added to 20 ml. of N/4 NaOH. Results were read on the Spekker absorptiometer using an Ilford No. 8 red filter. Standard curves were prepared as follows: into three tubes were added (1) 0.5 ml. of working standard and 1.5 ml. of water; (2) 1 ml. of working standard and 1 ml. of water; and (3) 2 ml. of working standard. To each tube were then added 20 ml. of N/4 NaOH and 3 ml. of dilute Folin and Ciocalteau reagent, which were mixed and read on the Spekker absorptiometer. Calculation was as described by Edwards *et al.* (1960).

Results

Accuracy of Method.—The 44 estimations made on 12 specimens of plasma gave a coefficient of variation of 17.8%.

Variation of Plasma Pepsinogen in Patient.—A total of 107 serial estimations were made on 26 patients on a two-hourly basis beginning at 7.30 a.m. The coefficient of variation proved to be 31%.

Random Levels on Patients.—The Table shows a frequency distribution of various levels of plasma pepsinogen in 521 patients with various diagnoses. The diagnosis of non-organic dyspepsia was established by radiographic exclusion of peptic ulcer, gastritis, and carcinoma in the presence of a long history of dyspepsia; duodenal and gastric ulcer were established by radiographic demonstration, gastritis by the typical radiographic appearance in the absence of demonstrable ulcer, and pernicious anaemia by blood and sternal marrow films. High levels did not occur in female non-organic dyspepsia or in pernicious anaemia. Male duodenal ulcer alone was distinguished by a different frequency distribution and had significantly more cases in the range of 200 to 400 units. Female duodenal ulcer was not so distinguished.

Frequency Distribution of Grouped Random Single Plasma Pepsinogen Levels in 521 Patients with Various Diagnoses

	Plasma Pepsinogen (units/ml.)					
	0–96	104–194	200–296	304–398	400–500+	Total
Non-organic dyspepsia: Male	53	46	17	3	1	120
Non-organic dyspepsia: Female	32	33	12	0	0	77
Duodenal ulcer: Male ..	25	58	52	23	3	161
Duodenal ulcer: Female ..	18	35	7	4	2	66
Gastric ulcer: both sexes	19	25	8	1	0	53
Pernicious anaemia: both sexes	14	5	1	0	0	20
Gastritis: both sexes ..	4	2	8	5	5	24

The group or two groups which include two-thirds or more of the cases in that diagnosis have been boxed in.

Discussion

Our coefficient of variation for the method of 17.8%, and for the patients over one day of 31%, compared unfavourably with the 8% average (range 2 to 22%) which Edwards *et al.* quoted Mirsky *et al.* as finding from hour to hour on any day on their patients, and the 12–18% which they claimed for daily specimens spread over a month. Our results were obtained under representative general diagnostic hospital conditions and showed the test to be unsuitable for use in these conditions. It is basically complicated and the results have not proved satisfactorily reproducible. Single estimations gave no reliable or decisive diagnostic information.

Summary

The Mirsky method of estimating plasma pepsinogen as proposed by Edwards *et al.* (1960) has not given satisfactory results when used under general diagnostic conditions. Single plasma pepsinogen levels did not give reliable or decisive diagnostic information.

We thank Dr. Robert Kemp for his interest and the use of his records.

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PENICILLAMINE AS LEAD-CHELATING SUBSTANCE IN MAN

BY

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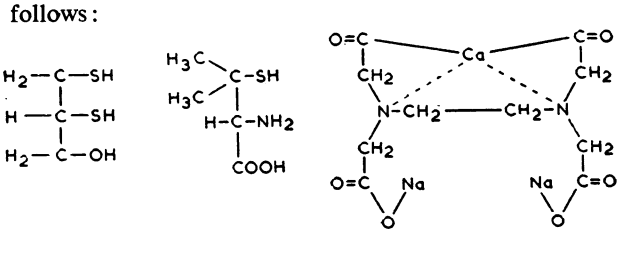
Ever since dimercaprol (B.A.L.) came into use in 1945 much interest has been devoted to chelating agents, not least in connexion with lead poisoning. After only a few years, however, it became clear that dimercaprol was not particularly suitable as an antidote to plumbism. About the same time the first favourable reports of treatment with sodium calciumedetate (calcium disodium ethylenediaminetetra-acetic acid; $CaNa_2$E.D.T.A.) were published (Belknap, 1952; Bessman *et al.*, 1952; Karpinski *et al.*, 1953; Ohlsson, 1954; Rieders *et al.*, 1955; Sidbury, 1955; Glömme and Swensson, 1956). But this substance also gave rise to disappointments; for several cases of renal damage, some of them fatal, occurred after excessive dosage (Dudley *et al.*, 1955; Foreman *et al.*, 1956; Moeschlin, 1957).

