LEAD INDUSTRIES ASSOCIATION
420 LEXINGTON AVENUE
NEW YORK 17, N. Y.

June 20, 1956

LEAD HYGIENE and SAFETY BULLETIN
No. 120

To Members of the Lead Industries Associations

"EDTA"

"A considerable amount of animal experimentation and a relatively small number of clinical tests have indicated that a new drug, calcium ethylenediaminetetraacetate or 'CaEDTA,' holds great promise for the treatment of heavy metal poisoning, including lead intoxication."

It was thus that the advent of a then quite new therapy for plumbism was announced in a bulletin issued by this office on August 22, 1952. In the intervening four years, the promise has been borne out and the wisdom of adhering to the abbreviation "EDTA" of the chemical name has found support in avoidance of the confusion incident to the several commercial names (Edathamil, Nullapon, Sequestrene, Versene and others) by which the drug has become known.

No estimate of the number of cases, chiefly of lead intoxication, thus far treated with EDTA is possible, but that it is very great is evidenced by the receipt of upwards of a hundred inquiries, mainly from domestic sources, but also from South American, European and Australasian countries, in this office alone.

Scientific and technical publications on EDTA have also been very numerous, and in following the developing experience with the drug, varying supplies of some twenty of these have been acquired. Insofar as quantity permits, these may be selected from a list which will be furnished on request, but of only two have we enough for enclosure with this bulletin.

Of the two reprints herewith, that with the title "Use of Calcium Ethylenediaminetetraacetate in Cases of Lead Intoxication" is one of the earliest on this subject, while that on the "Nephrotoxic Hazard from Uncontrolled Edathamil Calcium-Disodium Therapy" is one of the most recent.

As was inevitable with so effective a drug, its use as a preventive of lead intoxication has been advocated in some quarters. Condemnation of any such proposal as "disastrous" and "deplorable" was voiced by Dr. R. T. Johnstone in an editorial published in August, 1954, and as "rash and irresponsible" and "fraught with unknown potentialities" in a report by Dr. R. A. Kehoe in January of the following year. Certain of these adverse potentialities are now brought to light in the second of our two enclosures, in which Dr. Foreman and his colleagues for the first time established toxic side effects of the drug.

That carefully controlled treatment of lead intoxication by means of intravenous EDTA is a valuable form of therapy is no longer open to question. Physicians having the care of employees of our member companies who may wish guidance to competent medical sources of information on its use are invited to communicate with this office.

Manfred Bowditch
Director of Health and Safety

Enclosures: 2

Reprinted from The Journal of the American Medical Association
March 30, 1956, Vol. 160, pp. 1043-1048

NEPHROTOXIC HAZARD FROM UNCONTROLLED EDATHAMIL CALCIUM-DISODIUM THERAPY

Harry Foreman, M.D.
Camille Finnegan, B.S.
and
Clarence C. Lushbaugh, M.D., Los Alamos, N. Mex.

Edathamil calcium-disodium, the calcium chelate of ethylenediaminetetraacetic acid, a new drug finding widespread use in heavy metal poisoning,[1] has been considered generally a nontoxic material. The following report presents clinical and experimental data indicating that the compound has a previously unsuspected toxic potentiality. It is hoped that this new information will serve to promote caution in the use of the drug. The erroneous impression of lack of toxicity of the drug is based on many studies. Toxicity studies have been made and show[2] that there is a wide spread between the therapeutic dose of 75 mg. per kilogram of body weight and the L. D.$_{50}$ dose, which varies from 500 to 7,000 mg. per kilogram, depending on the species and the route of administration. Metabolic studies done in this laboratory[3] showed that the compound is not metabo-

From the Biomedical Research Group, Health Division, Los Alamos Scientific Laboratory, University of California.

This work was done under the auspices of the Atomic Energy Commission.

1. Foreman, H.: Use of Chelating Agents for Accelerating Excretion of Radioisotopes, J. Am. Pharm. A. (Assoc. Ed.) 40:479-482 (Oct.) 1951. Bruenner, L. P., Byrd, H. and Rubin, M.: Treatment of Lead Encephalopathy with Calcium Disodium Versenate, Report of Case, M. Ann. District of Columbia 20:313-319 (June) 1952. Hardy, H. L., and others: Use of Monocalcium Disodium Edetate During Twin Attacks of Lead Poisoning, J. A. M. A. 156:1171-1179 (April 2) 1954. Knelson, L. A., Utz, H. S. M., and Beam, J.: Successful Calcium Disodium Ethylene Diamine Tetra-Acetate Treatment of Lead Poisoning in Infant, New England J. Med. 250:336-340 (March) 1954.

