TREATMENT OF CHRONIC LEAD-POISONING WITH CALCIUM DISODIUM VERSENATE

BY

A. C. MARKUS, B.M., B.Ch.

AND

A. G. SPENCER, M.D., M.R.C.P.

(From the Medical Unit, University College Hospital, London)

The treatment of lead-poisoning by the use of intravenous calcium disodium ethylenediamine tetra-acetic acid (E.D.T.A.; versenate) has been described by several authors in America, both in children (Byers and Maloof, 1954; Bessman *et al.*, 1952, 1954; Karpinski *et al.*, 1953) and in adults (Belknap, 1952; Sidbury *et al.*, 1953; Hardy *et al.*, 1954). The first report from this country has been published recently (Giles *et al.*, 1955). All authors agree that treatment with versenate gives prompt relief from the distressing symptoms of lead-poisoning.

Calcium disodium versenate is a synthetic chelating agent that removes lead from the body by the formation of a soluble non-ionized lead complex which is excreted in the urine. The pharmacology and mode of action of versenate are described in detail by Karpinski *et al.* (1953).

In chronic lead-poisoning lead is stored in the bones, and this may subsequently be released by infection or metabolic stress and cause acute lead-poisoning (Aub *et al.*, 1925). It has been shown that lead can be mobilized by the administration of ammonium chloride or "para-thor-mone" (Hunter and Aub, 1927). However, such treatment may also cause a recurrence of the symptoms of lead-poisoning, and opinion is divided, therefore, on the wisdom of attempts to de-lead the skeleton (Cantarow and Trumper, 1944). It is possible that during treatment with versenate most of the lead excreted in the urine is derived from the body fluids and soft tissues, and that lead deposited in the skeleton is less readily accessible. For this reason, Giles *et al.* (1955) have tried treatment with versenate and ammonium chloride, but they did not obtain an increase in the rate of excretion of lead in the urine above that produced by versenate alone.

This paper describes the excellent results obtained by the use of versenate in three patients with chronic lead-poisoning. In addition, an attempt was made to mobilize lead stored in the bones and to increase its rate of excretion in the urine by the simultaneous use of para-thor-mone and versenate.

Case 1

A man aged 33, employed as a vitreous enameller for seven years, was referred to U.C.H. with complaints of anorexia, constipation, and headaches over the past year, and recently of repeated attacks of abdominal pain which lasted for hours and doubled him up. His doctor had successfully treated these attacks with intravenous calcium gluconate. The patient had also noticed an unpleasant taste in his mouth, that the grip of his left hand was weak, and that he had difficulty in concentration, as though "a cloud was over my brain." There was a well-developed lead line on the gums, but no distinct pallor of the skin. The B.P. was 140/80 and there were no objective abnormalities in

the central nervous system other than a coarse tremor of the hands. Before treatment he was observed to have several attacks of colicky pain in which the abdomen became rigid.

Laboratory Tests.—Hb, 80%. Punctate basophil count, 11,200 per million red blood cells. Urine: albumin +; sugar 0; no excess of amino-acids. Qualitative tests for coproporphyrins ++. Blood urea, 48%; urea clearance, 106%. Urine lead, 400 and 300 µg. a day.

Two four-day courses of versenate were given, separated by two rest days. Dosage: 2 g. of versenate dissolved in one litre of normal saline given intravenously over nine hours (0.3 g./30 lb. (13.6 kg.) body weight a day). Parathor-mone, 150 U.S.P. units a day intramuscularly, was given during the second course. The excretion of lead in the urine is shown in the Chart. The headaches, tremor, and colic were rapidly relieved. The lead colic returned on the third day of para-thor-mone injections, but was again relieved within 30 minutes of starting the versenate drip, and did not return. The excess urine coproporphyrins had disappeared by the end of treatment.

Two months later the patient said that he still felt better than he had for years, and had gained 12 lb. (5.4 kg.) in weight: Hb, 110%; punctate basophil count less than 1,000 per million red blood cells; qualitative tests for urine coproporphyrins, negative; no albumin.