Penicillamine (β-β-dimethylcysteine) was introduced by Walshe (1956) for the treatment of Wilson's disease. He reported good effects on the urinary excretion of copper when penicillamine was given intravenously or by mouth.

The formulae of these three chelating agents are as follows:



DIMERCAPROL PENICILLAMINE $CaNa_2$ E.D.T.A.

Since the beginning of 1957 penicillamine has been used at this hospital in the treatment of lead poisoning. At first administration was by intravenous drip—1 to 3 g. of penicillamine in 500 ml. of physiological NaCl over two to three hours. The effect on the urinary excretion of lead was compared with that of $CaNa_2$

E.D.T.A. The two substances were also given together to permit study of a possible synergistic action. Later on, penicillamine was given by mouth in dosage ranging from 0.3 to 1 g. daily. The results are now reported.

Case Reports

Case 1.—An 18-year-old youth had been employed in a printing works for three years. He was admitted to hospital for symptoms suspected to be caused by lead poisoning. However the amount of lead in the urine was normal (30 μg./24 hours), as was the concentration of lead in the blood (40 μg./100 ml.). On each of two successive days he received 2 g. of penicillamine intravenously. The 24-hour urinary lead excretion then rose to 180 and 150 μg., respectively, and the level in the blood fell to <10 μg./100 ml. (Table I).

TABLE I.—*Cases Treated with Intravenous Penicillamine and $CaNa_2E.D.T.A.$*

Date	Penicillamine I.V.	$CaNa_2$ E.D.T.A. I.V.	Daily Output of Lead in Urine (μg.)	Blood Level of Lead (μg./100 ml.)
		Case 1		
8/10/57			30	38
9/10/57	2 g.		180	19
10/10/57	2 ,,		150	10
11/10/57			30	
		Case 2		
24/1/57			170	
25/1/57		2 g.	2,000	
26/1/57		2 ,,	1,500	
27/1/57		2 ,,	1,900	
24/6/57			230	
25/6/57	1 g.		1,400	
26/6/57	1 ,,		1,100	
27/6/57	2 ,,		1,400	
28/6/57	3 ,,		1,200	
		Case 3		
23/4/57			230	
16/5/57	1 g.		420	
17/5/57	2 ,,		1,000	
18/5/57	2 ,,		870	
19/5/57			60	
20/5/57		2 g.	1,300	
21/5/57		2 ,,	640	
22/5/57		2 ,,	590	
		Case 4		
11/9/57			230	
12/9/57		2 g.	7,200	70* 50†
13/9/57		2 ,,	6,400	105 60
14/9/57	2 g.	2 ,,	6,500	80 30
15/9/57		2 ,,	4,400	60 28
16/9/57	2 ,,		1,200	55 55
17/9/57	2 ,,		2,300	60 16
18/9/57	2 ,,	2 ,,	3,600	19 17
19/9/57	2 ,,		1,900	29 15
20/9/57			360	
		Case 5		
15/10/57			210	124
16/10/57	2 g.		1,900	30
17/10/57	2 ,,		2,000	18
18/10/57	2 ,,	2 g.	4,000	25
19/10/57			410	
16/12/57			70	

* Before infusion. † After infusion.

Case 2.—A 50-year-old foundry worker, in whom periodic urine analyses had shown increased output of lead for about two years, was given 2 g. of $CaNa_2E.D.T.A.$ intravenously on three successive days in January, 1957. The 24-hour urinary lead excretion, which was 170 μg. prior to treatment, rose to 2,000, 1,500, and 1,900 μg. He was readmitted in June, 1957, when the 24-hour output of lead was 230 μg. In response to penicillamine for four days in intravenous doses of 1, 1, 2, and 3 g., the amount of lead excreted in the urine rose to 1,400, 1,100, 1,400, and 1,200 μg. (Table I).

