Bile and the Absorption of Strontium and Iron

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1. Strontium absorption was studied *in vivo* with loops of ileum in rachitic chicks and found to be increased by vitamin D₃, bile salts and sodium lauryl sulphate.
2. Bile salts and sodium lauryl sulphate rendered strontium soluble in butanol-benzene (1:1, v/v).
3. Bile was not concerned in the absorption of iron in rats from its water-soluble form, ferrous sulphate.
4. Ligation of the bile ducts in rats caused a decrease in the absorption of iron presented as its sparingly soluble phosphate.
5. The effect of bile on cation absorption is discussed.

In two papers (Webling & Holdsworth, 1965, 1966) it was shown that bile plays an important part in the absorption of calcium from the small intestine of the chick and the rat. This was separate from any effect of bile in aiding the absorption of vitamin D₃. The effect was seen with soluble solutions of calcium but was probably even more important in the absorption of calcium from its sparingly soluble salts. The effect of bile on calcium absorption was paralleled by an ability to make calcium soluble in lipid solvents. The question arises as to whether bile aids the absorption of other cations. Attempts to study the effect of bile on magnesium absorption by using chemical methods and atomic absorption gave very variable results and it was apparent that the problem would need the use of ²⁸Mg, which was not available to us. In the present paper, however, we report some effects of bile on the absorption of strontium and iron in chicks and rats.

MATERIALS AND METHODS

Experiments on chicks. The management of rachitic chicks, the experimental technique for studying absorption from ileal loops and the materials used in these experiments were as described previously (Webling & Holdsworth, 1965). In one experiment the gut was removed at the end of the period of absorption and the gut and its contents were dried and ashed. By counting the residual ⁹⁰Sr in the intestine, the amount absorbed was calculated. The ⁹⁰SrCl₂ (The Radiochemical Centre, Amersham, Bucks.) was diluted with carrier SrCl₂ to give a specific activity of 40 μCi/m-mole.

Relative solubilities in lipid solvents of the strontium complexes formed with bile salts or lauryl sulphate. The solutions used were those used in the ileal loop experiments and contained 0.02M-⁹⁰SrCl₂ in 0.15M-NaCl, in 0.007M-sodium lauryl sulphate, in 0.05M-sodium taurochenodeoxycholate, or in 0.05M-bile salts from rachitic chicks. The

solution was mixed with an equal volume of butan-1-ol-benzene (1:1, v/v) and shaken for 15 min. The mixture was centrifuged for 15 min. at 2000g and 0.050 ml. samples of the upper and lower phases were taken for scintillation counting. The partition coefficient of the strontium complex was calculated.

Experiments with rats. Normal male rats, Wistar hooded *Rattus norvegicus* (weight 150 ± 2 g.), were either sham-operated or had their common bile ducts tied as described by Webling & Holdsworth (1966).

For the study of iron absorption the ⁵⁹Fe was given by a metal cannula into the oesophagus. At 2 hr. the rats were anaesthetized with ether and a blood sample taken from the heart. They were then killed and liver, spleen and rib bone collected.

Form of ⁵⁹Fe used for absorption studies. The ⁵⁹FeSO₄ (The Radiochemical Centre) was mixed with a solution of FeSO₄·7H₂O, pH 4, to give a final specific activity 1 μCi/μmole. The soluble iron was given in this form, 1 μmole/rat in a total volume of 1 ml. containing sucrose (0.05 M). The insoluble iron was formed in a solution containing gum tragacanth (0.5%, w/v) and sucrose (0.05 M) by making the solution 2 mM with respect to Na₂HPO₄ at pH 7. The final 1 ml. used for dosing contained 1 μmole of Fe²⁺ in 2 mM-Na₂HPO₄. The solution was made immediately before the start of the experiment and stirred continuously by a magnetic stirrer.

Determination of ⁹⁰Sr. The methods used for determining ⁹⁰Sr in blood plasma and tibia were essentially those used for ⁴⁵Ca determination (Webling & Holdsworth, 1965) with the appropriate changes in discriminator setting for the more energetic β-radiation.

Determination of ⁵⁹Fe. No preparation of the tissues was necessary and they were counted directly in a well-type NaI-crystal scintillation counter (Ecko M 664A).

RESULTS

Effect of vitamin D₃ on strontium absorption. Vitamin D₃ had the expected effect of increasing the absorption of strontium from the gut (Table 1).

