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A BIOCHEMICAL STUDY OF THE URINARY PROTEIN OF MEN EXPOSED TO METALLIC MERCURY

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Proteinuria is not usually reported in chronic mercury poisoning. The urine of three men exposed to metallic mercury vapour was found to contain protein. In two, the proteinuria disappeared on removal from exposure. The urinary protein in two cases was investigated and found to be indistinguishable from serum protein by the criteria used. The bound carbohydrate values were similar to those found in nephritic urinary protein. One man was diagnosed clinically as a chronic nephritic over a year after removal from exposure. It would be premature to connect this condition with the preceding exposure to mercury.

Proteinuria is not a frequent finding in chronic mercury poisoning. None of a previous group of mercury workers examined for urinary amino-acids (Clarkson and Kench, 1956) showed proteinuria. A further small group of workers was subsequently brought to our notice all of whom were passing protein in the urine. The urinary protein was predominantly globulin on electrophoresis, exhibiting a pattern similar to that given by the low molecular weight cadmium urinary protein (Smith, Wells, and Kench, 1960). An examination of the mercury urinary protein was made to provide information on the modification of the normal protein economy of the body due to mercury.

Medical Data

Three men were exposed to metallic mercury vapour in their work over a period of two years, when it was discovered that their urine contained protein. In one of the men the amount was relatively small (M3) and rapidly disappeared on his removal from exposure. He was not examined further. The other two men behaved differently on removal from contact with mercury. One man (M1) continued to pass protein in small amounts for some three months. The other man (M2) continued to pass protein, which was different in electrophoretic pattern during and after exposure; the proteins were denoted M2A and M2B respectively (Fig. 1). This patient was investigated in hospital seven months after his exposure to mercury ceased. Clinical details are as follows:—

He complained of no symptoms but his urine contained protein which varied in quantity from day to day.

Blood pressure was 180/120 mm. Hg; it settled with rest to 160/105.

Urine was sterile, containing casts but no pus.

A straight radiograph of the urinary tract and an intravenous pyelogram excluded an opaque calculus. Both kidneys appeared to function well and outlined normally.

The serum sodium test was 141 mEq./litre, serum potassium 5·7 mEq./litre, serum Cl 104 mEq./litre, serum HCO_3 24 mEq./litre, blood urea 36 mg./100 ml., and urea clearance 79% of the average normal.

Materials and Methods

Twenty-four hour samples of urine were obtained from these men and transported to the laboratory with the least possible delay. The following investigations were carried out on the urine.

Urinary Mercury.—The concentration of mercury in the urine was determined by the permanganate oxidation and dithizone (diphenylthiocarbazone) method of Milton and Hoskins (1947).

Urinary Amino-acids.—These were analysed by the two-dimensional paper chromatographic procedure described by Clarkson and Kench (1956) using methanol : water : pyridine (80 : 20 : 4 by vol.) and ethyl methyl ketone: *tert*-butanol : water : diethylamine (40 : 40 : 20 : 4 by vol.).

Protein.—Urinary protein concentration was measured by a modification of the biuret method of Hiller, McIntosh, and Van Slyke (1927).

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The protein was prepared in a form suitable for analysis by dialysis in cellophane sacs overnight against running tap water and subsequently against distilled water for three to four hours. The protein was then obtained by freeze-drying. It was brown due to urinary pigment which was undialysable.

Electrophoresis.—The electrophoretic behaviour of the protein was examined by the ridgepole technique of Durrum (1950); the protein was located with azo-carmine B (Plückthun and Götting, 1951) and the profiles derived with an EEL "scanner".

The proteins were also examined by zone electrophoresis on acetyl cellulose columns (Campbell and Stone, 1956). The components thus separated from 150 mg. of freeze-dried whole protein were checked for homogeneity by paper electrophoresis and then prepared individually for analysis by dialysis and freeze-drying.

