97. THE ABSORPTION AND EXCRETION OF 'MINOR' ELEMENTS BY MAN

2. COBALT, NICKEL, TIN AND MANGANESE

BY NORMAN LESLIE KENT AND
ROBERT ALEXANDER McCANCE

From the Department of Medicine, Cambridge

(*Received 30 July 1941*)

IN a previous paper Kent & McCance [1941] described the results of their work on the absorption and excretion of Ag, Au, Li, B, and V. The experiments now to be described have been carried out in a similar way. As before, the subjects have been either patients or normal persons and the same spectrochemical apparatus and analytical technique have been used. The previous article should be consulted for information about the analytical methods; a full description of the normal subjects and of the metabolic organization has been given by McCance & Widdowson [1941].

Cobalt

Bertrand [1926] and his co-workers showed that Ni and Co were present in all samples of arable soil collected from European countries. They also found these elements in plants and in many human and mammalian organs [Bertrand & Macheboeuf, 1926; Bertrand & Mokragnatz, 1925]. Wohlwill [1907] reported that neither Ni nor Co was absorbed from the gut, but this must have been due to faulty analysis, for absorption has been demonstrated by others [Mascherpa, 1927; Simesen, 1939]. The facility with which 'coast' disease in sheep can be cured by a Co drench [Askew & Dixon, 1936; Wunsch, 1937] and the ease with which polycythaemia can be produced by the oral administration of Co [Josland, 1936] are excellent proofs that at any rate small quantities must be absorbed. If Co has once been absorbed or has been given parenterally, the literature suggests that it is excreted partly by the kidney and partly by the intestine and that the route depends to a large extent upon the nature of the compound and upon how it has been administered. Untersteiner [1931], for example, found that divalent Co was more rapidly eliminated than trivalent Co. Simesen [1939] recovered in the urine of the next 24 hr. 80% of the Co which he had injected as $[Co_3CO(NH_3)_4]Cl$ subcutaneously into rabbits. This compound was excreted unchanged by the kidney. Mascherpa & Perito [1931], who administered $CoCl_2$ to guinea-pigs by the same route, recovered from the urine during the following 10 days less than half the quantity injected. Le Goff [1927] injected 24 mg. of $CoCl_2$ intramuscularly into a man and recovered 6·8 mg. of the salt in the urine within the next 18 hr. He recovered a much smaller quantity (2·64 mg.) from another patient who was a diabetic. Unfortunately the faeces were not examined.

The presence of Co in bile was demonstrated long ago by Stuart [1884]. This was not confirmed by Mascherpa [1927], but has been substantiated by Caujolle [1936] after intravenous administration of the chloride.

The subject of the present experiment was a male hospital patient suffering from carcinoma of the stomach. His kidneys were functioning normally and he

did not vomit during the studies. Excluding the preliminary and after periods the experiment lasted 2 weeks. During the first, which served as the control, the patient was given a weighed diet, and urine and faeces were collected quantitatively. During the 2nd week the diet was repeated in every possible respect and urine and faeces were collected as before. On 5 of the 7 days of the 2nd week $CoCl_2$ was injected intravenously and in all 13 mg. of Co were administered in this way. The urine and faeces of both weeks were analysed for Co, but not the food. The results are given in Table 1. The 1st week's data show that the food

Table 1. *The metabolism of cobalt*

Week	Co injected intravenously mg.	Co excreted, mg.			Urine Co as % of total
		Urine	Faeces	Total	
1	0	0·21	1·04	1·25	17
2	13	2·36	1·78	4·14	57
Excess of 2 over 1	13	2·15	0·74	2·89	74

must have contained appreciable amounts of Co and that only 17 % of it passed through the kidney into the urine. During the week in which the injections were given there was a tenfold increase in the urinary excretion and a much smaller increase in the faecal excretion. Assuming the Co intake by mouth to have been the same in both weeks, the results show that of the 13 mg. which were injected intravenously 2·89 were excreted in the week, 74 % of this amount by the kidney. This single experiment confirms in broad outline the results of previous workers on animals and of Le Goff [1927] on man. It suggests that the gut is the main channel of excretion for the Co in natural foods, probably because relatively little is absorbed. Once Co has reached the tissues, however, it indicates that the processes of elimination are very slow, and that the kidney is the organ chiefly responsible. In the rate at which the Co was excreted the present results differ from those of Copp & Greenberg [1941], who administered radioactive Co to two rats and found that 90 % of the Co injected intraperitoneally into one animal was excreted within 4 days. This rapid elimination may be peculiar to the rat.