2. Pharmacology, Physiology, Biochemistry and Toxicity of Versenate, Framingham, Mass., Bersworth Chemical Co., 1952, pp. 25-32.

3. Foreman, H., Vaz, M., and Magee, M.: Metabolism of C^{14}-Labeled Ethylenediaminetetraacetic Acid in Rat, J. Biol. Chem. 186:1045-1051 (Aug.) 1950. Foreman, H., and Trueblin, T. T.: Metabolism of C^{14}-Labeled Ethylenediaminetetraacetic Acid in Human Beings, J. Lab. & Clin. Med. 43:566-571 (April) 1954.

lized but excreted rapidly and virtually completely, thus supporting the conclusions of the low toxicity of the drug. Histopathological studies [3] also support the conclusions of low toxicity, since no pathological changes in the tissues appeared after large doses were given.

Recently, however, there have been several cases in which it has appeared that the drug is not as innocuous as the above studies indicated. Dudley and others [4] recently reported two cases, both from the Massachusetts General Hospital, implicating edathamil as a toxic agent. A 30-year-old woman and a 4½-year-old child, both of whom had terminal cases, were given the sodium salt of the drug in attempts to lower elevated serum calcium levels. Autopsy examination revealed severe damage of the renal tubules and widespread engorgement of the reticuloendothelial cells, with coarse eosinophilic granules and extensive internal hemorrhage, all of which the authors felt could best be explained as the result of the edathamil therapy. Following is a report of a case that occurred at Los Alamos.

Report of a Case

Edathamil calcium-disodium was administered to a 40-year-old female chemical technician who had accumulated a small body burden of plutonium (approximately one-half tolerance dose) through a laceration in the thumb, caused by a broken piece of contaminated glassware.[5] Two and a half grams of edathamil calcium-disodium in 250 cc of saline solution was administered twice daily by intravenous drip over a half-hour period. The first treatment period was for 4 days and was followed by 2 days of rest, to the second period, the drug was administered for 12 consecutive days. It is estimated that approximately 25 to 30% of the body burden of plutonium was removed by the edathamil calcium-disodium during the course of treatment. On the 11th day of the second treatment period, the patient began to complain of mental dullness, lassitude, anorexia, nasal stuffiness, and low back pain. The following day the symptoms became intensified, and the patient complained, in addition to the above-mentioned symptoms, of dysuria and frequency, urgency, and

4 Dudley, H. R., Hawkins, A. C., Schilling, A., and Bubar, W. H.: Pathologic Changes Associated with Use of Sodium Edetate Disodium Type Acetate in Treatment of Hypercalcemia: Report of 2 Cases with Autopsy Findings, New England J. Med. 253: 331-337 (March) 1955.

5 Foreman, H., Pecaut, P. A., and Norwood, W. D.: Experimental Administration of Ethylenediamine-Tetraacetic Acid to Plutonium Poisoning, A. M. A. Arch. Indust. Hyg. 10: 220-231 (Sept.) 1954.

burning on urination. Therapy with the drug was stopped immediately, and investigation of the cause of her illness was undertaken.

Physical examination was noncontributory. Blood morphology and chemistry, including nonprotein nitrogen level, were within normal limits except for an elevated serum calcium level of 13 mg. per 100 cc. Urinalysis, however, showed 2+ albumin, numerous renal parenchymal cells, many fine granular casts, and occasional red and white blood cells. The signs and symptoms of the renal disturbance disappeared rapidly after the cessation of treatment. By the fifth day the urine was clear and the patient appeared entirely recovered. From the course of events, it appeared that the administration of the drug was related to the kidney disturbance.

Because of the unexpected association of renal damage with edathamil therapy in the three cases described above, it was decided to reinvestigate the toxicity of the drug to determine if renal pathological changes could be produced experimentally with edathamil in laboratory animals. It seemed to be particularly important to determine the dosage level required to produce the damage in relation to the amount of the drug that would have to be used for therapeutic effectiveness.

