tained in the crystalline state (needles), but is unstable even in the dry condition; the Na and K salts have been isolated but are extremely hygroscopic and therefore of little value; the Li salt is quite stable, but was difficult to prepare in good yield and could not be crystallized. However, it has been found that the Ba may be removed from BaPA by the addition of Na_2SO_4 and that after several washings of the $BaSO_4$ a quantitative recovery of the phosphoarginine may be obtained.

SUMMARY

1. A method is described for the isolation in good yield (10 g./kg.) of phosphoarginine from the muscle of crayfish.

2. The compound was isolated as a barium salt, free from contaminating material and corresponding to the formula $C_6H_{13}O_5N_4PBa,H_2CO_3,H_2O$.

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Urinary Excretion of Amino Acids by Men Absorbing Heavy Metals

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The paper describes an investigation of the urinary amino acids of industrial workers exposed to the metals lead, mercury, uranium and cadmium, and forms part of a study of specific biochemical lesions arising from absorption of these metals. An increased urinary amino acid excretion could arise either through disturbed intermediary metabolism with raised plasma levels or by impaired reabsorption in the renal tubular cells, and it was evident that the occurrence of amino aciduria would be important for an investigation of the toxic effects of these metals.

Much evidence has been accumulated over many years to show that kidney tissue is particularly susceptible to injury by heavy metals. Aub, Fairhall, Minot & Reznikoff (1926) reviewed the early work on gross renal damage by lead, and more recently the histological findings of Blackman (1936) and Wachstein (1949) together with glycosuria (McKhann, 1926; Zak & Finkelstein, 1952; Giamattasio, Bedo & Pirozzi, 1952; Millichap, Llewellin & Roxburgh, 1952) have located the injury in the proximal convoluted tubular epithelium. Further evidence for proximal tubular damage was supplied by Wilson, Thomson & Dent (1953) and Marsden & Wilson (1955), who were the first to record abnormal urinary amino acid patterns in lead-poisoned children. Evidence of similar damage by inorganic mercury was brought forward by Edwards (1942) and by Hepler & Simonds (1946). The toxicity of organic mercurials was well known, since blockage of sodium reabsorption formed the basis for their use as diuretics. Gross kidney damage in experimental uranium poisoning has been described by MacNider (1924). Laake (1945) has reported proteinuria and glycosuria, and Dounce (1949) suggested that cellular breakdown in the kidney was responsible for the presence of phosphatasuria (Breedis, Flory & Firth, 1943) and catalasuria (Dounce, Roberts & Wills, 1949). Raised urinary amino-nitrogen with concomitant normal plasma levels were recorded by Rothstein & Berke (1949) in animals suffering from severe uranium poisoning. Renal injury by cadmium has been described in animal experiments by Schwartze & Alsberg (1923), Prodan (1932) and Pancheri (1937). The only data relating to the human kidney were those of Friberg (1950), who found evidence of decreased renal efficiency in Swedish accumulator workers exposed to cadmium oxide dust.

The subjects of the present study showed no clinical evidence of renal disease. There were large increases of urinary amino-nitrogen in cadmium workers, with less profound changes in workmen absorbing lead, mercury and uranium. Characteristic amino acid patterns have been observed, in particular, marked increase of the hydroxy amino acids, threonine and serine, associated with intake of cadmium and to a less extent of uranium.

EXPERIMENTAL

Materials

The urinary specimens studied were entirely from men in industry and consisted of single specimens from each of fifty men in a light-engineering factory, and specimens from men variously exposed to lead, mercury, uranium and cadmium.

All fifty men in the light-engineering factory satisfied the following criteria as controls for this study: there was no past or present exposure; ages, and incomes (a rough guide to diet) were within the range of the heavy-metal workers and their work was similar with regard to the degree of physical exertion. Single specimens were collected from each of thirty-eight of the workmen at various times of the day, 3 months before examination of the heavy-metal workers. Rigorous controls were provided by the remaining twelve men, from whom urinary specimens were collected at the same time of the day (9 a.m.) and season of the year (January–March) as for the exposed workers. The specimens were collected directly in Pyrex bottles and preserved at 4° with a few crystals of thymol.

The lead workers were drawn from three different industries: a lead-accumulator factory where intake was mainly through inhalation of lead oxide dust; a smelting works in which the smelters were exposed to lead fume; and a lead chromate factory where the hazard consisted of acidic and basic lead chromates together with smaller quantities of lead oxide, nitrate and sulphate.

The mercury-containing specimens were in two groups; six were chosen from workers engaged in the repair of d.c. meters and exposed to mercury vapour, and four other specimens were from workmen inhaling organic mercurial compounds whilst manufacturing insecticides.

The eighteen uranium specimens were collected from men exposed to a gaseous compound of the metal. Ten of these specimens related to workmen currently exposed, and two specimens were from workers last exposed some 9 months before this study was made. In addition, there were six specimens from individuals who exhibited varying grades of proteinuria during the 3 months period before collection of the specimen.

Twelve workmen exposed to cadmium oxide dust in the alkaline-accumulator industry were selected from various parts of the factory known to present different degrees of hazard.

Methods

Creatinine was determined in all urines and also qualitative tests were carried out for reducing sugars, protein and bile pigments.

Creatinine. Creatinine was measured by the alkaline-picrate method of Bonsnes & Taussky (1945) and also by the procedure employing adsorption on Lloyd's reagent (Owen, Iggo, Scandrett & Stewart (1954).

Reducing sugars were detected by Benedict's qualitative reagent.

Protein. Trichloroacetic acid (25% w/v) (2 ml.) was added to 2 ml. of urine which had been filtered until clear. A just perceptible opalescence was recorded as trace (tr.), a definite opacity as positive (+) and a precipitate as strongly positive (+ +).

Bile pigments. After adsorption on a $BaSO_4$ precipitate, bile pigments were detected by the addition of Fouchet's reagent (1% w/v $FeCl_3$ in 25% w/v aqueous trichloroacetic acid).

The urinary concentration of the heavy metal constituting the hazard in each case was determined. Lead and cadmium concentrations were measured on the control series.

Lead. The method employed was a dithizone procedure (Kench, 1940) modified as follows. The acid digest of the dithiocarbamates, after addition of 0·2 ml. of acetic acid, was brought to pH 6 (yellow to phenol red) with ammonia and 2 ml. of 10% (w/v) KCN added. The solution was then rendered just alkaline with ammonia, the lead extracted into 15 ml. of CCl_4 by repeated shaking in the presence of slight excess of ammoniacal dithizone solution, and the optical density of the solution measured at 530 mμ. in a Unicam quartz spectrophotometer SP. 500.

By addition of KCN to the digest at pH 6, losses of lead in the subsequent dithizone extraction due to occasional high pH of the cyanide were avoided. At 530 mμ. the optical density of the lead dithizonate was maximal and that of the yellow dithizone oxidation product minimal. Consequently, the final 1% KCN wash of the lead dithizonate in CCl_4, originally described, could be dispensed with.

Thallium was the only metal found to interfere with recovery of urinary lead by this procedure and this metal was not encountered in this study. The reagent blank was 2 μg. and the range of normal lead up to 100 μg./l.

Mercury. The procedure consisted of acid-permanganate oxidation followed by extraction of mercury as dithizonate from its acid digest at approximately pH 1 (Hubbard, 1940; Milton & Hoskins, 1947; Varley, 1954). Optical densities of the $CHCl_3$ extract were measured at 490 and 600 mμ. in the spectrophotometer. The optical density of the mercury dithizonate $E_{1\,\text{cm.}}$ (490 mμ.) was corrected for the absorption at that wavelength of excess of dithizone by the expression

$$E_{1\,\text{cm.}}\,(490\ \text{m}\mu.)\,(\text{corr.}) = E_{1\,\text{cm.}}\,(490\ \text{m}\mu.)\,(\text{obs.}) - 0{\cdot}23E_{1\,\text{cm.}}\,(600\ \text{m}\mu.)\,(\text{obs.}).$$

Uranium. Uranium in urine was measured by the fluorophotometric method of Bloor, as modified by Bloor & Neuman (1949).

