

Iron retention by rats. Effect of cold-provoked increased ingestion of a lactose-rich diet

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Summary. The cold-provoked increased ingestion of lactose by rats favored iron retention by a number of organs, including the liver, spleen, kidneys and gastrocnemius muscle. The proportion of iron stored as haemosiderin was increased in the spleen. This increased retention might be explained by the positive action of monosaccharides, oligosaccharides and polyols on iron absorption. A slight hemolytic effect of the experimental diet, however, cannot be excluded.

Introduction.

Numerous studies have shown that oral administration of monosaccharides, oligosaccharides and polyols enhance iron retention in rats (Amine and Hegsted, 1971 ; Bouvet, 1970 ; Forth and Rummel, 1973 ; Herndon *et al.*, 1958). To our knowledge, no study has been done on the effects of increased amounts of ingested lactose on iron retention and its different types of storage in various organs. Food intake was augmented by subjecting certain animals to a + 5 °C temperature, since these animals ingest about twice as much as when kept at 28 °C. Iron stored in the liver and spleen was determined by assaying the ferritin and haemosiderin.

Material and methods.

In an initial experiment, 48 laboratory-bred male Wistar rats were randomly divided into two groups of equal size. The rats in the « starch » group received *ad libitum* the following diet : 69.5 p. 100 of wheat flour, 18 p. 100 of purified casein, 8 p. 100 of peanut oil, 3 p. 100 of salt mixture (Hubbel, Mendel and Wakeman, 1937), 1 p. 100 of vitamin mixture without vitamin D (Fischer, 1957), 0.5 p. 100 of TiO₂ and 50 IU of calciferol per 100 g of dry feed.

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In the second group, 20 p. 100 of the starch was replaced by an equal quantity of lactose. These rats did not receive calciferol but the presence of lactose prevented rickets (Fournier, Dupuis and Bescol-Liversac, 1959). Each diet contained 20 mg of iron per 100 g in the form of oxalate.

Each of the two groups was further divided into 4 groups of 6 animals each. Two groups were kept at 23 °C for 8 months ; one of these was then placed at 28 °C for 4 months and the other at 5 °C for the same period. Each of the remaining two groups was kept at either 28 or 5 °C for the entire 12 months.

The animals were killed at the end of experiment. The blood was collected and the liver, spleen, both kidneys and one gastrocnemius muscle were dissected out. These organs were washed with physiological saline and the liver was perfused with the same liquid. The iron content of the red blood cells and the organs was determined by combustion followed by a colorimetric orthophenanthroline assay (Hemmeler, 1951).

In a second experiment, 32 male rats were randomly separated at weaning into two equal groups and fed each of the above diets. Both groups were kept at 23 °C for 3 months and then subdivided, half of each group being kept for 9 months at 28 °C and the other half at 5 °C for the same period. The animals were killed at the end of the experiment and the livers and spleens were taken. The hemoglobin was removed, the ferritin and haemosiderin in the organs were extracted as described by Drysdale and Munro (1965), and the iron content of the two proteins was determined after combustion.

Results.

The results of the first experiment are shown in table 1. The means of body and organ weights are not given since they were not significantly different from one group to another.

After four months at 5 or 28 °C, the splenic iron content of the animals on the lactose diet was higher than in the « starch » diet group. Although the results suggest that cold has an additive effect, it could not be proven by statistical analysis.

After 12 months, the iron contents of the organs of lactose diet animals kept at 5 °C was higher than that of the other groups. This was particularly pronounced in the liver and the spleen. In the case of the spleen and the gastrocnemius muscle, however, an effect due solely to temperature cannot be excluded. This is supported by the finding in the « starch » diet group that the iron content of these two organs was higher when the rats were kept at 5 °C than at 28 °C. No notable difference could be detected in the erythrocyte iron content, and although rats on the lactose diet appeared to have slightly lower red cell iron contents, that difference was not significant.

Table 2 contains the results of the second experiment. After 9 months at 5 °C, the content of stored iron in the liver (ferritin + haemosiderin) of lactose rats was higher than that of the other groups, confirming the results noted after 12 months. The proportion of iron fixed as ferritin was practically constant in all the groups.