Case 3.—A 33-year-old man had worked for some weeks in breaking-up operations on a ship's hull. He was admitted to hospital with acute abdominal pain. The urinary output of lead was 230 μg./24 hours. Penicillamine was given intravenously for three days (1, 2, and 2 g.), whereupon the lead excretion rose to 420, 1,000, and 870 μg. After a treatment-free day, during which only 60 μg. of lead was excreted in the urine, $CaNa_2E.D.T.A.$ was administered intravenously for three days. The respective urine lead readings then were 1,300, 640, and 590 μg. (Table I).

Case 4.—A 34-year-old man had been engaged in scraping and repainting electric power-line poles for several weeks. He was admitted to hospital for acute abdominal pain. A 24-hour urine specimen contained 230 μg. of lead. Intravenous injection of $CaNa_2E.D.T.A.$ (2 g.) for two days raised the excretion of lead to 7,200 and 6,400 μg., respectively. On the following day 2 g. of penicillamine was given intravenously as well as 2 g. of $CaNa_2E.D.T.A.$; the urine contained 6,500 μg. of lead. The next day's treatment consisted of $CaNa_2E.D.T.A.$ alone (2 g.) and the urinary lead content was 4,400 μg. On the sixth and seventh days of hospitalization 2 g. of penicillamine was given alone and the lead excretion was 1,200 and 2,300 μg. Both chelating agents were administered on the eighth day and 2 g. of penicillamine alone on the ninth day; the respective urinary lead concentrations were 3,600 and 1,900 μg. The blood lead was measured daily before and after the infusions (Table I).

Case 5.—A 35-year-old man had scraped and repainted power-line poles for several weeks. On admission to hospital there were no clinical symptoms of lead poisoning. The 24-hour urinary excretion of lead was 210 μg. Intravenous infusion of 2 g. of penicillamine on each of two days raised this output to 1,900 and 2,000 μg. On the following day 2 g. of $CaNa_2E.D.T.A.$ was given in addition to the penicillamine and 4,000 μg. of lead was excreted (Table I).

Case 6.—A 50-year-old foundry worker was admitted to hospital in February, 1958. For two years tests had shown 24-hour urinary excretion of lead ranging from 200 to 250 μg., but he had no clinical symptoms of plumbism. On admission the 24-hour lead output was 100 μg. Treatment was begun with 2 mega units of benzylpenicillin by mouth on each of two days. After a 24-hour interval the same dose was given by intramuscular injection for two days. A second 24-hour interval was followed by 0.3 g. of penicillamine by mouth for two days, and after a third interval 2 g. of penicillamine was given intravenously for two days (Table II).

Case 7.—A 48-year-old foundry worker had repeatedly received $CaNa_2E.D.T.A.$ for chronic lead poisoning. On

TABLE II.—*Cases Treated with Penicillin and Penicillamine*

Date	Penicillin (Mega-units)		Peni-cillamine		Daily Output of Lead in Urine (μg.)	Blood Level of Lead (μg./100 ml.)
	Peroral	I.M.	Peroral	I.V.		
			Case 6			
18/2/58					100	70
19/2/58	2				150	85
20/2/58	2				100	95
21/2/58					110	65
22/2/58		2			140	80
23/2/58		2			90	120
24/2/58					90	130
25/2/58			0·3 g.		340	65
26/2/58			0·3 ,,		270	55
27/2/58					90	45
28/2/58				2 g.	900	45
1/3/58				2 ,,	1,100	55
2/3/58					240	30
			Case 7			
24/9/58					110	70
25/9/58	2				130	70
26/9/58	2				60	145
27/9/58					80	40
28/9/58		2			120	35
29/9/58		2			110	125
30/9/58					120	45
1/10/58					100	60
2/10/58			0·5 g.		170	35
3/10/58			0·5 ,,		250	25
4/10/58			1·0 ,,		490	20
5/10/58			1·0 ,,		450	15
6/10/58					100	
			Case 8			
8/10/58					150	40
9/10/58			0·5 g.		480	10
10/10/58			0·5 ,,		320	10
11/10/58					120	10
12/10/58			1·0 ,,		540	>10
13/10/58			1·0 ,,		780	
14/10/58					130	

admission to hospital in September, 1958, the 24-hour urinary lead excretion was 110 μg. and the concentration of lead in the blood was 70 μg./100 ml. Like Case 6, this man was first treated with benzylpenicillin by mouth and intramuscularly in doses of 2 mega units. After a 48-hour interval penicillamine was given by mouth—0.5 g. for two days and 1 g. for two days (Table II).