Cadmium. Cadmium was extracted as dithizonate from strongly alkaline solution containing KCN and measured as dithizonate in $CHCl_3$ at 506 mμ. in the spectrophotometer (Smith, Kench & Lane, 1955).

Separation of free amino acids from urine. Amino acids are present in urine both in free and bound forms, and the latter may account for 66% of the total urinary amino-nitrogen (Stein, 1953). Since the excretory mechanism for these bound compounds is not known, it is important to measure only amino acids that have been excreted in the free form. Some of the bound forms are labile to heat, and that of glutamic acid will break down even when stored at 4° to liberate the free amino acid. It is thus imperative to avoid procedures which involve raising the temperature of the urine. It is probable that the normal urinary amino-nitrogen value of 147 mg./l. obtained by the gasometric ninhydrin–CO_2 method (Van Slyke, Macfayden & Hamilton, 1943) was too high since it involved heating the urine to 100°. The mild conditions necessary for the isolation of free amino acids seemed best obtainable by the use of a sulphonated ion-exchange resin.

A column 6 cm. × 0·4 cm.^{2} of sulphonated ion-exchange resin Zeo-Karb 225 (mesh size 40/70, 4½% cross-linked), suitably supported by glass wool, was contained in a 10 ml. burette. A portion (5 ml.) of the urine specimen was added at the rate of 1 ml./30 min. to the resin prepared in the hydrogen cycle. A narrow dark-coloured band formed on the column owing to adsorption of pigments, including urochrome. Distilled water (50 ml.) was next passed down the column at a flow rate 1 ml./2 min. to remove any residual anions, organic acids and non-ionized compounds,

e.g. sugars. Amino acids retained by the column were now removed by a modification of the ammonia-displacement method of Partridge & Westall (1949). Ammonia (sp.gr. 0·880) was passed down the column at a rate of 1 ml./hr. The urochrome and amino acids were thereby displaced down the column together as a narrow dark-coloured band and their point of emergence was immediately apparent from the yellow colour of the urochrome. The effluent was collected in approximately 1 ml. fractions. The use of concentrated ammonia as displacing medium resulted in a five- to eight-fold concentration of free urinary amino acids and prevented chromatography on the column, which would have led to an alteration in the urinary amino acid pattern.

The effluent solution from the ion-exchange column was found to contain, besides free amino acids, urochrome, ammonia and small quantities of urea and peptides. The peptides were invariably detectable as blue spots with ninhydrin on the two-dimensional paper chromatogram to be described. Three spots were usually present with low R_F values in both solvents and could be dispersed by previous acid hydrolysis of the eluate from the column. The total ninhydrin colour formed by these peptides on the paper was always less than 5% of the total colour yield of the amino acids. The contribution from this source to the total amino-nitrogen in the fractions from the column was probably similar in magnitude.

Some hydrolysis of glutamine to glutamic acid took place on the ion-exchange resin. The extent of the conversion was measured for different quantities of glutamine added to the column, and was found to be constant for a given size of column and volume of urine added. When 5 ml. of urine were added to a column 6 cm. × 0·4 cm.^{2}, 36 ± 1% of the glutamine present was hydrolysed to glutamic acid. Glutamine and glutamic acid values have been corrected for this conversion on the ion-exchange column.

Great care was taken to avoid overloading the column as the concentrations of basic amino acids—lysine, histidine and methylhistidine—were then increased at the expense of glutamic acid, alanine and serine. This change in pattern was easily recognizable on a developed paper chromatogram and served as a useful check against overloading. No overloading occurred when the volume of urine added to a 6 cm. × 0·4 cm.^{2} column was 5 ml. or less.

Urinary constituents which interfered with the flow of amino acids in paper partition chromatography were removed completely or very much reduced in concentration by the ion-exchange separation. The solution could then be used for both total amino-nitrogen determination and for quantitative measurements of the amino acid chromatographic pattern.

Determination of total free amino-nitrogen. Amino acids in the fractions from the column were detected by a simple ninhydrin test on 2·5 µl. portions. Usually all the amino acids appeared in the early yellow-coloured fractions, the first of these containing 80% of the total amino-nitrogen. The volume of these fractions was measured with an accuracy of ±0·005 ml. in a graduated pipette before a 50 µl. portion was removed for analysis. For determination of α-amino-nitrogen in these portions the ninhydrin (trioxohydrindene hydrate) colorimetric procedure of Cocking & Yemm (1954) was followed. Owing to the presence of hydrindantin, the reduced form of ninhydrin, this method was particularly sensitive to free ammonia, and before the amino-nitrogen in the fractions from the column could be measured complete removal of ammonia was obligatory.

Table 1. *Recovery of added glycine–histidine from columns of Zeo-Karb 225*

All amino acid values are expressed as mg. of amino-nitrogen/l. of urine.

Glycine–histidine added to urine	Total amino-N in sample	Amount of added amino-N recovered	Recovery (%)
0	61·5	—	—
50	110	48·5	97
100	162	100·5	100·5
150	211	149·5	99·5
200	263	201·5	101
250	314	252·5	101
300	359	297·5	99

Table 2. *Six independent analyses of the amino acid content of the same urinary specimen*

All data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimen	1	2	3	4	5	6	Mean	Standard deviation	Standard error
Glutamic acid	<0·4	<0·4	<0·4	<0·4	<0·4	<0·4	<0·4	—	—
Lysine	2·56	3·42	2·46	2·63	2·04	2·53	2·61	0·45	0·21
Asparagine	5·91	5·35	4·96	5·35	4·10	5·50	5·18	0·62	0·30
Glutamine	11·62	11·15	11·50	11·53	10·23	11·09	11·19	0·38	0·18
Histidine	7·31	8·30	8·30	8·95	7·05	7·50	7·90	0·68	0·34
Glycine	28·80	23·80	22·0	25·60	23·70	23·40	23·85	0·91	0·45
Methylhistidine	2·08	2·76	2·48	2·94	2·71	2·21	2·53	0·31	0·16
Alanine*	7·16	10·10	7·15	7·70	7·60	8·80	8·08	1·05	0·52
Serine	6·44	6·71	6·36	7·00	6·81	7·44	6·79	0·42	0·20
Proline	1·60	2·70	1·61	1·50	1·45	2·27	1·86	0·47	0·23
Tyrosine	2·05	1·91	1·99	1·96	2·14	2·21	2·02	0·30	0·14
Valine	0·73	0·79	0·48	0·37	1·05	0·77	0·70	0·38	0·19
Leucine	0·93	0·92	0·78	0·41	1·69	0·67	0·88	0·40	0·19
Phenylalanine	1·33	0·51	0·86	0·36	1·24	<0·39	<0·86	<0·38	0·19
Threonine	2·94	2·22	3·33	2·40	3·14	3·12	2·86	0·40	0·19
Tryptophan	1·77	1·18	2·23	1·96	2·12	2·37	1·94	0·38	0·19
Cystine	0·96	1·16	0·79	0·96	0·97	1·05	0·98	0·27	0·13
Total amino-nitrogen	79·0	81·6	77·4	80·7	79·3	79·8	79·3	1·60	0·78

* Uncorrected for the presence of β-amino*iso*butyric acid.