Experimental Studies

Male Sprague-Dawley rats, weighing approximately 400 gm. each, were used in all of the studies. The first of the experiments was carried out with supralethal dosage levels, in order to be as certain as possible of the production of lesions. Two groups of six rats each were given 3,000 mg. per kilogram and 1,000 mg. per kilogram per day respectively. The animals were injected intraperitoneally once daily with a solution containing 10% edathamil calcium-disodium and 1% procaine (Novocain), which was added to control the discomfort of the injection. Control animals were injected with procaine alone in order to rule out the possibility of this agent influencing the lesions. Urine was collected and examined for changes in volume, pH, specific gravity, albumin, and sediment. All animals living after six days of injections were killed. Tissues were taken as soon as possible after death and fixed promptly by immersion

in a formaldehyde (Formalin)and mercuric chloride solution. Sections were prepared and stained by the standard hematoxylin-eosin method.

After it was demonstrated that kidney lesions did develop, further studies were carried out at lower dosage levels. The median effective dosage level (E. D_{50}), that is, the amount of the drug required to produce the first histological evidence of damage in 50% of the animals treated daily for 16 days, was determined. Four groups of five rats each were injected intraperitoneally daily with 5% edathamil calcium-disodium at dosage levels of 62.5, 125, 250, and 500 mg. per kilogram. Tissues for histopathological study were taken as in the previous experiments. Another group of rats was treated with 500 mg. per kilogram per day for 16 days and then allowed to recover for 30 days before sacrifice, in order to learn whether recovery was on a histological as well as a clinical basis. A sixth group of rats was injected with edathamil strontium-disodium at a dosage level of 250 mg. per kilogram per day for 16 days to find whether the strontium form of the compound produces lesions. An additional experiment was carried out in order to determine the effect of pH of the urine on the development of the kidney lesions. One group of six rats was given 0.25% ammonium chloride in the diet and another group 0.25% of sodium bicarbonate in the diet. Edathamil calcium-disodium, 3,000 and 1,000 mg. per kilogram, was used in this experiment.

An attempt was made to determine the pathogenesis of the renal lesion and to look for lesions in other organs. Two groups of 12 rats each were given 500 and 1,000 mg. per kilogram of the drug daily intraperitoneally. Two rats in each group were then killed by a blow on the head 6, 12, 24, 48, and 96 hours after the first injection. They were then examined as quickly as possible, and pieces of the heart, liver, and kidney were frozen in isopentane at −50 C and then dehydrated and embedded in paraffin under vacuum. Other tissues fixed in formaldehyde and mercuric chloride mixture of hematoxylin

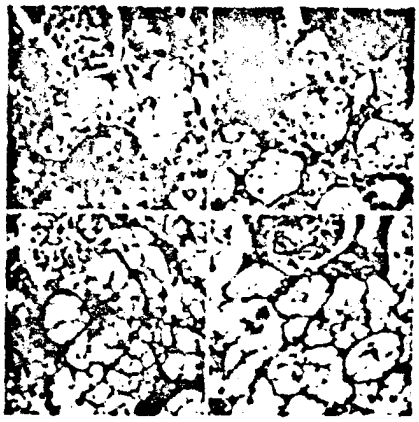
and eosin-stained paraffin sections were: thyroid, parathyroid, heart, liver, spleen, kidney, adrenal gland, small intestine, and colon. Kidney and total body weights were obtained at this time. The frozen and dehydrated tissues were studied for cytological changes in the proximal convoluted tubules, for changes in glycogen content with Best's carmine stain, and for fat with oil red O.