Carbohydrate Content.—Whole protein was hydrolysed with N H_2SO_4 in sealed tubes (Consden and Stanier, 1952). Descending chromatography (Jermyn and Isherwood, 1949) with ethyl acetate : pyridine : water (40 : 20 : 40 : by vol.) was employed and the sugars detected by spraying with aniline phthalate (Partridge, 1948). The sugars were measured by determination of the areas of the spots (Fisher, Parsons, and Holmes, 1949).

Protein-bound Hexose.—The distribution of the hexose between the electrophoretic components was measured by the orcinol (5-methylresorcinol) procedure of Weimer and Moshin (1953). A 2 : 1 mixture of galactose and mannose was employed as a standard.

Amino-acid Composition.—Each electrophoretic component was hydrolysed with 6N HCl according to Block (1951). The amino-acids thus derived were dissolved in 10% *iso*propanol and applied directly to the paper and analysed in the same way as were the free urinary amino-acids.

Ultracentrifuge Measurements.—Samples of the freeze-dried proteins were dissolved in phosphate-NaCl buffer *p*H 8·0 (K_2HPO_4 0·0044M, Na_2HPO_4 0·0652M, NaCl 0·15M) and dialysed for about 24 hours at 4°C. against a large volume of the same buffer. The refractive increment due to protein in the dialysed solutions was determined with a dipping refractometer (λ = 546 mμ). By diluting with buffer, solutions having a refractive increment due to protein of 0·00200 were prepared and these were examined in the Svedberg oil-turbine ultracentrifuge in a 12 mm. cell at approximately 250,000 g. Optical observations by the diagonal Schlieren method (Philpot, 1938) were photographically recorded on Ilford half-tone panchromatic plates, using a high-pressure mercury arc as a light source from which monochromatic light (λ = 546 mμ) was isolated by a suitable filter. Distances on the plates were determined with a measuring microscope reading to 0·001 mm. and sedimentation coefficients were computed by the method of Cecil and Ogston (1948).

Immunology.—This was carried out by the tube precipitin method of Oakley and Fulthorpe (1953) using rabbit antiserum prepared against proteins of whole normal human serum.

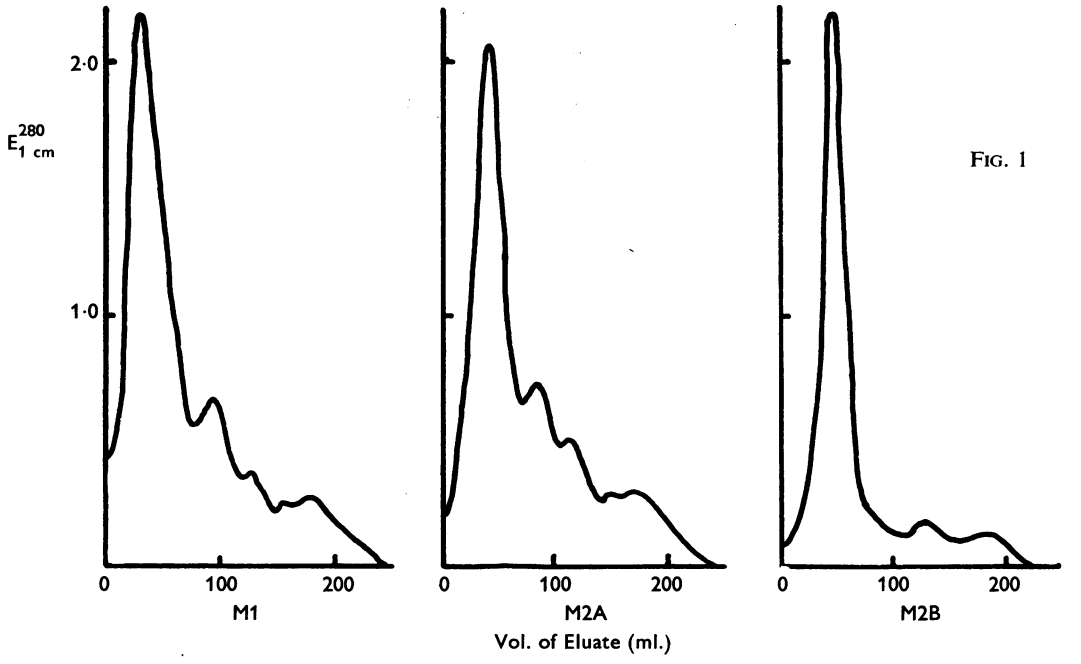

FIG. 1

Results

The concentrations of mercury and protein in the urine of the three men studied are presented in Table 1. The urinary proteins were completely coagulable by heat.

