt, E. 1974. The treatment of chronic liver disease with D-penicillamine. *Postgrad. Med. J.* 50:42-43.
80. Woodruff, C.W. 1956. Vitamin E deficiency in man. Biochemical evidence in a patient with xanthomatous biliary cirrhosis. *Am. J. Clin. Nutr.* 4:597-602.