Case 8.—A 50-year-old foundry worker who, when hospitalized in October, 1958, had a 24-hour urinary lead output of 150 μg. and a blood lead reading of only 40 μg./100 ml. Penicillamine was given by mouth; the dosage was 0.5 g. for two days and then, after a 24-hour interval, 1 g. for two days (Table II).

Discussion

In Cases 1–3 I.V. infusion of penicillamine thus produced a rise in the urinary excretion of lead of about the same order of magnitude as that caused by $CaNa_2E.D.T.A.$ Doubling or tripling of the dose of penicillamine brought about only a moderate increase in the output of lead when the penicillamine was given on consecutive days.

Cases 4 and 5 exemplify the simultaneous use of penicillamine and $CaNa_2E.D.T.A.$ in treatment of lead poisoning and the summation effect that may thereby be obtained. Of special interest was the finding from blood analyses that $CaNa_2E.D.T.A.$, in addition to its effect on urinary lead excretion, seemed to have a mobilizing action on lead stored in the body. This action was expressed as an increase in the blood lead after each infusion of $CaNa_2E.D.T.A.$ Similar observations were reported by Rieders *et al.* (1955). Penicillamine did not appear to have a corresponding effect.

In Cases 6 and 7 the urinary content of lead increased slightly in response to penicillin on the first day of therapy, but not on the second day. (This applied to peroral and intramuscular administration.) The second penicillin dose, on the other hand, was followed by a notable rise in the concentration of lead in the blood (Table II). The implications of this rise are as yet obscure.

Boulding and Baker (1957) stated that in two cases of lead poisoning they observed a significant increase of lead excretion in response to only 0.3 g. of penicillamine by mouth. Also in Cases 6, 7, and 8 of the present series low peroral dosage of penicillamine substantially increased the amount of lead in the urine. This effect was particularly striking if one takes into account the mildness of all three cases as judged from the blood content of lead—70, 70, and 40 μg./100 ml. respectively—before treatment was begun.

Summary

In eight men with varying degrees of exposure to lead, penicillamine (β-β-dimethylcysteine) was tested as a chelating agent. The results were encouraging and were comparable with those given by $CaNa_2E.D.T.A.$ The two agents were also used in combination. Complexes of lead with dimercaprol or sodium calciumedetate ($CaNa_2E.D.T.A.$) have proved under certain circumstances to be nephrotoxic. Whether or not compounds of lead and penicillamine may have a similar action has not yet been elucidated. In the trials hitherto made no side-effects of any kind have been observed.

A good effect on the excretion of lead was observed in response to peroral as well as to intravenous administration of penicillamine.

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Preliminary Communications

In Pursuit of the Vector of Kala-azar in Kenya

The search for the vector of kala-azar in Kenya has continued for nearly ten years and it is only now that we may have found it. This paper should give an idea of the complexity of the problem. Two areas are involved: the Kitui focus south of the Tana River, where thousands of cases have occurred, and further west, in the Rift Valley, where the disease is sporadic.

FIRST SUSPECTS

The first sandfly to be suspected was *Phlebotomus clydei*, long regarded as a possible vector in the Sudan, where it bites man readily and has a geographical distribution corresponding to that of the disease. In Kenya *P. clydei* occurs mainly in gerbil burrows, but is occasionally present in the shafts of termite hills and other habitats; it only rarely bites man. In Kauriro, south of the Tana, *P. clydei* is naturally infected with a leishmania of lizards (*L. adleri*), which in the sandfly is morphologically indistinguishable from *L. donovani*; this at first confused us considerably. We are now convinced that *P. clydei* is not a vector in Kenya, even though a few specimens were infected by feeding them on a hamster just after a heavy culture of *L. donovani* had been inoculated into its skin (Heisch, 1954).

The next sandfly studied was a *Sergentomyia* named *P. garnhami*, which appears in enormous numbers about three weeks after the onset of rain, in the shafts of termite hills round Kitui; it bites man, though not very readily. *P. garnhami* was also infected artificially by feeding it on a skin lesion on the leg of a patient which swarmed with leishmaniae; only a small proportion developed leptomonads (5%), the infections being very light though present in the "anterior" station (Heisch, 1955). Sometimes *P. garnhami* was found naturally infected with "anterior" leptomonads; these never "took" in hamsters, and, like the *Leishmania* isolated from *P. clydei*, were probably derived from lizards.