The 50 μl. portion from each fraction was added to 5 ml. of absolute ethanol. After the temperature had been raised to the boiling point, a stream of air was passed through for 2 min. The tube was cooled, any loss of volume corrected, 1 ml. transferred to a second test tube and evaporated down to approximately 0·1 ml. The amino-nitrogen was then determined by the method of Cocking & Yemm (1954).

Complete removal of ammonia was established by the following observations. The colour yield from ammoniacal amino acid solutions was similar to that from pure amino acids and samples from an effluent fraction which contained no amino-nitrogen were equal in colour to the distilled-water blank. As anions were absent, the necessity to raise the pH for removal of ammonia did not arise.

Twelve fractions could conveniently be measured simultaneously, accurate results being assured by checking the ninhydrin colour yield in each batch against a standard curve prepared by using a 1:1 mixture of glycine and histidine.

Table 1 gives the recoveries of amino-nitrogen by this method when different amounts of added glycine–histidine mixture (1:1) were run on six Zeo-Karb 225 columns. The total amino-nitrogen values presented in Table 2 are the results of six independent determinations on a single specimen of urine to which no amino acid had been added.

Paper-chromatographic separation of amino acids present in fractions collected from the ion-exchange column. On account of the large number of analyses in this study, a rapid, efficient means of separating amino acids was required. The two-dimensional paper chromatographic method described by Redfield (1953) appeared most suitable. By carrying out the second solvent run at a raised temperature some twenty different amino acids could be separated on 18 × 18 cm. papers with a total solvent-development time of only 7 hr.

Interfering substances in the chromatographic paper which cause amino acid losses (Thompson & Steward, 1951) were effectively removed by washing the papers for 24 hr. in running tap water. This was achieved by placing some 6–8 papers in a sink of slightly larger cross-section. The tap water was maintained at a level of about 4 in. by a constant-head device and the flow adjusted so that the papers were kept suspended in the water without folding. After being carefully removed from the sink, each paper was gripped by two glass rods held by rubber bands and suspended in air until dry.

Portions containing 5 and 10 μg. of amino-nitrogen were taken from the most concentrated fraction and added to the washed 18 × 18 cm. squares of Whatman no. 1 filter paper to give a spot of diameter less than 0·5 cm. A maximum of nine papers were affixed to a frame essentially similar to that described by Datta, Dent & Harris (1950). The frame was suspended in the chromatographic tank of size 28 × 20 × 25 cm. for 1 hr. before commencing the solvent run. The time of development in the first solvent (methanol–water–pyridine, 80:20:4 by vol.) was 2½–3 hr. and in the second solvent (ethyl methyl ketone–*tert.*-butanol–water–diethylamine, 40:40:20:4 by vol.) 3½–4 hr. at a temperature of 37°.

Measurement of individual amino acids on paper chromatograms. The two-dimensional chromatography described above separated very small quantities of amino acids (0·1–1·0 μg. of amino-nitrogen in each spot), for measurement of which many methods could not be applied, (Block, 1950; Boisonnas, 1950; Fowden, 1951). The only satisfactory procedure involved a reproducible ninhydrin reaction on the paper, followed by elution and measurement of the coloured product. The effects on this reaction of heat, moisture and atmospheric oxidation have been described (Fowden & Penney, 1950; Novellie, 1950; Brush, Boutwell & Barton, 1951; Thompson, Steward & Zacharius, 1951). A modification of the reagent described by Moore & Stein (1948) was found to be most suitable. The use of hydrindantin was involved and all traces of ammonia and diethylamine, therefore, had first to be removed from the paper.

The following procedure was adopted. After the second solvent run, the papers were allowed to stand in air until dry. The papers were washed with dry ether and each paper was then exposed to an atmosphere of dry steam for 10 min.

Ninhydrin reagent was prepared as follows: $SnCl_2,2H_2O$ (0·160 g.) was dissolved in 200 ml. of 0·2M citric acid buffer, pH 5. To 10 ml. of this solution, 1 g. of ninhydrin was added and the volume brought to 100 ml. with *iso*-propanol. Continuous shaking for 1 hr. was required to obtain a clear solution which was sufficient for the treatment of nine chromatograms. On account of the high sensitivity of the solution to atmospheric NH_3, the solution was prepared just before use.

The reagent was poured into a shallow porcelain bath and the steamed papers were dipped into it. The papers were immediately rolled into a cylindrical form and placed in a reaction vessel. This consisted of a gas-jar 30 × 8 cm. fitted with a perforated stopper, through which was passed a narrow glass inlet tube. Nitrogen saturated with 90% *iso*propanol was passed through the glass tube into the vessel for 2 min. and afterwards the vessel was placed in a hot-air oven at 90–95° for 20 min., after which time the reaction was complete.

All amino acids yielded blue spots, with the exception of histidine and methylhistidine, which gave a grey-blue colour, and cystine, which was decomposed during chromatography. The developed papers could be allowed to stand for 24 hr., but longer periods than this resulted in a rapidly increasing background coloration, rendering accurate spot analysis impossible.

The ninhydrin spots, along with a blank section, were cut out from each paper and weighed to the nearest mg. The papers were handled with forceps on account of the ninhydrin still present. Each paper spot was cut into small pieces, transferred to appropriate test tubes, and 3 ml. of 50% aqueous ethanol were added. The test tubes were shaken mechanically for 20 min. The suspended paper lint was removed from the coloured extract by centrifuging for 10 min. The optical density was measured at 570 mμ. in the spectrophotometer against the solvent ethanol–water (50:50, v/v). The background correction for each spot was calculated from its weight and the optical density for the blank section of the paper.The accuracy of such corrections was dependent upon an even background colour on the paper.

Figs. 1 and 2 are graphs in which the optical density of the eluate solution of each spot is plotted against the amount of the corresponding amino acid originally added to the paper chromatogram. The gradient gives the colour yield per unit weight of amino acid and shows a lower value for histidine (Fig. 2) than for the unsubstituted mono-amino-monocarboxylic acid glycine (Fig. 1). Tryptophan also showed a lower colour yield than glycine, whilst higher

values were found for arginine and lysine. By measuring in this way the colour yields [$E_{1\,\mathrm{cm.}}$ (570 mμ.)/μg. amino-nitrogen] of all amino acids encountered in urine, it was possible to calculate accurately the percentage of each amino acid in the original portion of the fraction from the ion-exchange resin. Separate procedures had to be adopted for cystine and β-amino*iso*butyric acid.

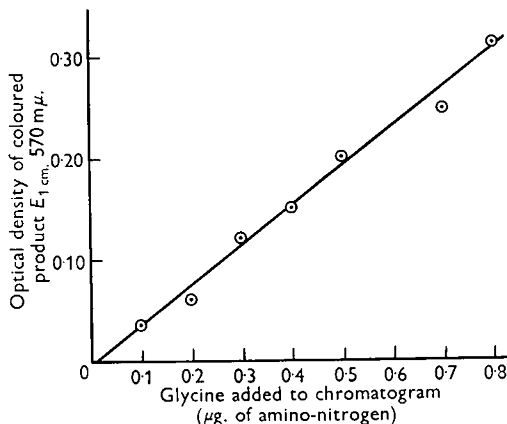

Fig. 1. Colour yield from glycine separated on a paper chromatogram, after reaction with a 1% (w/v) solution of ninhydrin in 90% (v/v) *iso*propanol containing $SnCl_2$ and 0·2M citric acid buffer, pH 5.