The group variations in stored iron levels (ferritin + haemosiderin) in the spleen were fairly comparable to those observed after 12 months, but were not significant.

TABLE 1
Effect of diet and temperature on the retention and distribution of iron in rats

		Iron content					
		Liver	Spleen	Kidneys	Gastrocnemius muscle	Erythrocytes	
	Temperature	($\mu\text{g}/\text{organ}$)	($\mu\text{g}/\text{organ}$)	($\mu\text{g}/\text{organ}$)	($\mu\text{g}/\text{g}$ fresh organ)	($\mu\text{g}/\text{g}$ dry weight)	
4 months *							
Starch	28 °C	1 447 ± 164	688 ± 26	233 ± 16	11 ± 1.6	—	
	5 °C	1 478 ± 61	976 ± 124	220 ± 30	16 ± 1.6	—	
Starch + Lactose	28 °C	1 624 ± 139	1 303 ± 132	207 ± 19.5	17 ± 2.4	—	
	5 °C	2 023 ± 178	2 390 ± 318	280 ± 9	19 ± 1.65	—	
12 months							
Starch	28 °C	1 408 ± 91	441 ± 55	243 ± 20	12 ± 0.8	2 566 ± 46	
	5 °C	1 356 ± 29	1 094 ± 127	267 ± 16	20 ± 1.6	2 576 ± 106	
Starch + Lactose	28 °C	1 539 ± 85	639 ± 110	184 ± 6.5	14 ± 1.6	2 492 ± 44	
	5 °C	3 850 ± 95	2 333 ± 198	323 ± 27	36 ± 5.3	2 269 ± 69	

Each result is the arithmetic mean ± SEM for 6 rats. The arrows indicate significantly different results. The degree of significance was determined by Fischer's test or by Mann and Whitney's non-parametric test (Schwartz, 1972) when the variances were different.

* These 4 months were preceded by 8 months at 23 °C.

TABLE 2
 Percent of iron fixed as ferritin in the liver and as haemosiderin in the spleen

Diet	Temperature	No of rats	Liver			Spleen		
			Total iron stored (ferritin + haemosiderin) ($\mu\text{g}/\text{organ}$)	Ferritin iron as p. 100 of total iron	Total iron stored (ferritin + haemosiderin) ($\mu\text{g}/\text{organ}$)	Total iron stored (ferritin + haemosiderin) ($\mu\text{g}/\text{organ}$)	Haemosiderin iron as p. 100 of total iron	
Starch	28 °C	6	1140 ± 85	74.3 ± 3.5	573 ± 73	55 ± 7.7	P < 0.05	
	5 °C	7	1143 ± 63	70.8 ± 1.9	648 ± 131	71.6 ± 4.6		
Starch + Lactose	28 °C	8	1250 ± 110	77.2 ± 2.5	708 ± 124	52.8 ± 7.5	P < 0.02	
	5 °C	8	1691 ± 88	76.4 ± 1.9	902 ± 101	76.1 ± 4		

The rats were subjected to various diets and temperatures for 9 months. Values are the mean ± SEM. The arrows indicate significantly different results. The degree of significance was determined by Fischer's test or by Mann and Whitney's non-parametric test (Schwartz, 1972) when the variances were different.

On the other hand, the increased lactose ingestion which accompanied lower ambient temperature resulted in a greater fixation of iron in the form of haemosiderin. This level was also elevated in the « starch » diet rats kept at 5 °C ; however, it is just significant when contrasted with similar values in animals on the same diet but kept at 28 °C.

Discussion.

Under the experimental conditions described here, the iron concentration in visceral organs was maximal in rats maintained at 5 °C for 12 months on a lactose-rich diet. The low ambient temperature led to an augmented feed consumption, and thus to increased quantities of ingested lactose and iron. It appears that a low temperature and increased iron ingestion are insufficient to augment the iron stores in the liver : the hepatic iron levels of rats on the « starch » diet at 5 °C were not higher than those of diet-paired animals at 28 °C. This is not the case of the spleen and the gastrocnemius muscle. In « starch »-fed rats, the increased ingestion, and thus the increased iron supply, in animals kept at 5 °C, caused the iron levels in those two organs to rise. The iron contents of the spleen and the muscle were even further augmented in rats on the « starch + lactose » diet.