Excretion of lead in urine in Cases 1, 2, and 3.

Case 2

A man aged 42, employed as a vitreous enameller for three years in the same factory as Case 1, complained of ill-health, lack of concentration, irritability, and headaches over the past six months. He had a bad taste in the mouth, a poor appetite, habitual constipation, and suffered from occasional attacks of abdominal pain, which were not severe. He was a thin, unhealthy-looking man with extensive gingivitis and a lead line on the gums. B.P. 140/70. The only abnormality found in the C.N.S. was a tremor of the hands.

Laboratory Tests.—Hb, 92%. Punctate basophil count, 4,000 per million red blood cells. Urine: no albumin, no sugar, no excess of amino-acids. Qualitative test for coproporphyrins +++. Blood urea, 33 mg. per 100 ml. Urea clearance, 94%. Urine lead, 540 and 540 µg. a day.

The treatment was as in Case 1, except that the dose of versenate was 4 g. a day, which was 1 g./30 lb. body weight a day. The excretion of lead in the urine is shown in the Chart. The symptoms were completely relieved by the end of the first course of treatment and there was then no excess of urine coproporphyrins. Six weeks later the patient still felt very well: Hb, 98%; punctate basophil count, less than 500 per million red blood cells; urine: no excess of coproporphyrins; no albumin.

Case 3

A man aged 47, a vitreous enameller employed for nine years in the same factory as Cases 1 and 2, had previously been treated for lead-poisoning in 1950 at the London Hospital. He attended U.C.H. for screening tests with other employees, but at that time had few spontaneous complaints. Because of a strongly positive qualitative test for urinary coproporphyrins he was offered treatment, but was unable to accept as his wife was ill. Subsequently he reported sick

with abdominal colic and was admitted for treatment. He then admitted to general malaise, constipation, and milder attacks of abdominal pain for the past four months. There was no pallor and no lead line, the patient being edentulous. The B.P. was 160/90; there was a coarse tremor of the hands, otherwise no abnormality in the C.N.S.

Laboratory Tests.—Hb, 88%. Punctate basophil count, 2,000 per million red blood cells. Urine: albumin +; no sugar and no excess of amino-acids. Qualitative tests for coproporphyrins + +. Blood urea, 38 mg. per 100 ml. Urea clearance, 140%. Urine lead, 560 and 610 μ g. a day.

The treatment was the same as in Case 2. Versenate dosage 4 g. a day (0.8 g./30 lb. body weight a day). The excretion of lead in the urine is shown in the Chart. The symptoms were completely relieved and the patient felt very well by the end of the second course of treatment: Hb, 97%; punctate basophil count, less than 500 per million red blood cells; qualitative test for urine coproporphyrins negative.

Methods

Lead.—Lead determinations were carried out at the M.R.C. Unit of Industrial Toxicology by the method recommended in the Report of the Lead Panel (1954). Normal values for lead excretion = <80 μ g. Pb a day. Upper values for lead workers = 250 μ g. Pb a day.

Coproporphyrins.—Three drops of hydrogen peroxide, 5 drops of glacial acetic acid, and 1–2 ml. of ether were added to 5 ml. of fresh urine in a test-tube and shaken. After ten minutes the ether layer was examined under Wood's lamp for the characteristic reddish-pink fluorescence.

Discussion

Calcium disodium versenate is the calcium complex of a synthetic amino-acid, ethylenediamine tetra-acetic acid:

The nitrogen atoms donate electrons to metals, completing their atomic configuration, and in this way form stable ring structures in which the metal is non-ionized. The bonds indicated by the arrows are responsible for the poor ionization. Metals such as lead, copper, and nickel are bound or chelated more firmly than is calcium and displace it from the complex. Fortunately, essential elements such as iron, copper, and cobalt are fixed even more strongly by the tissues, and the administration of calcium disodium versenate does not appreciably increase the rate of excretion of these metals from the body; furthermore, the plasma concentration of calcium, magnesium, and sodium is not significantly altered by this treatment (Cotter, 1954; Spencer *et al.*, 1952). The metal, when chelated, is freely soluble and is readily excreted in the urine, but as it is not ionized it does not exhibit its usual chemical and pharmacological properties (Cotter, 1954).