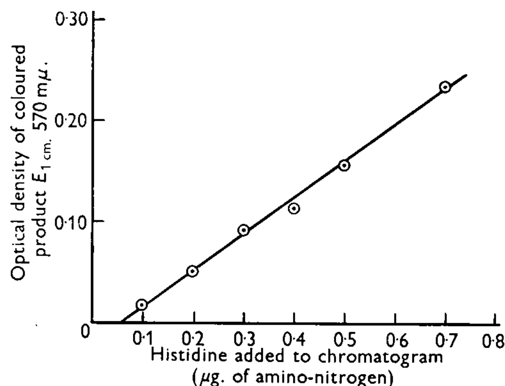

Fig. 2. Colour yield from histidine separated on a paper chromatogram, after reaction with a 1% (w/v) solution of ninhydrin in 90% (v/v) *iso*propanol containing $SnCl_2$ and 0·2M citric acid buffer, pH 5. Owing to incomplete separation on the two-dimensional chromatogram, some histidine is eluted with the glycine spot, and this accounts for the intercept of the histidine line on the abscissa.

Cystine. Cystine underwent decomposition in the two-dimensional solvent system employed in this work, to give a diffuse yellow spot with the ninhydrin reagent. However, its oxidation product, cysteic acid, which gave a satisfactory colour with ninhydrin, could be separated from other urinary amino acids by a one-dimensional solvent system

Table 3. *Amino acid content of urinary specimens obtained from workers in a light-engineering factory*

All data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimen	1	2	3	4	5	6	7	8	9	10	11	12	Mean m	Standard deviation s	Normal range $m\pm3s$
Glutamic acid	0·7	0·8	3·0	1·6	<0·3	1·2	1·0	3·7	2·6	<0·3	2·8	1·1	<1·6	0·9	0·0–4·3
Lysine	4·0	1·8	1·4	0·6	1·8	1·9	3·0	4·5	0·9	1·3	0·7	2·3	2·0	1·2	0·0–5·6
Asparagine	<0·4	<0·2	<0·3	<0·2	<0·3	<0·2	<0·3	<0·2	<0·3	3·1	0·4	5·3	<0·9	1·6	0·0–5·7
Glutamine	11·0	4·5	4·4	2·1	8·5	6·9	10·4	4·5	9·0	8·6	4·6	8·2	7·0	3·4	0·0–17·2
Histidine	10·4	4·8	5·8	3·6	5·7	3·9	6·2	6·1	4·4	4·5	3·5	3·9	5·2	2·3	1·4–12·1
Glycine	20·6	10·2	23·6	14·5	9·4	13·3	19·8	8·4	19·6	17·6	19·1	21·6	16·5	5·1	1·6–31·8
Methylhistidine	3·8	1·6	<0·5	2·3	0·5	2·1	2·4	3·0	1·7	<0·5	1·2	0·6	<1·7	1·1	0·0–5·0
Alanine*	7·6	4·6	8·1	6·1	7·5	4·6	6·4	6·3	7·5	6·5	5·6	5·7	6·4	1·1	3·1–9·7
Serine	5·8	2·8	3·6	2·3	5·1	3·4	3·8	4·6	5·5	4·5	4·7	4·8	4·2	1·1	0·9–7·5
Proline†	<0·4	<0·2	<0·3	<0·2	<0·3	<0·2	<0·3	<0·2	<0·3	<0·3	<0·3	<0·3	<0·3	—	—
Tyrosine	2·6	1·0	1·9	1·1	1·9	1·7	2·3	1·8	1·3	1·0	1·3	<0·3	<1·5	0·7	0·0–3·6
Valine	1·6	0·7	0·4	0·9	1·4	1·3	1·8	1·2	<0·3	0·9	0·9	<0·3	<1·0	0·4	0·0–2·2
Leucine	1·6	<0·2	<0·3	<0·2	1·5	<0·2	<0·3	2·1	<0·3	<0·3	3·3	<0·3	<0·9	1·0	0·0–3·9
Phenylalanine	1·3	<0·2	<0·3	<0·2	2·7	<0·2	<0·3	<0·2	<0·3	<0·3	0·8	<0·3	<0·6	0·7	0·0–2·8
Threonine	2·2	1·2	1·9	1·1	3·1	2·4	2·2	0·6	<0·3	1·0	1·8	3·1	<1·7	0·9	0·0–4·4
Tryptophan	0·5	0·6	<0·3	<0·2	<0·3	1·5	1·4	<0·2	<0·2	<0·3	1·4	<0·3	<0·6	0·5	0·0–2·1
Cystine	0·9	<0·4	<0·3	1·0	<0·5	1·1	0·9	0·7	0·6	<0·4	0·7	0·7	<0·7	0·3	0·0–1·6
Total amino-nitrogen	75·9	37·6	54·7	37·3	53·5	47·1	60·9	46·7	52·1	49·6	51·7	56·5	52·0	10·5	20·5–83·5

* Uncorrected for the presence of β-amino*iso*butyric acid.

† Since the proline concentration in these specimens was below the limit of measurement, a reliable normal range could not be calculated.

containing *n*-propanol and aqueous 0·1N-HCl (4:1, v/v). To oxidize cystine to cysteic acid in a portion of the eluate from the ion-exchange column containing 10 µg. of amino-nitrogen, 30 µl. of 100 vol. H_2O_2 was required. The one-dimensional chromatogram was standardized with known quantities of cystine and lysine, which was also separated as a distinct spot. By utilizing lysine as an internal standard in this system, and knowing the relative quantity of lysine from the two-dimensional chromatogram, a satisfactory determination of urinary cystine could be achieved.

β-Aminoisobutyric acid. β-Amino*iso*butyric acid and alanine travel together as one spot in the two-dimensional solvent system. The separation of β-amino*iso*butyric acid from other urinary amino acids was effected by a one-dimensional system, using *n*-butanol equilibrated with 10% aqueous acetic acid as the ascending developing solvent. Tyrosine also appeared as a separate spot and thus offered the opportunity of measuring β-amino*iso*butyric acid by using tyrosine as a reference standard. This separation was worked out only in the later stages of the investigation and the alanine values presented here include β-amino*iso*butyric acid. The evidence so far available suggests that the normal urinary excretion of β-amino*iso*butyric acid is intermediate in value between those of leucine and tyrosine.

Table 4. *Amino acid content of urinary specimens from workers exposed to lead*

Specimens marked F were from lead smelters and those marked P from a lead-accumulator factory. All amino acid data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimen	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	P_1	P_2	P_3	P_4
Glutamic acid	<0·6	3·6	3·5	<0·4	2·2	<0·3	0·9	2·2	4·6*	3·1	7·0*	4·6*
Lysine	8·2*	5·6	3·1	3·8	2·4	1·3	0·8	1·2	2·5	4·7	3·1	3·1
Asparagine	<0·6	<0·6	8·2*	<0·4	<0·3	<0·2	3·9	<0·2	<0·5	<0·5	5·9*	<0·4
Glutamine	7·8	11·3	13·8	5·2	8·2	10·5	6·3	6·4	15·7	11·2	7·7	9·7
Histidine	<0·6	11·6	11·5	6·2	3·5	3·6	2·9	2·4	6·8	7·7	4·4	7·1
Glycine	36·0*	25·3	21·1	32·8*	13·4	12·4	11·9	8·7	32·2*	29·0	24·6	23·4
Methylhistidine	9·0*	7·7*	7·2*	3·9	4·8	2·6	2·7	1·8	3·6	4·5	5·4*	4·7
Alanine†	17·2*	13·9*	11·2*	9·2	4·6	5·6	3·9	4·8	12·3*	10·5*	17·5*	14·6*
Serine	9·8*	8·1*	6·1	5·9	4·1	4·5	3·1	2·9	10·5*	9·6*	8·9*	7·3
Proline	<0·6	<0·6	<0·5	<0·4	<0·3	1·3	1·4	1·8	<0·5	2·7	1·7	<0·4
Tyrosine	5·6*	3·7*	4·5*	3·4	0·9	2·5	1·5	0·8	4·2*	4·0*	1·2	2·5
Valine	8·9*	2·9*	2·1	1·6	0·3	1·1	1·3	1·2	2·3*	2·2	1·6	0·8
Leucine	<0·6	2·2	1·2	<0·4	<0·3	<0·2	1·3	<0·2	<0·5	3·2	1·9	<0·4
Phenylalanine	<0·6	<0·6	<0·5	<0·4	<0·3	<0·2	<0·2	<0·2	<0·5	2·7	<0·5	<0·4
Threonine	8·1*	7·9*	4·6*	3·1	1·6	4·4	2·1	1·7	2·9	3·2	1·5	4·8*
Tryptophan	<0·6	5·4*	1·3	<0·4	<0·3	<0·2	1·4	<0·2	0·4	2·2	<0·5	<0·4
Cystine	1·1	1·6*	<0·5	<0·4	<0·3	0·6	0·5	<0·2	1·2	1·3	1·2	<0·4
Total amino-nitrogen	111*	109*	91*	75	53·6	49·7	39·1	35·6	104*	97·8*	93·2*	82·5
Lead (µg./l.)	134	160	124	52	165	120	165	75	170	120	290	280