Nickel

The occurrence and distribution of Ni in soil, plants and in human and animal organs was studied by Bertrand and his fellow workers [Bertrand & Macheboeuf, 1925; 1926; Bertrand, 1926]. They found that Ni and Co had similar distributions in nature, but that animals tended to contain more Co than Ni, plants and soils more Ni than Co [Bertrand & Macheboeuf, 1926]. Mascherpa [1927] and others have studied the absorption and excretion of Ni and its salts. Large doses, whether by mouth or injection, produce a muco-haemorrhagic enteritis, and there is general agreement that this metal is excreted into the intestine rather than by the kidney. This is thought to explain why early workers found that Ni was not absorbed after being taken by mouth. It has been demonstrated in the bile by Stuart [1884], Lehmann [1909] and Caujolle [1937]. Few records have been found of experiments on man, and none of the elimination of Ni after intravenous or subcutaneous administration. A general account of the absorption, excretion and pharmacology of both Ni and Co has been given by Hendrych & Weden [1934].

Two normal men were the subjects for the present studies. Each received daily intravenous injections of $NiCl_2$ during the second week of a long metabolism experiment. The results are shown in Table 2, and it will be seen that before the

Table 2. *The absorption and excretion of nickel*

Subject	Length of period days	Ni intake mg./period: Injected	Ni intake mg./period: In food	Ni excretion mg./period: In urine	Ni excretion mg./period: In faeces	Balance mg.	Ni 'recovered' mg.
E. B.	7		2·25	1·67	0·72	− 0·14	
	7	9	1·99	3·42	0·69	+ 6·88	3·8
	7		2·67	3·16	1·19	− 1·68	
N. K.	7		—	2·29	1·20	—	
	7	20	5·51	6·70	1·33	+17·48	
	4		2·21	2·88	0·59	− 1·26	
	7		3·43	3·48	2·11	− 2·16	7·4
	7		3·20	2·61	2·05	− 1·46	

injections were made there was more Ni in the urine than in the faeces. This suggests a reasonably good absorption of the traces of Ni normally present in food. E. B. was almost exactly in balance at this time. N. K.'s balance cannot be given since the figure obtained for his food suggests that it became contaminated with Ni from cutlery during its preparation for analysis. Great precautions were subsequently taken to work only with wooden utensils. During the period of injection the output of Ni rose in the urine of both subjects, so that they eliminated more by this channel than they took in with their food, and they continued to do so in later periods. Clearly Ni was being excreted in the urine. The faecal outputs did not rise during the period of Ni injections, and in subsequent periods they varied in quantity but tended to be slightly higher than they had been during the preliminary week. Only 42 % of the Ni injected into E. B. was recovered and 37 % of that given to N. K. Ni, like Co and Sn (*vide infra*), was excreted slowly and rather incompletely in these experiments and the organ mainly concerned was the kidney, not the gut.

Tin

The metabolism and pharmacology of Sn have been studied spasmodically since foods began to be preserved in cans. Most authors are in agreement that foods may become contaminated with Sn from tinned containers, but small quantities of the metal have also been found in fresh foodstuffs [Boyd & De, 1933; Bertrand & Ciurea, 1931]. Within recent years Sn salts have had a vogue in the treatment of furunculosis and most of the proprietary preparations are intended to be given subcutaneously. Apart, therefore, from the possibilities of industrial poisoning, it is evident that the body is frequently faced with the necessity of having to deal with small amounts of Sn salts. Buchanan & Schryver [1908], using human subjects, and Datta [1940], working with rats, came to the conclusion that tin was poorly absorbed and that small amounts taken by mouth were excreted mainly in the faeces. Sn given by subcutaneous or intravenous injection to animals has been reported to be rapidly removed from the circulation and to be excreted slowly and incompletely by the kidney [Ungar & Bodlander, 1887; Buchanan & Schryver, 1908; Salant *et al.* 1914; 1918; Salant, 1920]. No account has been found of the excretion of Sn after intravenous or subcutaneous administration to man.