Results

The studies showed that the drug could cause a toxic nephrosis consisting of severe hydropic degeneration of the proximal tubules (see figure). None of the other tissues examined showed pathological changes. All of the animals receiving dosages of 1 gm. per kilogram or higher developed kidney lesions if they received daily injections for a sufficient length of time. The animals that received 3,000 mg. per kilogram per day developed lesions in 48 hours; those that received 1,000 mg. per kilogram per day showed the first signs of kidney damage after four days of injection. The animals receiving dosages of 125, 250, and 500 mg. per kilogram per day developed lesions at some time between 4 and 16 days of treatment, but some of these did not develop lesions at all. None of the animals developed lesions with a daily dose of 62.5 mg. per kilogram given for the 16 days. It is of interest that rats have been given 40 injections of 100 mg. per kilogram per day without evidence of renal damage.[4] The E. D_{50} in animals treated daily for 16 days, as calculated by Thompson's moving average technique,[5] was 203 mg. per kilogram, with a 95% probability of the effective dose falling in the range between 131 and 314 mg. per kilogram. Animals that were treated with 500 mg. per kilogram per day for 16 days and then killed 30 days after injections were stopped showed complete histological recovery. The group treated with edathamil strontium-disodium de-

4. Thompson, W. R. Use of Moving Averages and Interpolation to Determine Median-Effective Dose. I. Fundamental Formulas, Estimation of Error, and Relation to Other Methods. Bact. Rev. 11:115-145 (June) 1947.

veloped the same lesion as the animals treated with edathamil calcium-disodium. Animals receiving procaine alone showed no kidney lesions. There were no differences between the urinary findings of the treated animals and of the controls in pH, volume, and albumin content, but urine of the treated animals showed many casts and epithelial cells.



Photomicrographs made after therapy with edathamil calcium-disodium (×175). *A*, severe hydropic degeneration of most proximal portions of proximal convoluted tubules of kidney of rat treated for three weeks with 250 mg. per kilogram. *B*, extension of degenerative process to almost one-half of proximal convoluted tubule of rat treated with 500 mg. per kilogram for 10 days. *C*, obstructive nephrosis (arrangement of proximal convoluted tubules with one of 1,000 mg. per kilogram for six days. *D*, disseminated degree of degeneration with one of 3,000 mg. per kilogram for six days. Many areas survivors of proximal convoluted tubules show complete loss of living cells and gross distortion. Distal convoluted tubule is seen unimpaired at upper right of field.

In animals receiving 3,000 mg. per kilogram, all on a normal diet or with ammonium chloride in their diet died within three to six days. Three out of five of the sodium bicarbonate group survived the full six days, but they

were terminally moribund at this time. All of the animals receiving the lower dosages survived the full experiment and were killed at that time. At the time of death all of the animals had lost between 10 and 15% of their starting weight.

Histopathology

In the animals treated with the lower doses, the pathological changes were limited to the proximal convoluted tubules. With treatment of 250 mg. per kilogram, occasional loops of the tubule adjacent to a glomerulus showed nuclear pyknosis and huge intracytoplasmic vacuoles with destruction of cell walls. Further along the proximal convoluted tubule the cytoplasm was distorted by innumerable smaller and irregularly sized vacuoles, which gave it a sieve-like appearance, as shown in the figure, *A*. There the nuclei were not altered. The other tubules appeared normal. With the 500 mg. per kilogram dose of the drug, this severe hydropic degeneration was found to have extended to about half of each proximal convoluted tubule. The rest of the tubular system showed fine droplet degeneration, as shown in the figure, *B*. In the rats that received 1,000 mg. of edathamil per kilogram, almost the entire proximal convoluted portion of the renal tubular system had undergone hydropic degeneration, with the formation of huge vacuoles and in some instances rupture of cell walls, as shown in the figure, *C*. Occasionally, a focus of obstructive nephrosis was seen associated with apparent occlusion of a collecting tubule with debris. In the rats receiving the higher dose (3,000 mg. per kilogram), the most severe changes were seen. The hydropic degeneration of the proximal convoluted tubules was fully developed, and in many areas (see figure, *D*) only the basement membranes remained; also, there was no sign of the preexisting cells or their component parts. In addition, there was parenchymatous degeneration of the distal convoluted tubules. This change appeared as nuclear pyknosis and chromatolysis and granular amorphous intratubular debris. In Henle's loops, edema of occasional cells was seen, causing the formation of so-

called bird-eye cells. In addition, there were hyaline and amorphous casts in the collecting tubules, and above these areas of obstruction dilatation of the more proximal portions of the tubules was seen. In some instances the glomerular tufts appeared edematous, and there was proteinoid precipitate in the glomerular capsule.