* Abnormally raised values $P = 0·003$.

† Uncorrected for the presence of β-amino*iso*butyric acid.

Table 5. *Amino acid content of urinary specimens from workers exposed to lead*

Specimens marked P were from workmen in a lead-accumulator factory and those marked C from a lead chromate works. All amino acid data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimens	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	P_{13}	P_{14}	P_{15}	P_{16}	C_1	C_2	C_3
Glutamic acid	0·5	3·5	4·4	3·1	2·3	1·9	3·1	0·9	2·2	1·1	2·5	1·1	<0·4	2·1	0·7
Lysine	2·1	2·5	2·5	1·1	1·9	5·6	1·7	1·8	3·4	1·4	1·6	1·4	3·1	0·7	0·7
Asparagine	3·0	5·5*	5·0	3·7	<0·3	<0·3	<0·3	<0·3	0·3	3·2	<0·2	3·0	5·0	3·1	2·0
Glutamine	13·2	2·9	5·3	6·3	10·3	6·4	3·3	2·4	4·8	7·2	5·9	2·5	12·0	7·3	7·4
Histidine	7·5	6·1	<0·6	4·4	4·3	5·8	4·6	4·0	5·3	4·0	<0·5	3·3	7·9	4·9	2·5
Glycine	16·7	18·8	18·6	21·1	18·5	19·6	23·8	28·8	18·6	13·6	12·3	12·7	24·0	8·0	9·9
Methylhistidine	2·8	4·4	4·0	2·7	2·1	5·9*	2·9	2·6	5·5*	2·7	4·1	2·3	2·4	1·4	0·6
Alanine†	9·6	10·3*	5·5	5·8	7·5	4·3	6·9	3·7	5·6	6·0	6·3	4·3	8·3	6·1	4·7
Serine	6·6	6·1	4·4	5·1	6·3	4·1	5·6	3·4	5·9	5·6	5·0	4·0	7·3	3·5	3·7
Proline	<0·4	2·1	1·0	0·7	<0·3	1·3	<0·3	<0·3	<0·3	<0·3	<0·2	1·4	<0·4	<0·2	<0·2
Tyrosine	4·6*	2·6	2·1	1·4	2·0	1·8	2·4	1·3	2·3	3·0	2·9	1·3	3·2	1·8	1·6
Valine	2·3*	1·7	0·6	1·0	1·4	0·9	1·1	<0·3	<0·3	2·2	2·6*	1·0	1·0	<0·2	1·1
Leucine	<0·4	2·1	<0·3	1·5	1·3	<0·3	<0·3	<0·3	<0·3	<0·3	<0·2	1·3	<0·4	<0·2	<0·2
Phenylalanine	<0·4	1·3	<0·3	<0·3	1·3	<0·3	<0·3	<0·3	1·5	<0·3	<0·2	0·8	<0·4	<0·2	<0·2
Threonine	4·4	1·8	1·9	3·2	2·5	2·0	2·4	4·4	1·3	2·4	<0·2	1·2	3·4	1·8	3·0
Tryptophan	1·3	0·8	<0·3	<0·3	<0·3	<0·3	<0·3	3·7*	1·1	1·1	1·6	<0·2	2·3*	0·5	1·2
Cystine	<0·4	<0·4	<0·3	0·7	<0·3	<0·3	0·8	<0·3	0·6	<0·3	0·6	0·5	<0·8	<0·4	<0·4
Total amino-nitrogen	77·0	70·5	62·5	61·2	60·3	58·2	57·6	56·8	55·6	53·2	44·6	41·4	78·5	41·0	39·0
Lead (µg./l.)	140	324	271	115	296	173	586	156	180	420	271	105	500	440	340

* Abnormally raised values $P = 0·003$.

† Uncorrected for the presence of β-amino*iso*butyric acid.

RESULTS

The data are expressed as mg. of amino-nitrogen excreted/g. of creatinine. This substance is not reabsorbed in the renal tubule and its daily excretion varies over a limited range. The ratio of the urinary concentration of amino acids to that of creatinine gives a more accurate measure of the reabsorptive efficiency of the tubule for amino acids during the period of collection, and provides a standard of comparison for amino acid data collected at different times.

The amino acid values presented in Table 3 are measurements made on urine specimens from group were therefore unimportant—a relevant finding since factory conditions precluded 24 hr. collection of urine.

Data relating to lead workers in three different industries are presented in Tables 4 and 5. The values for urinary lead, as for other metals quoted in the tables, have not been related to urinary creatinine, since the mechanism of heavy-metal excretion was not known. Similarly, Table 6 refers to specimens from mercury workers, and Tables 7 and 8 to workers exposed to uranium. Workmen exposed to cadmium oxide dust (Table 9) in an alkaline-accumulator industry were chosen from various parts of the factory with a varying cadmium hazard.

Table 6. *Amino acid content of urinary specimens from workers exposed to mercury*

Specimens marked I were from workers exposed to mercury vapour and those marked O from workers inhaling organic mercurial compounds. All amino acid data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimens ...	I_1	I_2	I_3	I_4	I_5	I_6	O_1	O_2	O_3	O_4
Glutamic acid	4·9*	4·2	4·4	3·3	4·5*	3·8	<0·2	<0·2	<0·1	<0·1
Lysine	4·2	2·1	2·0	1·9	3·1	1·8	1·5	2·0	0·9	0·9
Asparagine	<0·6	<0·4	<0·4	<0·4	<0·4	5·1	0·5	3·4	2·9	2·7
Glutamine	11·5	10·2	5·2	7·1	9·5	5·7	5·9	6·1	5·4	5·0
Histidine	12·2*	4·7	7·6	6·9	9·9	9·7	2·6	4·4	3·3	3·4
Glycine	55·6*	37·2*	25·2	38·0*	18·2	15·4	8·9	8·3	6·2	4·6
Methylhistidine	2·5	4·6	15·7*	3·9	3·2	2·7	1·5	1·9	0·9	2·0
Alanine†	7·4	5·5	6·8	5·6	11·2*	8·7	3·7	1·7	2·4	1·9
Serine	5·3	8·6*	5·5	1·0	6·4	5·1	2·6	2·0	2·1	1·7
Proline	<0·6	<0·4	0·9	<0·4	<0·4	<0·4	<0·2	<0·2	<0·2	0·7
Tyrosine	2·0	1·3	2·5	1·4	5·7*	3·1	1·2	0·5	0·9	1·9
Valine	2·9*	0·7	1·4	0·3	<0·4	1·6	1·0	0·2	0·4	0·6
Leucine	<0·6	<0·4	<0·4	<0·4	<0·4	1·1	1·5	0·4	0·3	<0·2
Phenylalanine	<0·6	<0·4	<0·4	<0·4	<0·4	1·2	<0·2	0·1	<0·2	<0·2
Threonine	4·5*	2·8	2·0	3·4	<0·4	3·7	1·6	0·5	0·8	1·1
Tryptophan	<0·6	1·2	1·2	1·0	<0·4	1·2	0·9	0·3	0·7	0·6
Cystine	1·2	<0·8	1·2	<0·7	0·8	0·9	<0·4	<0·4	0·3	<0·3
Total amino-nitrogen	114*	83·4	81·5	73·5	72·0	71·0	35·8	31·8	27·5	26·8
Mercury (μg./l.)	383	200	194	310	410	607	102	660	234	102

* Abnormally raised values $P = 0{\cdot}003$.
† Uncorrected for the presence of β-amino*iso*butyric acid.