Two normal men acted as subjects for the present investigations and each received a total of 28 mg. of Sn as 'Stanoxyl' by daily intravenous injection during the 2nd week of a 21-day metabolism experiment. The 1st week served as a control, during which the absorption and excretion of the Sn present in the food was followed. The results are given in Table 3. Both men were roughly in

Table 3. *The absorption and excretion of tin*

Subject	Length of period days	Sn intake mg./period: Injected during 2nd week	Sn intake mg./period: In food	Sn output mg./period: In urine	Sn output mg./period: In faeces	Balance mg./period	Sn recovered mg.
E. B.	7	0	14·4	7·2	6·6	+ 0·6	
	7	28	13·1	12·6	7·3	+21·2	19·7
	7	0	12·4	13·4	11·9	−12·9	
R. M.	7	0	12·8	8·8	2·9	+ 1·1	16·7
	14	28	30·5	36·6	10·6	+11·3	

balance during period 1 and were excreting 52 and 75 % of their whole outputs of Sn in the urine. These figures are much higher than those of Datta [1940] or of Buchanan & Schryver [1908]. During subsequent periods the injected Sn was slowly excreted. 70 % of the amount administered was recovered from E. B. and 60 % from R. M. The kidney excreted most of this extra Sn, although the amount in the faeces also rose slightly. The part played by the kidney can be appreciated more fully if the amounts in the food and urine are compared before and after the injections were given.

Manganese

The regular occurrence of Mn in plants and animals—and hence in food—has been a well-established fact for many years [Bertrand, 1939], and many tables have been published showing the usual range of Mn concentration in various foodstuffs [Lindow & Peterson, 1927; Skinner & Peterson, 1928; Davidson, 1929; Remington & Shiver, 1930; Richards, 1930; Peterson & Skinner, 1931]. Perla *et al.* [1939] found that rats might retain little or none of the Mn naturally present in the food, practically all of it passing out in the faeces. When inorganic Mn was added to the food so that the intake was raised from 0·096 to 0·165 and later to 13·45 mg./rat/day, 25 % of the dose was absorbed and retained. Skinner *et al.* [1931] obtained somewhat similar results, but found much higher percentages of Mn normally excreted in the urine.

The kidney has never been found to play an active part in the elimination of Mn [Harnack, 1901]. Normally, only a very small part of the Mn in the food is excreted by this organ, and the figure of 20 %, which Skinner *et al.* [1931] obtained, seems very high in the light of other work. Mn taken by mouth may increase slightly the amount in the urine, but the proportion so excreted always falls [Perla *et al.* 1939; Skinner *et al.* 1931]. Reiman & Minot [1920] showed that these conclusions held good also for human subjects, but in their opinion part at least of the Mn in the faeces represents Mn that has been absorbed and subsequently eliminated. These workers found that after a man had taken 8 g. of franklinite, containing 0·77 g. of Mn, the blood Mn might rise from 0·012 to 0·024 mg./100 ml. within an hour, and that after patients with biliary fistulae had taken 5 g. of franklinite by mouth the Mn in the bile might rise to 10 times its previous level. They held therefore that at least a part of the Mn in the faeces represented metal which had been absorbed, only to be re-excreted. Few references have been found to experiments in which Mn was injected into animals and none to such experiments on man. Cahn [1884] injected toxic doses of Mn salts into rabbits, killed the animals shortly afterwards and analysed their organs. He concluded that Mn so administered was eliminated mainly by the intestine. Quite recently Greenberg & Campbell [1940] have used a radioactive

isotope, Mn^{54}, to follow the fate of Mn in the body. 1 mg. of Mn^{54} was injected intraperitoneally into 1 rat and during the subsequent 4 days 90·7 % of it was recovered in the faeces. The result would have been more convincing if the Mn had been given intravenously or subcutaneously rather than intraperitoneally. The quantity excreted in the urine was too small to have any significance and the remaining 9·3 % of the injected Mn^{54} was found in the bodily organs.