In the treatment of heavy metal poisoning the calcium complex is used, as versenate alone combines with calcium in the plasma and if given in large doses may cause hypocalcaemic tetany and death (Popovici *et al.*, 1950; Spencer *et al.*, 1952). Animal experiments have shown that calcium disodium versenate is relatively non-toxic and has a high therapeutic index (Bauer *et al.*, 1952). The intravenous LD₅₀ for rats is 3,000 mg. per kg., compared with the effective therapeutic dose of less than 50 mg. per kg. per day. In patients few toxic reactions have been reported from the use of calcium disodium versenate. Transient depression of the bone marrow occurred in one case (Belknap, 1952), and T-wave changes in the electrocardiogram were demonstrated by Bradley and Powell (1954).

Calcium disodium versenate can be given by mouth and does not to any extent chelate heavy metals present in the gut (Bradley and Powell, 1954; Cotter, 1954). However, it is much more effective when given intravenously. When versenate labelled with ¹⁴C was given intravenously 90% was excreted within six hours (Foreman *et al.*, 1953b). This suggests that the most effective way of giving versenate is by a slow continuous intravenous infusion.

Dosage has largely been empirical, most workers following the recommendations of Foreman *et al.* (1953a)—namely, 1 g./30 lb. body weight/day at a maximum rate of 0.5 g./30 lb./hour intravenously, and not exceeding 5 g./30 lb./week. Since 1 mol of versenate can bind 1 mol of lead, the administration of 0.5 g. of versenate would, if 100% efficient, promote the excretion of 260 mg. of lead. However, the maximum observed excretion of lead during treatment with versenate has been 4 mg. a day in children (Giles *et al.*, 1955) and 10 mg. a day in adults (Hardy *et al.*, 1954), an efficiency of only about 1%. Bessman *et al.* (1954) have suggested that the reason for this low efficiency is that versenate has to compete for lead with other binding agents such as the sulphhydryl group of proteins.

The three patients in this series responded very quickly to treatment with disodium calcium versenate, as did those previously reported. Relief from lead colic and from cerebral symptoms such as headache and irritability occurred within an hour of starting treatment, and the patients were completely free of symptoms by the end of two four-day courses. There were no toxic effects. The excess of coproporphyrins disappeared from the urine, the punctate basophil count gradually fell, and the haemoglobin returned to normal levels. The excretion of lead in the urine was greatly increased during the infusion of versenate and reached a maximum of 5 mg. of Pb a day.

The first patient who was given versenate in a dosage of 0.3 g./30 lb./day excreted much less lead in the urine than did the second and third patients, who were given 0.8 and 1 g./30 lb./day. The higher dose caused no ill effects and was much more effective in promoting lead excretion, probably because it was given in a very slow intravenous infusion over nine hours. Bessman *et al.* (1954) had reported that increasing the dose of versenate did not increase the excretion of lead, but in their observations it was given over a shorter period of time, and much of it may have been excreted in the urine before coming into equilibrium with lead in the tissues. It should be noted that the smaller dose of versenate was just as effective in relieving the acute symptoms of lead-poisoning as was the larger dose, and there was no recurrence of symptoms in this patient in the six months of observation following the period of treatment. It does not seem necessary to attempt the removal of all the lead stored in the body, and indeed this would probably need 10–20 courses of treatment.

Para-thor-mone and ammonium chloride have both been used to remove lead from the skeleton (Hunter and Aub, 1927). In the present series, combined treatment with calcium disodium versenate and injections of para-thor-mone did not cause a sustained rise in the excretion of lead in the urine greater than that obtained with calcium disodium versenate alone. However, there may have been an initial increase in the excretion of lead in the stools as in the cases reported by Hunter and Aub (1927). Giles *et al.* (1955) have reported that the administration of ammonium chloride and versenate to one patient did not result in an increased excretion of lead in the urine above that produced by versenate alone. The high rate of excretion of lead in the urine and the excellent clinical results obtained by treatment with calcium disodium versenate alone are so uniformly satisfactory that the use of acidifying agents or of para-thor-mone does not seem justified.

