chlorure est apparemment la somme de l'effet produit par les anions HCO_3^- et Cl^- pris séparément (130) mais c'est un effet faible comparé à celui du maléate (172) pourtant non totalement cumulatif. Ces résultats montrent donc que le processus d'activation par deux anions est plus complexe qu'il n'apparaît et qu'il ne saurait être expliqué par un simple phénomène d'additivité des activations dues à chaque anion.

Une étape dans la compréhension de cette double activation sera de déterminer les corrélations existant entre les activités enzymatiques et les anions effectivement transportés. A ce jour, on a déjà montré un transfert net de Cl^- contre un gradient de concentration[10] chez *Sphaeroma* comme cela a été fait pour les branchies de poissons[2].

Conclusion. La présence dans la fraction microsomale des endopodites 4 et 5 de *Sphaeroma serratum* d'une activité ATPasique sensible au bicarbonate et stimulée par l'addition d'un autre anion permet de supposer que cette enzyme pourrait être une ATPase stimulée par deux anions. L'existence d'une ATPase (HCO_3^-–Cl^-) ne peut être encore déduite de ces résultats mais ceux-ci constituent néanmoins un début de vérification de l'hypothèse émise pour rendre compte de la sécrétion chlorhydrique de l'estomac[3], de la secrétion pancréatique[4] ou de la régulation ionique chez les poissons euryhalins[2]. En effet, puisque ces différents phénomènes de transferts postulent un échange actif HCO_3^-:Cl^- par un mécanisme similaire à celui du transport couplé de Na^+:K^+, il est normal de penser que la corrélation mise en évidence ci-dessus entre une activité ATPasique et 2 anions dont un serait le bicarbonate constitue une étape non négligeable dans la compréhension de ces mécanismes de transfert.

Summary. An Mg^{2+}-dependent ATPase activity stimulated by the anions is found in the microsomal fraction prepared from a homogenate of the endopodits of pleopods 4 and 5 of *Sphaeroma serratum*. This enzyme has an optimal activity for 25 m*M* whatever the anion and for pH 7.6. Its inhibition is almost complete for 10^{-3} *M* of thiocyamate. The stimulation of the enzyme by 2 anions is cumulative if HCO^-_3 is one of these anions. This anion-stimulated ATPase could be involved with the (Na^+–K^+) ATPases in the osmoregulation mechanism of this isopod.

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Fatty Livers Induction by Orotic Acid Contained in Skim Milk Powder

Orotic acid, a normal intermediate of pyrimidine biosynthesis, was demonstrated to produce fatty livers and hepatic lesions in many animals[1]. Lipid accumulation in rats is periportal, more severe in females than in males and dependent on the diet[2]. 10 mg of orotic acid added to 100 g of feed altered liver metabolism in chicken[3], and orotic acid fed to lactating goats markedly decreased milk secretion[4]. Orotic acid is, however, a normal constituent of ruminant milk and found only in negligible amounts in human milk[5]. We have reported that dried milk powders contained relatively high amounts (100–180 mg) of orotic acid per 100 g[6]. We decided to investigate to what extent

Table I. The effect of experimental diets on food intake, rat weight and liver weight of rats on diets

Diet	Duration of feeding (days)	No. of rats	Food intake[a] per day (g)	Rat weight[a] (g)	Liver wt./100 g rat wt.[a] (g)
Control	3	5	12.82 ± 1.1	176.56 ± 7.2	3.34 ± 0.17
Control	8	5	11.68 ± 2.3	182.60 ± 8.3	3.38 ± 0.31
Group A[b]	3	5	11.20 ± 2.9	171.20 ± 4.5	3.45 ± 0.24
Group A[b]	8	5	9.94 ± 2.1	186.00 ± 12.8	3.53 ± 0.13
Group B[c]	3	5	9.33 ± 1.7	168.70 ± 4.2	3.72 ± 0.16
Group B[c]	8	5	8.75 ± 2.0	173.50 ± 5.1	3.89 ± 0.21

[a] Figures represent mean ± standard deviation. [b] Group A diet contained 0.15% orotic acid added to the balanced diet. [c] Group B diet consisted of an adequately supplemented powdered skim milk diet which was assayed to contain 0.15% orotic acid.

