3. *L*(+)-Arginine decarboxylase can be resolved into apo-enzyme and codecarboxylase by precipitation with ammoniacal ammonium sulphate solution. *L*(+)-Ornithine decarboxylase resolves spontaneously on standing into apo-enzyme and codecarboxylase. It has not been possible to resolve *L*(+)-glutamic acid decarboxylase and there is no evidence that this enzyme contains codecarboxylase.

4. The action of common enzyme inhibitors on six amino-acid decarboxylases in a cell-free state is

described. The four enzymes containing codecarboxylase are markedly more sensitive to inhibition by iron and hydrazine than the two in which it has not been possible to demonstrate the presence of codecarboxylase.

The authors are indebted to the Medical Research Council for grants towards the cost of these investigations. They wish to thank Prof. A. C. Chibnall, Dr M. Stephenson and Dr M. Dixon for criticism, and Miss H. M. R. Epps for permission to quote certain results.

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The Lead Content of Urinary and Biliary Calculi and Gall-bladder Bile

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(Received 16 October 1944)

The report by Trumper & Gordy (1941) of a renal calculus containing 3% of lead, and a subsequent paper by Wood (1941), who examined a number of urinary calculi, both recent and from a collection made in 1850-60, and found lead present spectroscopically in all except two, suggested a further investigation of the lead content of urinary calculi. Two groups were examined, one of 17 stones removed at operation over the last few years, the other of 16 stones, a representative selection from a pre-1900 collection of bladder calculi in the museum of the Pathology Department, one of the seven dated stones belonging to 1830, the remaining six to 1860-80. In view of the possible importance of the bile as a channel of lead excretion (Aub, Fairhall, Minot & Reznikoff, 1926), biliary calculi removed at operation or *post mortem*, and bile obtained from the gall-bladder *post mortem*, were also analyzed.

EXPERIMENTAL

The stones were dried, powdered, and a portion taken for qualitative analysis. From 1 to 3 g. of the remainder were analyzed for lead by the method previously described (Tompsett & Anderson, 1935), with the modification advised for materials containing considerable calcium phosphate (Tompsett, 1939).

Lead was estimated in bile by the method described for blood (Tompsett & Anderson, 1935, 1939).

The results of the analyses of urinary calculi are shown in Table 1, of gall-stones in Table 2, and of bile in Table 3. The stones in Table 1 are grouped

by the results of qualitative analysis according to the scheme given by Harrison (1937).

Table 1. *Lead content of urinary calculi*

Type	Lead content (mg./100 g.) of	
	Recent stones	Old stones
Renal calculi:		
Oxalate	7.5, 5.4, 3.7, 11, 10.5	—
Oxalate and phosphate	7.6, 10.5, 9	—
Phosphate and carbonate	11, 29, 8.4	—
Vesical calculi:		
Uric acid or urate	0.66, 0.33	1.6, trace, 0.44
Phosphate	5.5	—
Phosphate and urate	2.7	3.5, 3.6, 3.2
Phosphate, urate and carbonate	1.3, 11	—
Phosphate and oxalate	—	7.7, 19, 9.65, 5.2, 6.3, 2.7
Phosphate, oxalate and urate	—	10.8, 9.2, 10.6
Urate and oxalate	—	3.7
Mean, excluding pure uric acid or urate stones	8.9	7.3
Mean, all stones excluding pure uric acid or urate stones	8.2, standard deviation 5.15	

Table 2. *Lead content of biliary calculi*

Type of calculus	Lead content (mg./100 g.)
Multiple faceted	0.61, 2.2, 0.45, 4
Cholesterol-pigment	0.3, 11, 6.1
'Pure' cholesterol	1.1
'Pure pigment'	65, 13.5