Table 7. *Total amino-nitrogen of urinary specimens, from workers exposed to uranium, as determined by the copper-complex method of Albanese & Irby* (1944)

S_1–S_6 refer to specimens from workers currently exposed, and B_1–B_6 to those previously but not currently exposed to uranium.

Specimens	S_1	S_2	S_3	S_4	S_5	S_6	
Amino-nitrogen (mg./g. of creatinine)	346	232	405	363	338	234	319 (mean)
Specimens	B_1	B_2	B_3	B_4	B_5	B_6	
Amino-nitrogen (mg./g. of creatinine)	366	266	242	218	140	208	240 (mean)

twelve workers in the light-engineering factory, concurrently with urine from heavy-metal workers. The range of the amino acids is similar to that in specimens collected, as described above, from thirty-eight workmen at various times of the day, 3 months previously. Diurnal and seasonal variations of urinary amino acid concentration in this

Observations on the tests for reducing-sugar and proteins are presented in Table 10. Specimens not included in this table gave negative results for both. Specimen J was from a worker in whom biochemical evidence indicated an apparently symptomless diabetes mellitus. Bile pigments were absent from all specimens.

Table 8*a*. *Amino acid content of urinary specimens from workers exposed to a gaseous uranium compound*

Specimens marked X were from workers exhibiting proteinuria. All amino acid data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimen	X_1	X_2	X_3	X_4	X_5	X_6
Glutamic acid	0·6	4·2	2·0	<0·2	1·5	<0·2
Lysine	9·6*	6·4*	1·1	0·7	0·7	1·9
Asparagine	17·5*	<0·4	2·8	2·4	2·0	0·2
Glutamine	35·0*	8·9	6·4	5·1	3·2	6·6
Histidine	28·8*	9·3	6·6	3·2	2·8	3·3
Glycine	29·2*	21·1	15·5	8·4	6·6	13·0
Methylhistidine	18·5*	3·8	2·7	2·9	2·4	4·3
Alanine†	19·6*	9·4	5·1	3·5	3·1	6·1
Serine	13·3*	7·1	5·1	3·2	3·0	4·8
Proline	1·0	<0·4	<0·3	<0·2	1·8	<0·2
Tyrosine	7·2*	3·0	2·0	0·9	1·4	1·5
Valine	5·1*	<0·4	1·4	0·3	1·0	1·2
Leucine	1·0	<0·4	3·1	<0·2	<0·2	<0·2
Phenylalanine	1·0	<0·4	0·8	<0·2	<0·2	<0·2
Threonine	7·2*	6·0*	1·8	1·3	1·5	2·1
Tryptophan	0·4	2·2*	0·6	0·9	<0·2	<0·2
Cystine	2·6*	1·1	0·6	0·4	<0·3	<0·4
Total amino-nitrogen	192*	79·1	56·7	32·8	29·4	44·2
Uranium (μg./l.‡)	50	10	30	40	10	20
Uranium (μg./l.§)	20	20	20	40	20	20

* Abnormally raised values $P = 0{\cdot}003$.
† Uncorrected for the presence of β-amino*iso*butyric acid.
‡ Uranium content of the specimen.
§ Average uranium excretion over 3 months before the collection of the specimen.

Table 8*b*. *Amino acid content of urinary specimens from workers exposed to a gaseous uranium compound*

Specimens marked S were from workers currently exposed, and those marked B from workers previously but not currently exposed. All amino acid data are expressed as mg. of amino-nitrogen/g. of creatinine.

Specimen	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	B_1	B_2
Glutamic acid	<0·6	<0·5	6·0*	5·9*	<0·5	2·4	2·1	<0·3	2·4	1·1	0·8	2·3
Lysine	8·9*	2·9	1·6	3·7	13·8*	2·0	2·9	2·9	1·4	2·3	7·7*	1·1
Asparagine	<0·6	<0·5	<0·5	<0·5	<0·5	5·8	<0·3	4·6	1·9	<0·2	4·3	2·9
Glutamine	27·6*	19·2*	4·4	3·0	6·5	10·0	3·0	6·7	6·2	4·1	4·1	5·7
Histidine	12·1	8·4	3·0	4·2	14·7*	6·3	13·4*	7·2	3·9	2·7	5·1	3·3
Glycine	22·7	25·8	57·5*	44·7*	23·4	19·7	20·8	17·9	16·9	11·0	25·8	10·6
Methylhistidine	3·4	4·9	3·9	1·0	4·2	3·6	6·3*	3·3	3·1	2·7	1·8	2·4
Alanine†	17·0*	12·5*	21·3*	13·0*	9·5	7·3	6·4	4·7	5·0	3·8	3·2	4·1
Serine	13·6*	11·1*	4·4	4·5	6·8	5·7	3·7	3·9	3·6	3·5	1·8	4·7
Proline	1·6	2·1	<0·5	0·9	<0·5	1·4	1·2	<0·3	<0·3	1·7	1·1	<0·2
Tyrosine	2·4	2·4	<0·5	2·6	5·3*	2·0	2·3	1·3	0·9	1·5	2·3	1·5
Valine	2·1	1·2	<0·5	0·6	1·7	1·5	1·9	1·1	<0·3	1·6	<0·3	1·0
Leucine	0·6	1·6	<0·5	0·7	0·5	1·7	<0·3	<0·3	<0·3	<0·2	<0·3	1·6
Phenylalanine	2·6	0·5	<0·5	<0·5	<0·5	0·4	<0·3	<0·3	<0·3	<0·2	<0·3	<0·2
Threonine	7·2*	5·5*	<0·5	3·8	5·0*	1·8	2·7	4·7*	2·4	2·3	2·4	<0·2
Tryptophan	2·2*	1·5	<0·5	1·4	<0·5	0·8	0·7	3·0*	1·6	1·3	1·3	2·2*
Cystine	1·2	1·1	1·0	1·0	1·4	0·9	0·7	<0·6	0·5	<0·4	<0·8	0·4
Total amino-nitrogen	119*	102*	99·0*	98·1*	98·4*	70·9	67·4	60·3	49·3	38·0	61·3	40·6
Uranium (μg./l.‡)	50	40	40	40	30	40	10	20	40	20	10	10
Uranium (μg./l.§)	45	76	40	30	40	40	50	30	60	40	10	10

* Abnormally raised values $P = 0{\cdot}003$.
† Uncorrected for the presence of β-amino*iso*butyric acid.
‡ Uranium content of the specimen.
§ Average uranium excretion over 3 months before the collection of the specimen.

Table 9. *Amino acid content of urinary specimens from workers exposed to cadmium in an alkaline accumulator factory*

All amino acid data are expressed in mg. of amino-nitrogen/g. of creatinine.