Effect of bile salts and lauryl sulphate on strontium absorption. Chick bile salts, the chief bile salt of

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Table 1. *Effect of vitamin D₃ on strontium absorption in the chick*

Ileal loops of chick intestine were established *in vivo*. Each loop received 3.48 mg. of ⁹⁰Sr (final concn. 0.04 M) as SrCl₂ in 0.5 ml. of 0.15 M-NaCl. Chicks given vitamin D₃ received 200 i.u. orally 16 hr. before the experiment. Blood samples were taken at 20 and 60 min. The gut and its contents were removed and residual ⁹⁰Sr was counted. Tibiae were taken at 60 min. and ashed and the ⁹⁰Sr in the ash was counted. The results are means $\pm$ S.E.M. for three birds.

Group	Plasma		Tibia	Sr absorbed (mg./hr.)
	(counts/min./ml. at 20 min.)	(counts/min./ml. at 60 min.)	(counts/min. at 60 min.)	
Rachitic	2570 $\pm$ 410	3580 $\pm$ 890	7820 $\pm$ 661*	1.08 $\pm$ 0.22
Vitamin D ₃ -treated	8700 $\pm$ 530	7150 $\pm$ 1290	25748 $\pm$ 2143*	2.17 $\pm$ 0.21

* Significantly different ($P < 0.01$).

Table 2. *Effects of sodium lauryl sulphate and bile salts on strontium absorption*

Ileal loops of rachitic chick intestine were established *in vivo*. Each loop received 1.74 mg. of ⁹⁰Sr (final concn. 0.02 M) in 0.5 ml. of 0.15 M-NaCl (saline) or in 0.5 ml. of 0.007 M-sodium lauryl sulphate, 0.05 M-sodium taurochenodeoxycholate, or 0.05 M-bile salts from rachitic chicks. Blood samples were taken at 20 and 60 min. Tibiae were taken at 60 min. and ashed and the ⁹⁰Sr in the ash was counted. The results are means $\pm$ S.E.M. for four birds.

Test substance	Plasma		Tibia
	(counts/min./ml. at 20 min.)	(counts/min./ml. at 60 min.)	(counts/min. at 60 min.)
Saline	1570 $\pm$ 380	1110 $\pm$ 318	4714 $\pm$ 340
Lauryl sulphate	6050 $\pm$ 1680*	3750 $\pm$ 1320*	12801 $\pm$ 965*
Taurochenodioxicholate	5770 $\pm$ 1240*	3350 $\pm$ 1470	12912 $\pm$ 1400*
Chick bile salts	4020 $\pm$ 930*	2410 $\pm$ 405	10815 $\pm$ 1520*

* Significantly greater than saline control ($P < 0.02$).

Table 3. *Butanol-benzene/saline partition coefficients of strontium-bile salt complexes*

Samples (1 ml.) of the solutions used in Table 2 were shaken with 1 ml. of butan-1-ol-benzene (1:1, v/v) at 20° for 1 hr. The liquid was centrifuged, 0.05 ml. of each layer counted and the partition coefficient calculated.

Test substance	Butanol-benzene/saline partition coefficient
Saline	0.00021
Lauryl sulphate	0.088
Taurochenodeoxycholate	0.14
Chick bile salts	0.29

lauryl sulphate greatly increases the solubility in the organic phase (Table 3).

Effect of bile on iron absorption. Rats were used in these studies and the effect of the absence of bile was studied in a group that had had their common bile ducts ligated 48 hr. before the experiment. The results in Table 4 show that the spread of results in each group was high and therefore the significance of the results is doubtful. It seems possible that, when iron is presented as a sparingly soluble salt, bile causes a greater uptake of iron (Table 4).

DISCUSSION

chick bile (sodium taurochenodeoxycholate) and sodium lauryl sulphate all increased the absorption of strontium from ileal loops of the rachitic chick. Significant results were obtained with the plasma ⁹⁰Sr at 20 min. and in the tibia (Table 2).

Solubilities of strontium complexes in butanol-benzene. Very little ⁹⁰Sr appears in the butanol-benzene phase from a saline solution of strontium chloride but the presence of bile salts and sodium

It was first reported by Greenberg (1945) that vitamin D increased the absorption of strontium in rachitic rats, and a similar effect was observed with rachitic chicks (Wasserman, 1962). The results reported in Table 1 confirm these findings and are needed for comparison with the study of the effects of bile salts. Taurochenodeoxycholate, the principal bile salt of chick bile, caused an increased absorption of strontium of similar magnitude to that caused by vitamin D₃ and this occurs immediately, whereas

Table 4. *Effect of bile on iron absorption*

Normal rats were either sham-operated or had their common bile ducts tied 48 hr. before the experiment. Then $1 \mu\text{mole}$ of Fe^{2+} ($1 \mu\text{C}$ of ^{59}Fe) was given by stomach tube in a total volume of 1.0 ml. either as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ or as a suspension of the phosphate in 0.5% gum tragacanth. After 2 hr. 0.5 ml. of blood was taken by heart puncture, the rat was killed and liver, spleen, and 1 g. of rib from the first and second pairs of ribs were taken for counting of ^{59}Fe . The results are means $\pm$ S.E.M. for four rats.