One woman and two men—all normal persons—were the subjects of the present experiments. Each received 4–7 injections of Mn butyrate during the 2nd of a 4-period metabolism experiment. E. B. received a diet designed to contain very little Mn; A. M. and P. S. took 40–50 % of their calories in the form of white flour. In other respects the latter's diets were freely chosen. A. M. (the woman), who received 14·3 mg. of Mn, and E. B., who received 19·8 mg., excreted little or none of the injected dose within the time of observation. P. S., however, who received 31·5 mg., probably excreted 16·1 mg. in the faeces during the last 3 weeks of his experiment, but the balances were somewhat irregular and unconvincing. The full results are to be found in Table 4.

Table 4. *The absorption and excretion of manganese*

Subject	Period no. and length	Mn intake mg./period: By mouth	Mn intake mg./period: Injected	Mn excretion mg./period: Urine	Mn excretion mg./period: Faeces	Balance mg./period	Mn recovered mg.
A. M.	1 (7 days)	15·5		0·7	13·7	+ 1·1	
	2 (7 days)	19·7	14·4	0·4	17·5	+16·1	
	3 (7 days)	23·4		0·3	24·9	− 1·8	3·1
	4 (7 days)	15·8		0·9	18·0	− 3·1	
E. B.	1 (6 days)	10·3		0·3	10·3	− 0·3	
	2 (6 days)	13·4	19·8	0·2	14·5	+18·5	
	3 (6 days)	12·5		0·4	12·0	+ 0·1	0
	4 (6 days)	14·6		0·4	8·2	+ 6·0	
P. S.	1 (7 days)	46·5		1·2	48·0	− 2·7	
	2 (7 days)	43·5	31·5	0·3	59·0	+15·7	
	3 (7 days)	32·4		0·1	37·6	− 5·4	16·1
	4 (7 days)	30·5		0·3	25·1	+ 5·1	

In no subject was there any evidence that any of the injected Mn was excreted in the urine. So far as the urine is concerned these results are essentially in agreement with those of most previous workers. It is evident, however, that Mn is not excreted freely, if at all, by the human bowel when it is injected in the amounts used for these experiments. The partial excretion shown by P. S. may have been the result of the larger doses he received, or of the smaller storage capacity which he possibly possessed. At any rate the difference is thought to have been genuinely one of metabolic behaviour and not to have arisen from contaminations or analytical inconsistencies. The Mn in the foods and faeces of P. S. and in some of the specimens of A. M. was determined chemically by the periodate method of Willard & Greathouse [1917]. The results agreed satisfactorily with those obtained spectrographically, and actually the figures given for P. S. in Table 4 were those obtained by the chemical method.

Table 4 shows that the Mn intakes may vary considerably on natural foods. One of the easiest ways of raising them is to eat a large amount of brown bread, for bran is very rich in Mn. 100 g. of 92 % flour were found to contain 2·15 mg. of Mn and 100 g. of 69 % flour only 0·49 mg. Table 5 shows the balances of 2 persons when they were deriving 40–50 % of their calories from 69 % flour and from 92 % flour.

Table 5. *Manganese intakes and excretions on diets containing large amounts of white and brown flour*

Subject	Diet	Mn intake in food mg./week	Mn excretion, mg./week			Balance mg./week
			Urine	Faeces	Total	
A. M.	White flour	15·5	0·7	13·7	14·4	+1·1
	Brown flour	59·6	0·2	56·2	56·4	+3·2
E. W.	White flour	18·8	0·4	19·2	19·6	−0·8
	Brown flour	61·8	0·6	60·8	61·4	+0·4

Taking the results in Tables 4 and 5 together, they show that the urinary excretion of Mn, like that of Fe, is negligibly small, whatever the intake by mouth or injection. The fact that injecting Mn in these doses did not necessarily provoke any excretion of the metal by either kidney or gut recalls that the human animal has been shown to react to injections of Fe in exactly the same way [McCance & Widdowson, 1938], and illustrates the biochemical and pharmacological affinities of the two metals, some of which were pointed out long ago by Cahn [1884].

SUMMARY

Metabolism experiments on men and women, combined with intravenous injections of Co, Ni, Sn and Mn salts, have shown that

(1) One man excreted about 20 % of his food Co in the urine. Injected Co was excreted slowly, mainly by the kidney.

(2) Two men excreted 60–70 % of their food Ni in the urine. Injected Ni was excreted slowly, mainly by the kidney.

(3) Two men excreted between 50 and 80 % of their food Sn in the urine. Injected Sn was excreted very largely by the kidney.