The urinary proteins from both men exhibited similar electrophoretic behaviour on paper with a preponderance of globulin. This changed markedly in the case of M2, following removal from exposure, to a pattern showing little globulin. A similar picture was presented by acetyl cellulose column electrophoresis (Fig. 1), except that a minor component, not fully resolved, appeared in the β-globulin region.

TABLE 1

CONCENTRATIONS OF MERCURY AND PROTEIN IN URINE OF MEN EXPOSED TO METALLIC MERCURY

Workman	Mercury (μg./l.)	Protein (g./l.)
M 1	180	1·3
M 2A	430	1·6
M 2B	—	0·8
M 3	990	0·3

The urinary mercury concentrations of men with no known exposure to mercury were given as 0-90μg./24 hr. by Buckell, Hunter, Milton, and Perry (1946).

The nature of the protein-bound carbohydrate was investigated by paper chromatography of hydrolysates of the total urinary protein. Galactose, glucose, mannose, fucose, and ribose were detected and their presence confirmed by the use of alternative solvent systems. The results are presented in Table 2. The distribution of hexose between the electrophoretic fractions is shown in Table 3.

In every case the urinary free amino-acids determined were within the normal limits as found by Clarkson and Kench (1956).

The amino-acid composition of the electrophoretic components is given in Table 4. For comparison only the eight most accurately measured amino-acids were taken and each value expressed as a percentage total of eight.

In the ultracentrifuge the total urinary proteins (M1, M2A, M2B) each sedimented as a single symmetrical peak having the following sedimentation coefficients respectively ($S_{20, w}$, approximately 1% solution), M1, 4·03S; M2A 4·16S; M2B, 4·04S. These values correspond closely with the value given by a 1% solution of human serum albumin.

TABLE 3

HEXOSE CONTENT OF ELECTROPHORETICALLY SEPARATED COMPONENTS OF MERCURY URINARY PROTEINS

Nature of Protein*	Total Protein	Electrophoretic Component†			
		1	2	3	4
M 1	41	19	38	24	—
M 2A	37	17	38	21	—
M 2B	29	15	—	19	12
UN 1	31	14	31	22	12
UN 2	33	12	—	24	13

*As in Table 2.
†The hexose values were determined by reference to a galactose-mannose 2 : 1 standard and are expressed as mg./g. protein (biuret). Each figure is the mean of two readings.

Immunologically the mercury urinary proteins exhibit multiple precipitin reactions with rabbit anti-total human serum protein. This was assumed to indicate similarity of antigenic structure to serum and nephritic urinary proteins which gave an equally complex pattern.

Discussion

The urinary protein investigated was associated with absorption of mercury. It is known that none of the men exhibited proteinuria before exposure to mercury and removal from exposure resulted in the cessation of proteinuria in two cases and in a reduction of the protein excretion in the third. The last case also showed a marked change in the electrophoretic pattern of the protein, most notable being the disappearance of the large component with the mobility of α_2 globulin. The mercury urinary protein was separable on electrophoretic analysis into five components. The first three, corresponding to albumin, α_2, and β globulin, were sufficiently well differentiated to allow their isolation

TABLE 2

RELATIVE PROPORTIONS OF MONOSACCHARIDES BOUND TO URINARY PROTEINS FROM MERCURY WORKERS AND FROM NEPHRITIC PATIENTS

Nature of Protein	No. of Analyses	Galactose	Glucose	Mannose	Fucose	Ribose
M 1†	7	32*	9	23	30	6
M 2A	6	30	9	25	29	7
M 2B	6	35	9	21	30	5
UN 1	6	38	11	19	27	5
UN 2	5	36	9	20	30	4