Specimen	J	L	A	M	R	D	K	O	Q	P	H	N
Glutamic acid	8·1*	57·9*	1·5	<0·8	3·7	0·6	2·3	1·8	1·1	1·2	<0·6	3·7
Lysine	45·3*	7·9*	10·5*	31·6*	2·3	1·9	4·0	2·5	9·4*	2·0	2·2	2·2
Asparagine	2·4	1·9	1·5	<0·8	<0·5	8·9*	<0·5	6·3*	3·9	4·2	5·1	4·8
Glutamine	49·7*	40·6*	62·5*	23·6*	15·2	19·0*	14·1	13·4	14·6	14·6	7·2	6·2
Histidine	34·9*	34·4*	13·7*	10·1	5·5	15·2*	4·9	11·0	17·5*	7·0	11·3	7·9
Glycine	64·5*	133·0*	104·0*	53·5*	48·6*	26·3	28·1	19·0	21·0	19·4	23·5	13·9
Methylhistidine	22·6*	1·9	6·2*	<0·3	3·0	<0·5	<0·5	8·0*	3·9	3·2	5·7*	3·7
Alanine†	12·0*	64·5*	16·7*	14·4*	7·5	9·3	12·4*	7·7	10·7*	9·5	8·0	5·2
Serine	40·2*	21·6*	40·0*	20·0*	8·6*	10·0*	11·3*	10·0*	9·4*	7·9*	2·4	4·0
Proline	17·5	1·9	3·4	<0·8	<0·5	<0·5	1·1	1·9	0·9	2·7	<0·4	<0·3
Tyrosine	16·8*	21·7*	10·9*	3·1	1·7	3·4	4·1*	3·0	2·4	5·8*	2·1	2·7
Valine	13·0*	1·9	3·8*	2·7*	2·9*	0·9	1·0	3·1*	1·2	2·2	0·3	1·1
Leucine	23·2*	1·9	4·9*	3·5	1·4	<0·5	1·1	1·2	0·7	2·4	<0·4	1·7
Phenylalanine	9·3*	1·9	1·5	<0·8	<0·5	<0·5	<0·5	1·0	<0·5	2·2	<0·4	1·2
Threonine	57·5*	56·0*	41·5*	11·3*	6·5*	5·1*	8·0*	2·5	0·8	5·0*	4·9*	2·2
Tryptophan	4·8*	4·6*	3·0*	1·1	<0·5	0·8	1·7	0·9	<0·5	2·7	<0·4	3·8
Cystine	4·8*	4·6*	3·1*	1·7*	1·4	1·3	<0·9	<0·9	<0·9	1·2	<0·7	<0·6
Total amino-nitrogen	475*	380*	299*	165*	107*	102*	94·0*	94·0*	89·1*	83·2	72·5	63·1
Cadmium (μg./l.)	36	38	335	30	18	20	62	37	12	26	139	10
Protein (mg./g. of creatinine)	1470	1690	1280	560	0	48	1300	10	10	25	43	0

* Abnormally raised values $P=0{\cdot}003$.

† Uncorrected for the presence of β-amino*iso*butyric acid.

Table 10. *Quantitative observations on reducing-sugars and proteins in urine of men absorbing lead, uranium and cadmium*

The letters P, S, X, H and J have the same significance as in Tables 4, 5, 8 and 9. The symbols −, tr., + and + + refer respectively to negative, trace, positive and strongly positive reactions. Specimen J was from a worker in whom biochemical evidence indicated an apparently symptomless diabetes mellitus.

	Lead		Uranium								Cadmium	
	P_1	P_7	S_9	S_{10}	S_{11}	S_{12}	X_1	X_3	X_5	X_6	H	J
Reducing sugars	−	−	−	tr.	+ +	+ +	−	−	+	tr.	tr.	+ +
Protein	tr.	tr.	+	+	−	−	tr.	tr.	+ +	−	−	tr.

DISCUSSION

The mean total amino-nitrogen/g. of creatinine of the controls in the present work was 52 mg. (Table 3), a result in good agreement with that of 74·3 mg./l. obtained by the specific sulphonated ion-exchange resin procedure (Stein, 1953). This latter method measured taurine but not glutamine, whereas in our investigations the reverse was true.

The amino acid pattern observed in the controls was in many respects similar to previous descriptions (Dent, 1951; Walshe, 1953; Stein, 1953; Dustin, Moore & Bigwood, 1955). Of the simpler neutral amino acids, glycine and alanine occurred in larger quantities than valine and leucine. Stein observed that the concentration of glycine was uniformly three times that of alanine, but we did not observe such a constant relationship, possibly because our measurements of alanine include β-amino*iso*butyric acid. There was always more serine than threonine and more tyrosine than phenylalanine. The urines were comparatively rich in histidine, but the levels of methylhistidine, of dietary origin, were low and the values less than those recorded by Stein.

The observations on glutamine were of particular interest as it constitutes a large proportion of the plasma amino-nitrogen (Krebs, Eggleston & Hems, 1949) and its amide group furnishes a ready source of ammonia both in the kidney and elsewhere. The urinary glutamine was of the same order as that recorded by Archibald (1944), but the levels varied over a wide range (S.D. 3·4 mg. of amino-N/g. of creatinine) in accord with its behaviour in the plasma (Krebs *et al.* 1949; Boulanger & Osteux, 1949; Hamilton, 1945) where quantitatively it is the most variable amino acid.

The concentration of free glutamic acid in freshly voided urine was so low as often to be beyond detection. This fact, already observed by Stein, is surprising in view of its high plasma level (Krebs *et al.* 1949). Other amino acids virtually

absent from our control urinary specimens were methionine, aspartic acid, hydroxylysine, ornithine, citrulline, arginine, proline and hydroxyproline.

Against this background of data on the excretion of amino acids in the control series, observations relating to absorption of lead, mercury, uranium and cadmium can be considered. Three of the eight specimens from workers in the F series exposed to lead fume (Table 4) contained increased amino-nitrogen values. Alanine, threonine, methylhistidine and tyrosine were all present in abnormal quantities in these three urines. Of sixteen specimens from workers exposed to lead oxide dust (series P, Tables 4 and 5), three contained elevated total amino-nitrogen, and elevated individual amino acids; alanine (in five specimens) and serine (in three specimens) appeared to be most frequently affected. The three C specimens from workers exposed to acidic and basic lead chromates were normal. Since figures for alanine include any β-amino*iso*butyric acid, these increases could be due to either or both compounds. One-dimensional chromatograms of F_1 and P_3 indicated that both alanine and β-amino*iso*butyric acid participated in this increase and probably are the two amino acids most frequently influenced by lead absorption.

Only one of the six specimens from workers exposed to mercury metal (series I, Table 6) had a raised amino-nitrogen. The amino acid patterns were peculiar, however, in that glycine excretion was greater than normal in three specimens, particularly no. 1. Series O of workers exposed to organic mercurial compounds was normal.

The results presented in Table 7 obtained by using the copper-complex method of Albanese & Irby (1944) indicate that the total amino-nitrogen values of workers currently exposed to uranium (series S) were significantly higher than those of workers previously but not currently exposed (series B). These results are in accord with the findings by the ninhydrin method (Tables 8*a* and *b*). In currently exposed workers (series S) five out of ten specimens contained abnormal amounts of total amino-nitrogen, whilst the two specimens of series B (previously exposed workers) were within the control range. Specimen S_1 was notable for elevated levels of glutamine, alanine, serine and threonine, in contrast with specimens S_3 and S_4, in which 80 and 69% respectively of the total amino-nitrogen was excreted as glycine and alanine. One-dimensional chromatograms have established that the β-amino*iso*butyric acid content is low as compared with alanine and that the high values in the conjoined alanine spot are due mainly, if not entirely, to increases in urinary alanine concentration. Specimens B_1 and B_2 exhibited little abnormality in their excretion patterns. X_1 and X_2 were the only specimens with raised total amino-nitrogen in the X series. X_1 was obtained from a worker who exhibited proteinuria consistently over the previous three months. The amino acid pattern was grossly abnormal, glutamine being greatly augmented, and also to a less extent alanine, valine, serine, threonine, asparagine, tyrosine, lysine, histidine and methylhistidine. A notable increase in threonine was also obtained in X_2.