Table II. The total lipids and fat/protein ratio in livers from rats on experimental diets

	3 days feeding (g/100 g liverwt.)[a]		8 days feeding Fat/protein ratio
Control	3.49 ± 0.30	3.19 ± 0.24	0.16
Group A[b]	5.49 ± 1.0	7.10 ± 0.7	0.35
Group B[c]	6.55 ± 1.0	7.24 ± 2.2	0.36

[a] ± Standard Deviation. [b] Rats received control diet +0.15% orotic acid. [c] Rats received skim milk powder diet.

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Table III. The fatty acid[a] composition of total liver lipids extracted from rats on various experimental diets (molar compositions %)

Diet	C14:0	C16:0	C18:0	C18:1	C18:2	C20:4
Control						
3 days feeding	0.31	28.7	25.0	11.8	14.8	19.2
8 days feeding	0.2	28.4	25.0	10.3	16.3	19.4
Test diet A[b]						
3 day feeding	0.3	24.3	18.3	17.3	15.4	24.0
8 days feeding	0.3	22.7	19.5	19.3	18.7	23.2
Test diet B[c]						
3 days feeding	0.1	28.9	20.2	12.6	16.4	22.4
8 days feeding	–	18.4	26.2	10.0	20.0	27.3

[a] C14.0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C20:4, arachidonic acid.

[b] Group A rats received control diet + 0.15% orotic acid.

[c] Group B rats received a skim milk powder diet which was assayed to contain 0.15% orotic acid.

orotic acid in milk powders influences liver lipids in laboratory rats.

Females Sprague Dawley rats weighing 155 to 165 g were selected for study and divided into three groups of 10 and housed in air-conditioned cages with access to cool water ad libitum. Control rats were fed 50% sucrose, 32% vitamin free casein, 4% corn oil (Mazola), 4% Hegstead salt mix[7] and 1% standard vitamin mix. Group A rats were fed a normal skim milk powder diet containing 4% corn oil (Mazola), 4% Hegstead salt mixture[7], 1% standard vitamin mixture and 1% sucrose. The orotic acid content of this diet was found to be 0.15% by a previously described method[6]. The 3 diets were isocaloric and contained identical casein protein content. Groups A and B contained identical orotate concentrations. Five rats were sacrified from each group after 3 and 8 days respectively. Livers were removed and weighed, and aliquots were analyzed for protein by the Kjedahl method and for lipid content by the method of FOLCH[8]. Extracted lipids were analyzed for component fatty acids by gas liquid chromatography[9].

Although steatosis was visible in orotic-acid-treated rats, there was only a small, not highly significant, hepatic hypertrophy. This is in contrast to a pronounced hypertrophy in both Wistar and Sprague Dawley weanlings fed 1% orotic acid[10]. The low concentration of orotic employed, combined with the use, in the present study, of mature rats might account for the lack of drug-induced hepatic hypertrophy. There was a marked increase in total lipid content of the liver which increased progressively with orotic acid feeding (Table II). This increase is in good agreement with the reports of others[1,2,10,11]. The effect of the diets utilized on fatty acid composition of liver lipids is shown on Table III. Except for a progressive decline in palmitic acid and an increase in arachidonic acid in orotic-acid-treated rats, no clear picture was obtained as to the effect of the diets on fatty acid composition of the livers.

Although WINDMUELLER et al.[11] have reported that there was no change in plasma lipids in patients receiving orotic acid for gout, it is proper to speculate on possible implications of orotic acid ingestion in normal humans. It is noteworthy that whereas human milk contains no orotic acid, ruminant milk, and especially powdered milk and whey concentrates, contain relatively high amounts of this compound[6]. Our studies show that the presence of calcium and lactose in the powdered milk does not protect against fatty livers induced by orotic acid was previously reported[12]. FINK[13] reported cases of extensive liver necrosis due to feeding skim milk in powdered form to rats. Our present study may provide a partial explanation for these initial observations. It is indeed ironic that an essentially fat-free product such as dried skim milk should contain chemicals capable of inducing fatty livers.

Résumé. Chez des rats nourris avec 0.15% d'acide orotique ou de la poudre de lait écrémée contenant 0.15% d'orotate le foie s'enveloppe de graisse sans s'hypertrophier. On constata seulement de légers changements dans la composition des graisses acides du foie des rats traités à l'acide orotique. Ces résultats mettent en discussion l'emploi répandu de produits laitiers séchés destinés à l'alimentation humaine.

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