*All figures are expressed as a percentage of the total monosaccharide found in the unfractionated protein and are arithmetic means of the results of the individual analyses. The standard deviation of the readings was 1·6 with 80 d.f.
†M 1 and M 2A = urinary protein from workmen during exposure to mercury
M 2B = urinary protein from M 2 after removal from exposure
UN 1 and UN 2 = urinary protein from two nephritic patients

TABLE 4

AMINO-ACID COMPOSITION OF ELECTROPHORETIC COMPONENTS OF MERCURY URINARY PROTEIN

Source of Protein	No. of Analyses	Electrophoretic Component	Arginine*	Lysine	Aspartic Acid	Glutamic Acid	Histidine	Glycine	Serine	Alanine
M 1	5	1	9·6	12·9	18·8	25·7	2·0	4·3	6·6	20·7
M 2A	2	1	9·2	14·4	20·3	25·4	2·7	5·6	6·3	16·2
M 2B	4	1	7·2	13·2	16·1	29·4	1·5	5·8	5·9	21·1
UN 1	3	1	8·7	14·6	18·3	24·4	2·1	7·1	8·5	16·4
UN 2	2	1	7·1	12·1	17·9	24·5	3·0	6·1	7·4	21·7
M 1	4	2	11·2	10·3	15·5	20·2	2·0	9·8	10·4	20·0
M 2A	3	2	9·6	10·2	17·4	19·0	2·5	12·1	11·0	18·2
UN 1	2	2	10·8	8·8	22·3	17·7	3·1	14·2	10·9	12·2
M 1	4	3	13·4	9·5	12·4	20·3	—	12·8	12·5	20·0
M 2A	2	3	11·8	10·3	14·8	15·6	—	13·7	13·5	19·2
M 2B	2	3	12·9	13·0	17·7	17·9	2·0	13·2	10·3	13·0
UN 1	2	3	9·0	10·4	18·1	17·2	2·3	12·1	18·4	12·4
UN 2	3	3	9·1	8·5	17·3	20·4	2·1	12·0	13·2	17·4
M 2B	2	4	9·9	8·3	16·4	16·1	—	13·1	19·3	16·9
UN 1	2	4	8·6	9·1	14·0	17·9	2·3	16·1	16·7	15·3
UN 2	2	4	9·9	8·6	14·3	22·9	2·0	14·0	14·5	11·2
Error†			1·11	1·41	1·45	1·13	0·29	0·83	0·99	1·62
Variance			0·85	0·79	1·35	1·46	0·44	0·43	0·71	1·33

*The values for each amino-acid are expressed as a percentage of the total of the eight amino-acids measured in terms of α-amino nitrogen.
†Mean difference between 17 pairs (histidine 13 pairs) of duplicate readings.

for chemical analysis but the two components of lowest mobility, present only in small amounts, were not completely resolved and further examination was not feasible.

The molecular weight of the mercury urinary protein as deduced from ultracentrifugal analysis was very near to that of serum albumin. The component of electrophoretic mobility comparable with albumin was the biggest fraction. Amino-acid analysis of the components showed that each was indistinguishable in this respect from the components of nephritic urinary protein of corresponding electrophoretic mobility. The bound carbohydrate values were also very close to those for nephritic urinary protein and immunological similarities were apparent.

This evidence leads to the conclusion that in the men studied exposure to mercury resulted in the appearance of serum proteins in the urine. This protein had presumably traversed the kidney but it is not possible to confirm the mechanism by which this occurred. However, as the proteinuria ceased in two of the cases following removal from the mercury hazard the lesion would appear to have been, on this occasion, readily reversible.

The third man (M2) was diagnosed clinically as a chronic nephritic over a year after removal from exposure and the predominance of albumin in the urinary protein was in accordance with this finding. The evidence derived from the ultracentrifuge and amino-acid, carbohydrate, and immunological analyses is totally consistent with chronic nephritis. It would be premature to connect this condition with the preceding exposure to mercury.

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