Table 3. *Lead content of gall-bladder bile*

No.	Age (years)	Sex	Occupation	Post-mortem diagnosis	Lead content of bile ($\mu\text{g./100 ml.}$)
1	53	M.	Hammerman	Bronchitis, bronchopneumonia	27
2	63	M.	—	Carcinoma of stomach	14
3	2½	M.	Child	Delayed chloroform poisoning	31
4	16	M.	Pithead worker	Peritonitis	43
5	48	F.	Housewife	Thrombocytopaenic purpura	30
6	63	M.	Works foreman	Pelvic cellulitis	40
7	37	M.	Driller	Chronic nephritis	32
8	16	M.	Shop assistant	Sympathicoblastoma	71
9	65	F.	Housewife	Peritonitis	14
10	63	M.	Miner	Infarct of myocardium	8
11	73	M.	Retired	Carcinoma of intestine	24
12	13	M.	Schoolboy	Rheumatic endocarditis	38
13	30	M.	Steel-worker	Gunshot wound	70
14	54	F.	Housewife	Chronic pulmonary tuberculosis	97
					Mean 36

DISCUSSION

It will be seen that appreciable amounts of lead were found in all the urinary calculi examined, with the exception of the pure uric acid or urate stones, which contained only very small amounts. This was not unexpected, as the conditions of formation of phosphatic calculi would appear to be suitable for the deposition of lead. Entrapment by the precipitation of alkaline phosphates or calcium oxalate is one of the methods used for the separation of lead from urine. There are no striking differences in lead content between the recent calculi and those of the older group. It is of interest that the amounts of lead found in the urinary calculi (excluding the pure uric acid and urate stones) are of the same order as those found in bone. The 28 calculi with a range of lead concentration from 1.3 to 29 mg./100 g. (mean weight, 8.2 mg. and standard deviation 5.15) can be compared with 19 femurs analyzed by Tompsett (1936), which on a wet weight basis ranged from 1.8 to 10.8 mg./100 g. (mean weight, 4.8 mg.).

The lead content of the gall-stones varied considerably, and the concentration appeared to bear a direct relation to the amount of pigment. It would appear as if copper behaved in a similar manner, for it was present in considerable amounts in the more pigmented stones, colouring the ash blue. No quantitative estimations of copper were made. Judd & Dry (1935) found 0.063–1.07 mg./100 ml. of copper in gall-bladder or fistula bile, while Meunier & Saint-Laurens (1926) found 300 mg./100 g. in pigmented stones.

The lead content of the bile is much less than that of the calculi, being in $\mu\text{g.}$ instead of mg./100 g. The figures are of the same order as those found for lead in blood (Tompsett & Anderson, 1935, 1939), and are all less than 100 $\mu\text{g./100 ml.}$, which is the upper limit for lead in normal blood. In two in-

stances, both bile and accompanying gall-stones were analyzed. Bile no. 5, with 30 $\mu\text{g./100 ml.}$ of lead, contained a cholesterol-pigment stone with 11 mg./100 g. of lead, and bile no. 6, with 40 $\mu\text{g./100 ml.}$, contained a number of 'pure' pigment stones with 65 mg./100 g. There is, however, a considerable excretion of lead by the liver, for the concentration in gall-bladder bile, with a mean of 38 $\mu\text{g./100 g.}$, is approximately 10 times that found in normal urine. The occupations given in Table 3, and noted for 12 of the recent cases in Table 1, do not suggest any abnormal lead hazard. In the case of the steel-worker dying of a gunshot wound, the bullet did not remain in the body. No information is available for the old urinary calculi.

SUMMARY

1. The lead content of 17 recent urinary calculi, and of 16 calculi all more than 45 years old, was determined. In five calculi composed of uric acid or urates only, the lead content ranged from a trace to 1.6 mg./100 g. In the remaining 28 mixed calculi, lead ranged from 1.3 to 29 mg./100 g., mean 8.2 mg. There was no obvious difference in lead content between the recent calculi and the old. The amounts of lead found were of the same order as those found in long bone.

2. In ten gall-stones the lead content varied from 0.3 to 65 mg./100 g., the highest concentration being found in 'pure' pigment stones.

3. In 14 samples of gall-bladder bile obtained *post mortem* the lead content varied from 8 to 97 $\mu\text{g./100 ml.}$, with a mean of 38 $\mu\text{g.}$

I am much indebted to Prof. J. W. S. Blacklock for the opportunity to analyze the old calculi in the museum collection, and for his interest in this work. Part of the cost of this investigation was defrayed from a grant from the Rankin Fund of the University of Glasgow.