Summary

The use of calcium disodium versenate in the treatment of three patients with chronic lead-poisoning is described. This treatment rapidly relieved the symptoms

of lead-poisoning and promoted the excretion of large amounts of lead in the urine. No toxic effects were observed.

The administration of para-thor-mone during treatment with versenate did not increase the rate of excretion of lead in the urine above that obtained by versenate alone.

The chemistry, pharmacology, and method of use of versenate are discussed.

We wish to thank Dr. Donald Hunter, Dr. Lesley Bidstrup, and Mr. B. J. Biles, of the Medical Research Council Department for Research in Industrial Medicine, for the lead estimations; and Mr. V. K. Asta for the chart.

REFERENCES

- Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P. (1925). *Medicine (Baltimore)*, 4, 1.
 Baucr, R. O., Rullo, F. R., Spooner, C., and Woodman, E. (1952). *Fed. Proc.*, 11, 321.
 Belknap, E. L. (1952). *Industr. Med.*, 21, 305.
 Bessman, S. P., Reid, H., and Rubin, M. (1952). *Med. Ann. Distr. Columbia*, 21, 312.
 — Rubin, M., and Leikin, S. (1954). *Pediatrics*, 14, 201.
 Bradley, J. E., and Powell, A. M. (1954). *J. Pediat.*, 45, 297.
 Byers, R. K., and Maloof, C. (1954). *Amer. J. Dis. Child.*, 87, 559.
 Cantarow, A., and Trumper, M. (1944). *Lead Poisoning*, p. 195. Williams and Wilkins, Baltimore.
 Cotter, L. H. (1954). *J. Amer. med. Ass.*, 155, 906.
 Foreman, H., Hardy, H. L., Shipman, T. L., and Belknap, E. L. (1953a). *Arch. industr. Hyg.*, 7, 148.
 — Vier, M., and Magee, M. (1953b). *J. biol. Chem.*, 203, 1045.
 Giles, H. McC., Moore, C. J., and Still, B. M. (1955). *Lancet*, 1, 183.
 Hardy, H. L., Elkins, H. B., Ruotolo, B. P. W., Quinby, J., and Baker, W. H. (1954). *J. Amer. med. Ass.*, 154, 1171.
 Hunter, D., and Aub, J. C. (1927). *Quart. J. Med.*, 20, 123.
 Karpinski, F. E., Rieders, F., and Girsh, L. S. (1953). *J. Pediat.*, 42, 687.
 Popovici, A., Geschickter, C. F., Reinovsky, A., and Rubin, M. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, 74, 415.
 Report of Lead Panel (1954). *Analyst*, 79, 397.
 Sidbury, J. B., Bynum, J. C., and Fetz, L. L. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, 82, 226.
 Spencer, H., Vankinscott, V., Lewin, I., and Laszlo, D. (1952). *J. clin. Invest.*, 31, 1023.

TRICHOMONAS VAGINALIS INFECTION IN THE MALE

BY

W. E. COUTTS, M.D.

R. VARGAS-SALAZAR, M.D.

EDNA SILVA-INZUNZA, M.D.