In the sections from the rats killed sequentially during the administration of 500 and 1,000 mg. per kilogram of the drug, the renal lesion was seen to develop at the end of four days with the larger dose. No lesions were seen in the other organs examined. The special stains for glycogen in the frozen and dehydrated tissues failed to show the accumulation of this substance in either the kidney or heart or the loss of it from the hepatic parenchyma. Stains for fat were similarly noncontributory. The hematoxylin-eosin-stained sections of the quickly frozen kidneys, however, showed that the hyaline droplets of the cytoplasm of the proximal convoluted tubules increased greatly in size with time until the vacuoles developed. These droplets seemed to be highly water-soluble, since the ordinary method of histological dehydration appeared to remove them, leaving the sieve-like appearance of the renal cytoplasm.

Comment

The unfailing appearance of kidney lesions following repeated administration of edathamil calcium-disodium at high doses definitely establishes the nephrotoxic potentiality of the drug and supports the opinion that the renal changes described in the cases above resulted from edathamil administration. Repeated doses are necessary before lesions can be demonstrated. Possibly a single dose could produce lesions, but this dose would have to be so high that it would be lethal. The results show that there is an interrelationship between the size of the dose and the length of time required to produce the kidney damage. It is reasonable to expect that, because of the reversible nature of the lesion, there is a threshold dose—the dose below which lesions never develop no matter how long the drug is administered. This threshold

would be the amount of the drug from which the rate of recovery would be greater than the rate of production of injury. This same effect would obtain with higher-than-"threshold" doses by allowing longer periods of time for recovery. Interspersing rest periods into any prolonged treatment regimen would, therefore, seem very advisable. The fact that repeated doses are necessary for development of lesions might explain the lack of histopathological lesions in the studies reported.[8] Probably edathamil was not given for a long enough time to produce the renal lesions.

The data obtained from our experiments with rats afford an indication of doses that might be expected to produce lesions in man and also provide a comparison with therapeutic dosage levels. On the basis of the $E.D._{50}$ for rats treated daily for 16 days, it might be expected that 50% of patients weighing 150 lb. (68 kg.), who received 14 gm. a day for 16 days, would develop kidney lesions. Less than 2.5% of those given 9 gm. of the drug under the same conditions would develop nephrosis, while only 2.5% of individuals given 22 gm. would be expected to escape such damage. In order that development of lesions occur in 4 days, 70 gm. would have to be given daily to a 150-lb man, although such a person could be given 4.5 gm. daily for at least 16 days without difficulty. The recommended therapeutic dosage level of 5 gm. a day, given not more than five days in succession and followed by a two-day rest period,[1] would seem to be well within the desirable safety limits.

In the fatal cases reported by Dudley and others,[6] the adult patient who weighed 122 lb. (55.3 kg.) received 5 to 6 gm. on two successive days, followed by two days' rest, and then 18 to 22 gm. (330 to 440 mg. per kilogram) for nine successive days. On the basis of the data given above, this individual had a high probability

[8] Monsen, O. L., in Use of Calcium Ethylenediaminetetraacetate in Treating Heavy Metal Poisoning: Report of a Conference Held at Massachusetts General Hospital, A. M. A. Arch. Indust. Hyg. 7:140 (Feb.) 1953.

of developing renal lesions. In a similar manner, it might have been predicted that the other patient had only a fair probability of developing a nephrosis from treatment with 2 gm. for three days followed by 3 gm. (200 mg. per kilogram) for five days. In the Los Alamos case the low dose, equivalent to 7 gm. daily for a 150-lb. man, would not have been expected to cause trouble in more than 2% of the cases so treated. In the experiment reported here we could not find any synergistic factors, such as urinary pH, that could explain this unexpected enhancement. It is possible, though, that such factors as general status of health, endocrine balance, renal disease, and/or abnormal renal function could enhance the nephrotoxic action.

Both the calcium chelate and the sodium salt of edathamil cause kidney damage. The sodium form of the compound converts to the calcium chelate on contact with serum calcium, and, hence, the administration of either the calcium or sodium derivative results in the calcium chelate as the form passing through the kidney. Dudley and others [4] postulated that, when the urine pH falls to 5 or below, there is a dissociation of the calcium chelate, with release of free calcium ion in the tissue fluid of the kidney, which could bring about the injury. Data from the experiments reported here do not support this hypothesis. If the lowering of the pH in the tubular fluids leads to a chain of events that result in kidney injury, then, the animals that receive sodium bicarbonate in their diet would have been expected to show either no kidney lesions or at least lesser injury than the animals receiving ammonium chloride in their diet. There was no difference histologically, however, between the damaged kidneys of the two groups of animals. Moreover, the strontium chelate produced kidney damage.