Tissue	^{59}Fe counts/min.			
	FeSO_4		FeHPO_4	
	Sham-operated	Bile duct tied	Sham-operated	Bile duct tied
Blood (1 ml.)	1431 $\pm$ 662	3093 $\pm$ 1008*	1232 $\pm$ 403	606 $\pm$ 143†
Liver (whole)	2963 $\pm$ 1329	5344 $\pm$ 1622*	2541 $\pm$ 998	1420 $\pm$ 518†
Spleen (whole)	1008 $\pm$ 493	1372 $\pm$ 471	799 $\pm$ 282	334 $\pm$ 198†
Rib (1 g.)	162 $\pm$ 82	228 $\pm$ 115	133 $\pm$ 54	39 $\pm$ 20†

* Greater than sham-operated control ($0.1 < P < 0.2$).

† Less than sham-operated control ($0.1 < P < 0.2$).

vitamin D_3 has to be given several hours before the experiment (Sallis & Holdsworth, 1962). Sodium lauryl sulphate, which we showed had a marked effect on calcium absorption (Webbing & Holdsworth, 1965), had a similar effect on strontium absorption. Whereas there was a close correlation between the effect of bile salts on calcium absorption and their effect of making calcium soluble in butanol-benzene (Webbing & Holdsworth, 1966), this was not so with strontium. Sodium lauryl sulphate was more effective than chick bile salts in increasing absorption of strontium but had less effect on making strontium pass into the butanol-benzene phase. Thus the simple explanation that substances that make strontium lipid-soluble increase the transport of strontium across cell membranes is not likely to be the complete explanation of the phenomena. It does appear the calcium and strontium are similarly affected by the presence of vitamin D_3 and bile.

The study of the absorption of iron has been the subject of a large number of papers over the past 50 years, yet the factors controlling iron absorption are largely unknown. The subject was reviewed by Moore & Dubach (1962). In the present work no attempt was made to make an extensive study of this complex subject but rather to see if bile was involved at some stage. An early paper (Smith & Crandall, 1941) found that bile had no effect on ^{59}Fe absorption except when the diet contained neutral fat, which was alleged to cause precipitation of the iron. Similarly Wheby, Jones & Crosby (1964) found that ligation of the bile duct did not affect the uptake of ^{59}Fe when it was given in a soluble form. The results obtained in the present work

(Table 4) show that, in the normal rat, bile is not required for the absorption of soluble ferrous iron, and indeed that absorption tended to be better in the absence of bile. When the iron was given as its sparingly soluble phosphate to rats with ligated bile ducts, the amounts absorbed were diminished compared with the corresponding sham-operated group. In rats with intact bile ducts there was no difference between the amount of iron absorbed when the iron was given in a soluble or in the sparingly soluble form. Thus the behaviour of iron differs from that of calcium (or strontium), where bile increases the absorption of the soluble ionic form. However, it appears that bile may be important in the absorption of sparingly soluble forms of iron just as it helps the absorption of sparingly soluble forms of calcium (Webbing & Holdsworth, 1966).

REFERENCES

- Greenberg, D. M. (1945). *J. biol. Chem.* **157**, 99.
- Moore, C. V. & Dubach, R. (1962). In *Mineral Metabolism*, vol. 2B, p. 287. Ed. by Comar, C. L. & Bronner, F. New York: Academic Press Inc.
- Sallis, J. D. & Holdsworth, E. S. (1962). *Amer. J. Physiol.* **203**, 497.
- Smith, P. W. & Crandall, L. A. (1941). *Amer. J. Physiol.* **135**, 259.
- Wasserman, R. H. (1962). *J. Nutr.* **77**, 69.
- Webbing, D. D'A. & Holdsworth, E. S. (1965). *Biochem. J.* **97**, 408.
- Webbing, D. D'A. & Holdsworth, E. S. (1966). *Biochem. J.* **100**, 652.
- Wheby, M. S., Jones, L. G. & Crosby, W. H. (1964). *J. clin. Invest.* **43**, 1433.