(4) Only a very small part of the Mn in the food was excreted in the urine, and there was no increase after the intravenous injections of Mn salts. Two persons retained the whole of the injected Mn, a third excreted about 50 % by the bowel.

The authors are grateful to Prof. Norrish for placing a spectrograph at their disposal and to Dr W. C. Price for technical advice. The work could never have been undertaken without the help and co-operation of the subjects, and also of Miss B. Alington and Dr E. M. Widdowson.

The greater part of the expenses were covered by a grant made by the Medical Research Council.

REFERENCES

Askew & Dixon (1936). *N.Z. J. Sci. Tech.* **18**, 73.
Bertrand (1926). *Science*, **64**, 629.
—— (1939). *Ergebn. Vitamin u. Hormonforsch.* **2**, 192.
—— & Ciurea (1931). *C.R. Acad. Sci., Paris*, **192**, 780.
—— & Macheboeuf (1925). *Bull. Soc. Chim. Fr.* 4th Ser., **37**, 934.
—— —— (1926). *Bull. Soc. Chim. Fr.* 4th Ser., **39**, 942.
—— & Mokragnatz (1925). *Bull. Soc. Chim. Fr.* 4th Ser., **37**, 554.
Boyd & De (1933). *Indian J. med. Res.* **20**, 789.
Buchanan & Schryver (1908). *Local Gvt. Bd* (*Med. Dept.*), *Rep. Inspector Foods*, No. 7.
Cahn (1884). *Arch. exp. Path. Pharmak.* **18**, 129.

Caujolle (1936). *Bull. Soc. Chim. biol., Paris*, **18**, 1081.
—— (1937). *Bull. Soc. Chim. biol., Paris*, **19**, 342.
Copp & Greenberg (1941). *Proc. nat. Acad. Sci., Wash.*, **27**, 153.
Datta (1940). *Indian J. med. Res.* **28**, 451.
Davidson (1929). *Cereal Chem.* **6**, 128.
Greenberg & Campbell (1940). *Proc. nat. Acad. Sci., Wash.*, **26**, 448.
Harnack (1901). *Arch. exp. Path. Pharmak.* **46**, 372.
Hendrych & Weden (1934). Handbuch exp. Pharmak. 2 Teil, 3 Band. Berlin: Julius Springer.
Josland (1936). *N.Z. J. Sci. Tech.* **18**, 474.
Kent & McCance (1941). *Biochem. J.* **35**, 837.
Le Goff (1927). *C.R. Soc. biol., Paris*, **97**, 21.
Lehmann (1909). *Arch. Hyg., Berl.*, **68**, 421.
Lindow & Peterson (1927). *J. biol. Chem.* **75**, 169.
McCance & Widdowson (1938). *J. Physiol.* **94**, 148.
—— —— (1941). In the Press.
Mascherpa (1927). *Arch. exp. Path. Pharmak.* **124**, 356.
—— & Perito (1931). *Arch. int. Pharmacodyn.* **40**, 471.
Perla, Sandberg & Holly (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 372.
Peterson & Skinner (1931). *J. Nutrit.* **4**, 419.
Reiman & Minot (1920). *J. biol. Chem.* **45**, 133.
Remington & Shiver (1930). *J. Ass. Off. Agr. Chem.* **13**, 129.
Richards (1930). *Biochem. J.* **24**, 1572.
Salant, Rieger & Treuthardt (1914). *J. biol. Chem.* **17**, 265.
—— —— —— (1918). *J. biol. Chem.* **34**, 463.
—— (1920). *J. industr. Hyg.* **2**, 72.
Simesen (1939). *Arch. int. Pharmacodyn.* **62**, 347.
Skinner & Peterson (1928). *J. biol. Chem.* **79**, 679.
—— —— & Steenbock (1931). *J. biol. Chem.* **90**, 65.
Stuart (1884). *Arch. exp. Path. Pharmak.* **18**, 151.
Ungar & Bodlander (1887). *Z. Hyg. InfektKr.* **2**, 241.
Untersteiner (1931). *Arch. int. Pharmacodyn.* **41**, 410.
Willard & Greathouse (1917). *J. Amer. chem. Soc.* **39**, 2366.
Wohlwill (1907). *Arch. exp. Path. Pharmak.* **56**, 403.
Wunsch (1937). *Chem. and Ind.* **56**, 855.