The deleterious reaction noted above is more likely brought about through the chelation properties of the compound, and it is probably the small portion of an administered dose, noted in metabolism studies to turn over very slowly, that is involved. The drug could very well bind with a trace metal necessary for the function

of an essential enzyme system. Since this must be a metal forming a chelate with a higher stability constant than the calcium chelate, magnesium is unlikely to be involved. Iron, and perhaps manganese, are more likely metal ions. The interruption of a metabolic chain by inactivation of an essential enzymatic function could explain the development of nephrosis. A break in the metabolic pathway could cause metabolites to accumulate, which would increase the cytoplasmic osmotic pressure, leading to imbibition of water by the cell and the type of hydropic degeneration that occurred in the rats. The location of the lesion in the proximal convoluted tubules and the gradient of decreasing damage in the distal portions of the renal tubule would seem to implicate a metabolite excreted by the glomerulus and reabsorbed by the proximal convoluted tubule. The histochemical studies made here fail to give a basis to this hypothesis or to show an accumulation of glycogen and closely related sugars. Further studies, however, of the mode of action of edathamil in producing this nephrosis are in progress. The renal lesion in the rat is remarkably similar to that occurring in man.[5] The eosinophilic amorphous debris in the reticular macrophages of the tissues found at autopsy of the two patients was not found, however, in the rats.

The demonstration of the hazard of renal damage occurring with the therapeutic use of edathamil should not be considered as a cause for stopping clinical use of the drug. Edathamil has proved too useful clinically to envision any curtailment in its use. The reversibility of the lesions and the prolonged administration of large doses of the drug required to produce lesions offer obvious means of using edathamil safely. As an additional safety factor, it is recommended that the drug be used in doses approximating 50 mg. per kilogram per day, given for five days, followed by two days of rest. Its use should always be attended by daily urinalysis and should be stopped at once with the onset of signs of renal tubular alteration. Whether the existence of various stages of acute and chronic nephritis precludes use of the drug is not known, but logically it would seem

necessary to proceed with extreme caution in the presence of a nephritis. Toxic nephrosis, such as mercurial poisoning, would certainly seem to exclude the use of edathamil because of the danger of synergistic enhancement of the nephrotic damage with ordinary safe dosage levels of the drug.

Summary and Conclusions

The occurrence of toxic nephrosis in a woman under therapy with edathamil calcium-disodium for mobilization of plutonium prompted experimental reinvestigation of the toxicity and histopathology of the drug. Numerous experiments done on rats demonstrated for the first time that edathamil is a potentially hazardous drug, since, on repeated administration of moderately large doses, it produced nephrosis. The specific nature of the lesion was severe hydropic degeneration of the proximal tubules. The lesion was found to be reversible and was cleared within a few days after cessation of administration of the drug. None of the other tissues of the body that were examined appeared to be affected. The E. D_{50} (amount of the drug required to produce the first histological evidence of damage in 50% of the animals treated daily for 16 days) was 203 mg. per kilogram of body weight per day. With a dose of 62.5 mg. per kilogram per day, no kidney lesions were demonstrated.

The results of these experiments should promote caution in use of the drug. The reversibility of the lesions and the prolonged administration of large doses of the drug required to produce lesions offer an obvious means for using the drug safely. It is suggested that the drug be used in doses of approximately 50 mg. per kilogram per day, given for five days, and followed by two days of rest before the regimen is repeated. Daily urinalysis should be done during the treatment period, and, with onset of unusual urinary findings, administration of the drug should be stopped. The presence of acute or chronic renal disease should probably cause reluctance in use of the drug.