The greatest divergence from normality was observed in urinary specimens of workmen absorbing cadmium oxide (Table 9). The total amino acid concentration in ten of the twelve specimens was increased, particularly in J, L, A and M. Raised urinary-protein concentration was notable in these cases. The high total amino-nitrogen output was reflected in abnormal excretion patterns and in a greater number of amino acids in supernormal quantities. Specimens J, L, A and M contained excessive quantities of glycine, alanine, glutamine, serine, threonine, tyrosine, lysine, histidine and methylhistidine. The hydroxy-amino acids, serine and threonine, were remarkable in that they were increased in ten specimens in some of which the total amino-nitrogen was well within the normal range. The urinary concentration of threonine and serine reached peak values 33 and 9·5 times the corresponding mean values of the controls.

A number of conclusions can be drawn from these data. Uranium and cadmium are far more potent agents in the production of amino aciduria than mercury and lead. Raised urinary amino-nitrogen occurred with only 20 μg. of uranium and cadmium/l. of urine, whereas lead and mercury excreted in quantities up to 500 μg./l. may not alter the amino acid pattern. Although these figures are not a measure of the quantity of heavy metal remaining and producing effects in the tissues and, with lead at least, most of the absorbed metal is permanently retained, nevertheless it is evident that the reabsorptive pathways for amino acids in the renal tubules are not markedly sensitive to lead or mercury. Indeed many workmen in this series in whom no amino aciduria was present showed characteristic effects of lead absorption as in abdominal colic and high punctate basophilia. The changes noted were mainly confined to increases in alanine by lead and glycine by mercury.

It is clear that varying grades of amino aciduria may occur in workmen exposed to heavy metals in industry. This fact established, we feel justified in proceeding with the second part of this study, which involves taking blood from the workmen for simultaneous measurements, with the same precise technique, of blood and urinary amino acids. It will then be possible to decide whether these heavy metals cause amino aciduria through a general

metabolic disturbance leading to elevated blood amino acid concentration, or, as would appear more likely from the accumulated evidence presented in the Introduction, whether the phenomenon results from diminished reabsorption in the renal tubule. If the latter alternative is true, the data already collected lend support to the concept of differing pathways of reabsorption of the amino acids suggested by the work of Pitts (1944) and Beyer, Wright, Skeggs, Russo & Shaner (1947). When gross amino aciduria is present, practically all the amino acids are increased to some extent, but specific pathways can be differentiated by their relative sensitivity to toxic heavy-metal ions. The pathways for reabsorption of threonine and serine, for example, are easily blocked by cadmium and, to a less extent, by uranium. This work is being continued to obtain further information on these aspects of the problem.

SUMMARY

1. The effects of absorption of lead, mercury, uranium and cadmium on urinary excretion of amino acids have been investigated.

2. Cadmium and uranium produced more profound changes than did lead and mercury in the total urinary amino-nitrogen levels and in the amino acid patterns.

3. The urinary concentrations of the hydroxyamino acids threonine and serine were particularly increased by absorption of uranium and cadmium.

4. A relative increase in alanine was found in five out of fifteen lead-containing urines, and in glycine in three out of six mercury-containing urines.

5. The observations are discussed in relation to the causation of the amino aciduria and the possible role that heavy metals may play in the elucidation of the reabsorptive pathways in the renal tubular cells.

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A Hand-operated Tissue Chopper

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The mechanical chopper devised by McIlwain & Buddle (1953) for the preparation of tissue slices marks an important advance in manometric technique. The machine prepares slices more rapidly and more uniformly than any previous device; furthermore, it allows small and cheap animals to be used; for example, mouse liver and kidney may be chopped as easily as those of the rat. It seems likely that the tissue chopper in one form or another will be widely adopted.

The chopper of McIlwain & Buddle (1953) was designed to allow quick adjustments, a principle leading to an ingenious but intricate design. In consequence their machine is available only to those laboratories which command the services of skilled mechanics. In practice, frequent adjustment of the tissue chopper is not essential; once the machine has been set to cut slices of a given thickness, for example, months may elapse before it is desired to alter the slice thickness; further, there is little virtue in being able to make an adjustment in 1 sec. instead of, say, 10. This paper describes a chopping machine, based on the principle of McIlwain & Buddle (1953), but designed to be as simple as possible, so that skilled labour is not required for its construction, and of heavy construction, so that many years of trouble-free service will be obtained. The first requirement has been achieved at the expense of simple and rapid adjustment. The second principle leads to the use of ferrous metals and brass throughout, so that, apart from being of assured durability, the machine absorbs its own vibration.

One feature of the technique of using the chopper merited investigation. In their instructions McIlwain & Buddle (1953) recommend that the tissue be weighed, chopped and then transferred to the experimental vessel. If, as a general principle, the rate of oxygen consumption of tissue slices is referred to the wet weight before rather than after chopping, it is implied that a negligible proportion of the cells are injured in chopping; this seemed unlikely so the point was investigated.

CONSTRUCTION OF THE MACHINE

The main features of the machine are shown in Figs. 1–5.

The body is of bright mild-steel flats, sides $3 \times \frac{5}{16} \times 8$ in., ends $3 \times \frac{1}{4} \times 3\frac{3}{8}$ in. The base is a selected cast-iron retort-stand base 9×12 in. All shafts are of $\frac{3}{8}$ in. diameter silver steel. The steel screw, obtained from a government surplus dealer, is $\frac{5}{16}$ in. $\times$ 10 threads/in., square thread, with a brass nut $\frac{1}{2} \times \frac{1}{2} \times \frac{7}{8}$ in.; the brass ratchet wheel is 64 diametrical pitch, 100 teeth, so that each tooth corresponds to 0·001 in. (25·6 μ.) movement of the table; the driving wheel was made from a 3 in. diameter model locomotive-wheel casting. The table and connecting-rod ends were made from brass, B.S. 249:1940; the cam and plates of the ratchet mechanism were brass, B.S. 251:1940. Fixings were high-tensile steel hexagon head B.A. bolts with spring washers. The chopping lever was forged from a bright mild-steel bar $\frac{3}{4} \times \frac{1}{4}$ in. The drawings are largely self-explanatory. Provision was made for the lubrication of all bearings on the principle shown in Fig. 5; the oil holes (not generally shown) were 0·066 in. in diameter, tapped 8 B.A. to receive $\frac{1}{4}$ in. screws as dust excluders. Other features not indicated in the drawings are: (i) the fixing of the body to the base, by means of six 4 B.A. bolts; (ii) the attachment of the ratchet wheel to the screw spindle by a $\frac{1}{32}$ in. silver-steel pin; (iii) the connecting-rod halves were brazed into their ends; (iv) the driving pawl was brazed to its spindle; (v) the silver-steel pin for the razor blade was press-fitted into the lever. Extensive use has been made of the principle of the clip boss, which was used to attach not only the wheels but also the levers and collars to shafts.

Potential difficulties have been eliminated as follows: (i) the necessity for aligning the connecting-rod ends and obtaining the optimum length was avoided by making the connecting rod in two parts joined by a pair of bosses, so