R. OLMEDO, M.D.

R. TURTELTAUB

AND

J. SAAVEDRA

(From the Urological Clinic, Jose Joaquin Aguirre Clinical Hospital, School of Medicine, Chile University, and Anti-venereal Policlinics of the National Health Service of Chile)

In recent years fungi, viruses, spirochaetes, and protozoa have tended to displace the gonococcus in the causation of acute and chronic inflammation of the lower genito-urinary tract in both sexes, and for this reason more examples of such infections are now seen at urological clinics. Some authors believe that the types of trichomonads described—*Trichomonas vaginalis* (genital), *T. hominis* (intestinal), and *T. tenax* (buccal)—belong to one species only, their morphological differences arising from their particular human "habitat" (*Standortsvarietäten-Flaskamp*). But Wenrich (1947) suggests that the different trichomonads infesting man belong to distinct species. Despite occasional reports of trichomonal infection of the male urethra, such infection has been regarded for many years as affecting women only. However, with the increasing occurrence of residual post-gonococcal urethritis following adequate penicillin therapy, and the higher incidence of non-

gonococcal urethritis in the male, *T. vaginalis* infection with its complications in both sexes is gaining wider recognition as a definite entity. Some authors (Pomeroy, 1948; Freed, 1948) refer to it as the "seventh venereal disease."

The Parasite and its Recognition

T. vaginalis is a pear-shaped, ovoid, or round pentagellated protozoon, 10–20 μ long by 8–10 μ broad, though much smaller forms, measuring 2 to 3 μ , are not uncommon. Four free flagella arising at the blepharoblast, part of the neuromotor system, emerge from the upper pole of the body of the parasite. The so-called fifth flagellum is attached to one of the borders of the body and forms part of the undulating membrane. Even the smallest forms have flagella, though occasionally only a single but very long, thin flagellum is present. Small, non-flagellated trophozoites have been observed; these are degenerative forms which recover their properties when they change habitat (Wenyon, 1926). Our own observations have led us to conclude that they represent evolutive forms of the life cycle.

In fresh preparations examined under dark-field illumination the free extremity of one or more flagella can be seen bending and unbending, inwards and occasionally outwards (Feinberg). This phenomenon can also be seen in fixed, unstained smears (Fig. 1). Occasionally in such preparations the flagella appear segmented. If fresh preparations are observed under dark-field illumination with interposed polarizing filters (Silva-Inzunza's technique), flagella appear coloured red. The flagellum of the undulating membrane is without colour, and for this reason we believe it to be an independent structure. Thus the parasite is really a tetra-flagellate (Silva-Inzunza and Coutts, 1954). When fixed, unstained smears are examined under dark-field illumination a cystostome is clearly visible in the upper pole of the body of some parasites, the common axis from which flagella branch off emerging from the borders of this opening (Fig. 2). At the lower pole another opening may be seen, through which the axostyle, a pointed, more or less rigid structure of doubtful significance, is probably ejected. Intracytoplasmic structures—nucleus, blepharoblast, and parabasal body—are too well known to need description. Little is known about multiplication and development of *T. vaginalis*; multiplication may be by simple binary division or by budding (Fig. 3). Cysts and pseudocysts have also been described.

The presence of *T. vaginalis* in secretions or urinary sediment can be established by examination of fresh preparations or of unstained smears fixed in 10% formol. This last type of preparation has the advantage that the sample can be collected anywhere and sent to a laboratory for diagnosis. Fresh preparations can be viewed with simple, dark-field, or phase-contrast microscopy; fixed, unstained smears are best studied under sunlight or lamplight dark-field. Less satisfactory results are obtained with stained smears. In fresh preparations treated with 5% aqueous solution of fluorescein (vital stain) all living forms of the parasite rapidly assume a brilliant green colour (trophozoites, cysts, etc.). This technique is very useful when the material contains numerous pus cells and few trophozoites (Coutts and Silva-Inzunza, 1954).

Pathogenicity

The subject of the pathogenicity of *T. vaginalis* has been much discussed (Rodecurt, 1952). Some authors doubt or deny that it has pathogenic properties, others favour a facultative pathogenicity, while still others stress the influence of co-infectors (anaerobes, viruses, spirillae). The experiments of Trussell and Plass (1940), of Hesseltine and others in women, and those of Lanceley and McEntegart (1953) in men, however, have established beyond doubt the pathogenicity of *T. vaginalis*.

In adult males contagion is usually of a sexual nature. We have seen a few cases in prepubertal boys, mostly vagabonds living promiscuously. We have seen pederasts fortuitously infected by another who shortly before had been