P. O. Box 1663 (Dr. Foreman).

Printed and Published in the United States of America

Reprinted from the A. M. A. Archives of Industrial Hygiene and Occupational Medicine
February 1953, Vol. 7, pp. 141-151

USE OF CALCIUM ETHYLENEDIAMINETETRAACETATE IN CASES OF LEAD INTOXICATION

H. FOREMAN, M.D.
LOS ALAMOS, N. MEX.
H. L. HARDY, M.D.
BOSTON
T. L. SHIPMAN, M.D.
LOS ALAMOS, N. MEX.
AND
E. L. BELKNAP, M.D.
MILWAUKEE

IN RESPONSE to the considerable interest which has been expressed in calcium ethylenediaminetetraacetate (Ca EDTA),[1] a new drug which shows promise in the treatment of heavy-metal poisoning, we are suggesting a protocol for the use of the drug in lead intoxication.

The purpose of suggesting a uniform mode of treating patients with this drug is to promote the use of case material as efficiently as possible in gathering information about the drug. It is hoped that during these initial stages the trial of the drug will be limited to experienced clinical investigators with adequate facilities for observation and follow-up of the patients.

As an aid to such investigators, we have summarized the experience in the use of the drug in humans to date and are indicating a dosage schedule based on toxicity studies in animals. These studies were carried out by Dr. George Maison of the Department of Pharmacology, Boston University School of Medicine.[2]

Mr. Manfred Bowditch, Director of Health and Safety of the Lead Industries Association, has agreed to hold a registry of such protocols of cases of lead poisoning treated with Ca EDTA in his office at 420 Lexington Ave., New York 17. It is planned by this means to make the information collected centrally available for study.

1. (*a*) Braunman, S. P.; Reid, H., and Rubin, M.: Med. Ann. Dist. Columbia, June, 1952. (*b*) Sudbery, J. B.: Personal communication to M. Rubin. (*c*) Belknap, E. L.: Industr. Med. 21:205, 1952. (*d*) Van Ordstrand, H. S.: Personal communication to the authors. (*e*) Proescher, F.: Proc. Soc. Exper. Biol. & Med. 70:619, 1951. (*f*) Lassle, D., and Schilling, A.: Personal communication to M. Rubin. (*g*) Gellhorn and Schaglen-Edwards: Personal communication to M. Rubin. (*h*) Grant, W. M.: Personal communication to M. Rubin. (*i*) Gebers, R. F., and Raymond, S.: J. Urol. 66:474, 1951. (*j*) Abrahmsen, B., and Weinberg, T.: J. Urol. 66:316, 1951. (*k*) Kinsin, B., and Natelson, S.: Science 113:307, 1950. (*l*) Popovici, A.; Geschickter, C. F.; Reinovsky, A., and Rubin, M.: Proc. Soc. Exper. Biol. & Med. 74:415, 1950. (*m*) Popovici, A.; Geschickter, C. F., and Rubin, M.: To be published. (*n*) Geschickter, C. F.: Effect of Chelating Compounds on Body Distribution of Radio Calcium, read before meeting of Bio-Medical Program Directors, New York, Feb. 20, 1951.

2. Ca EDTA may be obtained by writing Riker Laboratories, Inc., 8480 Beverly Blvd., Los Angeles.

PROTOCOL FOR THE USE OF Ca EDTA IN LEAD INTOXICATION

Aims

1. To test the efficacy of Ca EDTA in accelerating the excretion of lead in humans
2. To determine the toxic effects of Ca EDTA, if any
3. To obtain data for establishing an optimum dosage
4. To determine the best mode of administration of the drug

Necessary Clinical Data

Name, sex, and age
Home address
Physician in charge
Hospital
Occupational history in the case of an adult
Exposure history in the case of a child
Medical history and examination
1. Character of onset
2. Clinical course, including response to previous therapy
3. Pretreatment physical examination, with particular attention to
   (a) Lead line of the gums
   (b) Involvement of the peripheral nerves by weakness or palsy of extensor muscles
   (c) Visible jaundice
   (d) Involvement of central nervous system, i. e., encephalopathy

Medical examination during treatment
1. Evidence of side, or toxic, actions in
   (a) Cardiovascular system
   (b) Gastrointestinal tract
   (c) Genitourinary tract
   (d) Central nervous system
   (e) Peripheral blood and bone marrow studies where possible
2. Appraisal of effects, both objective and subjective

Medical examination after treatment
1. Careful observation for possible reactions for at least a month and at intervals thereafter, depending on circumstances