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The Routine Estimation of Haemoglobin as Oxyhaemoglobin

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(Received 16 October 1944)

It has become evident that visual (subjective) colorimetric estimations of haemoglobin are, perhaps even for clinical purposes, not sufficiently accurate because of the wide scatter in determinations by the same observer and the still greater variations between the results of different observers using samples of the same blood. Photoelectric colorimeters, on the other hand, have no subjective errors and do not cause visual fatigue. Moreover, they are now cheaper (Bell, 1944) than colorimeters of the Duboseq type. A number of procedures suitable for photoelectric colorimetry have already been closely investigated, viz. the alkaline haematin method (Clegg & King, 1942; Peterson & Strangeways, 1944), the cyanmethaemoglobin method (Stadie, 1920; Austin & Drabkin, 1935; King, Gilchrist & Delory, 1944) and the pyridine haemochromogen method (Rimington, 1942). Although these three methods seem to be the most favoured they are moderately complicated, and the latter two use dangerous or objectionable chemicals. The use of oxyhaemoglobin, the most easily prepared derivative, has been suggested by Szigeti (1940), Karr & Clark (1941), Bell & Guthmann (1943) and Reeve (1944). This paper describes our experiences with the oxyhaemoglobin method using the balanced modification of the photoelectric colorimeter described by Bell & Guthmann (1943).

METHODS

Measurement of optical densities of haemoglobin derivatives with various light filters. The densities of oxyhaemoglobin and of the three pigments mentioned above were measured in the green, blue and violet regions of the spectrum. The oxyhaemoglobin solution was made by diluting 0.2 ml. of oxalated venous blood to 25 ml. with approximately 0.007 N-NH₃ (0.04 ml. ammonia solution, sp.gr. 0.88, to 100 ml. with water (Szigeti, 1940)); the other three pigments by

the methods quoted in the introduction. The final concentration of blood was the same in all cases and the results have been referred to oxyhaemoglobin as 100 (Table 1).

Measurement of optical densities with green light filters. The green region of the spectrum was investigated by means of a number of filters (Ilford Micro 3 and Spectrum 604; and the Wratten nos. 40, 44, 53, 58A, 60P, 61N, 63, 65, 65A and 67; cf. Ilford Ltd. (1944) and Eastman Kodak Co. (1940)) in the hope of finding a region where all the pigments likely to be found in circulating blood had approximately the same density. The results for three of these filters are given in Table 2.

The first four pigments were prepared from each of eight blood samples. Sulphaemoglobin (four samples) was prepared by bubbling H₂S through the oxyhaemoglobin solution for 1½ min., centrifuging and measuring the density before any precipitate of colloidal sulphur appeared. A marked change in colour was seen; there was a dense absorption band in the red (c. 620 mμ) with faint bands of residual haemoglobin; the band in the red persisted after treatment with Na₂S₂O₄. The haemoglobin was largely (probably over 80%) converted to sulphaemoglobin (cf. Drabkin & Austin, 1935).

Comparison of oxyhaemoglobin method with colorimetric iron analyses. The accuracy of the oxyhaemoglobin method was investigated by the Wong (1928) thiocyanate method, but it was found that duplicate estimations did not agree closely. A slight alteration of Ponder's (1942) modification was suggested to us by Dr A. B. Anderson and the final procedure was as follows:

To 0.5 ml. of blood in a 50 ml. flask were added gradually 2 ml. conc. H₂SO₄, swirled to mix and allowed to stand overnight. Potassium persulphate (2 ml. saturated K₂S₂O₈ solution) was added, mixed and allowed to stand 1 hr. Distilled water was added to approximately 25 ml. and mixed. Sodium tungstate (2 ml. 10%) was added, mixed and allowed to stand 1 hr. The volume was made to 50 ml., the fluid mixed and filtered. To 20 ml. of the filtrate were added 1 ml. saturated K₂S₂O₈ solution and 4 ml. 3 N-KCNS. The density of the Fe(CNS)₃ colour was read exactly 3 min. later, with a Micro 2 light filter (Ilford).