Necessary Laboratory Data
1. If feasible, starting one week before the beginning of treatment, collect daily urine specimens and/or blood samples for lead assay; continue daily assays for two or three days following cessation of treatment; when possible, coproporphyrin studies should be done on the urine
2. Hematological tests to be made include hemoglobin values, white blood cell count, differential count, stippled cell count, reticulocyte count, hematocrit reading, bleeding and clotting time, and bone marrow biopsy (or direct smears if removal of marrow for biopsy is deemed impractical)—one set of values in pretreatment
3. Routine urine analysis and Addis count
4. Further studies should include serum calcium and phosphorus levels, if possible, total protein, albumin-globulin ratio, nonprotein nitrogen level, prothrombin time, and presence of urine urobilinogen

For close follow-up of the patient, it is urged that certain laboratory tests should be made daily during treatment. These tests include hemoglobin values, white blood cell count, differential count, stippled cell count, serum calcium and phosphorus levels, prothrombin time, and presence of urine urobilinogen, blood lead and/or urinary lead, and primary coproporphyrin, if quantitative test is available. Other tests should be made as frequently as seems practical or as

circumstances indicate. This follow-up should continue for three to seven days after the last administration of the drug and then for longer intervals as seems wise to the investigator.

Suggested Dosage

1. Route by intravenous drip only; dose of not more than 0.5 gm. per 30 lb. (13.6 kg.) of body weight per hour
2. Total dose limited to 1 gm. per 30 lb. of body weight for 24 hours
3. Maximum total dose per week (7 days) of 5 gm. per 30 lb. of body weight in divided doses
4. Individual courses of treatment limited to 10 days, with a one-week rest period between; maximum dose of 7.5 gm. per 30 lb. of body weight over 10 days

COMMENT

The use of ethylenediaminetetraacetic acid (EDTA) in humans is still so recent that very few reports have reached the stage of publication. Most of the information contained in the accompanying table was obtained by personal communication with those presently engaged in the work. Because of the current nature of the work, the compilation necessarily is not complete. It covers, however, most of the cases up to July, 1952. It is surprising that, in view of the recent application of this agent to biological problems, there have already been more than 100 instances in which the drug or its chelates were administered to humans.

Of the eight patients with lead poisoning treated with Ca EDTA, five were children and three adults. In the four children whose cases were acute there was marked alleviation of symptoms within several hours of treatment. In every patient in whom urinary Pb assays were determined, there was considerable enhancement of Pb excretion following treatment, up to a factor as high as 20 in one person. There was no evidence of toxicity from the use of the drug, nor were there symptoms associated with the mobilization of Pb. The highest single dose of Ca EDTA administered was 10 gm. given to a patient with beryllosis. This patient received a total of 94.9 gm. over a period of 22 days. In a very large series, the uncomplexed acid was used as an anticoagulant for transfusions into patients. Even in persons receiving multiple transfusions there was no evidence of ill effect. In other patients the EDTA was given experimentally to study Ca metabolism. In one striking incidence, a patient with terminal carcinoma with osteolytic lesions, in whom the serum Ca levels were as high as 25 mg./100 cc., a single dose of 20 gm. of EDTA was administered intravenously in 20 minutes, and the serum Ca level was brought down to 8.8 mg./100 cc. in one hour. In other experimental studies, the Ni and Mg chelates were administered. The use of the Mg chelate resulted in appreciable depression of the serum Ca and P levels. This is to be expected in view of the fact that Ca will displace Mg from combination with EDTA and will be removed from the body as the chelate of the EDTA. Other human applications included such uses as ureteral irrigation to dissolve calculi and eyewashes to remove calcific deposits. Topical applications in the form of water-soluble ointments indicate that EDTA is readily carried through the skin.

The low toxicity of Ca EDTA is explained by the findings in studies using the C^{14}-labeled material. The material is excreted unchanged. All of the drug can be accounted for in the excreta. Sixty to 70% of the administered drug is present in the urine after 6 hours, 95 to 99% after 25 hours. The remainder is in the feces. Distribution studies reveal no unusual localization of the drug in any organ during the time the material is in the body.

Printed and Published in the United States of America

Various Uses of Ethylenediaminetetraacetic Acid (EDTA)
(Complexing of Ions)