These findings confirm previous observations (Amine and Hegsted, 1971 ; Bouvet, 1970 ; Forth and Rummel, 1973 ; Herndon *et al.*, 1958), indicating that iron retention is favored by monosaccharides, oligosaccharides and polyols. The majority of authors explain this phenomenon by admitting that these compounds act by forming complexes with iron, thus facilitating its absorption (Charley *et al.*, 1963 ; Davis and Deller, 1966 ; Saltman, 1965). We believe, however, that other hypotheses may also be invoked. Thus, Fournier and Piette (1960, 1961a, b, c) showed that a lactose-rich diet, given to rats of various ages, had relatively slight hemolytic effects, among which were a slight reticulocytosis, increased serum iron and increased splenic contents of iron and haemosiderin. We observed the latter two effects in the spleens of rats on a lactose diet and kept at 5 °C. In this group of animals, erythrocyte iron after 12 months was slightly, although non-significantly, lower than that of the other groups. Even though we noted no appreciable difference in hematocrits, a slight hemolytic effect or a possible shift of red cell iron to the organs cannot be excluded.

It thus appears difficult at present to determine the exact contribution of these two phenomena to the increased iron retention observed in the organs of rats ingesting large quantities of a lactose-rich diet for prolonged periods of time.

Reçu en avril 1980

Accepté en juin 1980.

Résumé. Une augmentation par le froid de la consommation de lactose chez le Rat favorise la rétention du fer dans de nombreux organes, comprenant le foie, la rate, les reins et le muscle gastrocnémien. La proportion du fer stocké sous la forme d'hémosidérine est accrue dans la rate. Cette rétention améliorée peut être expliquée par l'action positive des monosaccharides, des oligosaccharides et des polyols sur l'absorption du fer. Cependant, on ne peut pas exclure un léger effet hémolytique du régime expérimental.

References

- AMINE E. K., HEGSTED D. M., 1971. Effects of diet on iron absorption in iron deficient rat. *J. Nutr.*, **101**, 927-932.
- BOUVET D., 1970. Influence de la nature du composé glucidique ingéré simultanément avec ^{59}Fe sur la rétention et l'utilisation de cet élément. *C. R. Soc. Biol.*, **164**, 2452-2455.
- CHARLEY P. J., SARKAR B., STITT C. F., SALTMAN P., 1963. The chelation of iron by sugars. *Biochim. biophys. Acta*, **69**, 313-321.
- DAVIS P. S., DELLER D. J., 1966. Prediction and demonstration of iron chelating ability of sugars. *Nature (London)*, **212**, 404-405.
- DRYSDALE J. W., MUNRO H. N., 1965. Small-scale isolation of ferritin for the assay of the incorporation of ^{14}C -labelled amino-acids. *Biochem. J.*, **95**, 851-858.
- FISCHER J. E., 1957. Effects of feeding diets containing lactose, agar, cellulose, potatoe starch, arabinose on the dry weights of cleaned gastro-intestinal tract organs in the rat. *Amer. J. Physiol.*, **188**, 550-554.
- FORTH W., RUMMEL W., 1973. Iron absorption. *Physiol. Rev.*, **53**, 724-792.
- FOURNIER P., DUPUIS Y., BESCOL-LIVERSAC J., 1959. Observations relatives aux effets ostéogènes du lactose. *C. R. Acad. Sci. Paris*, **249**, 2836-2838.
- FOURNIER P., PIETTE M., 1960. Effets hématopoïétiques de l'ingestion de lactose au cours de l'évolution du rachitisme du rat. *C. R. Acad. Sci. Paris, sér. D*, **251**, 1565-1566.
- FOURNIER P., PIETTE M., 1961a. Variations du nombre des hématies au cours de crises réticulocytaires du Rat consécutives à l'ingestion de lactose. *C. R. Acad. Sci. Paris, sér. D*, **252**, 942-944.
- FOURNIER P., PIETTE M., 1961b. Etude sur le rat des effets hémolytiques du lactose : hyperbilirubinémie et accroissement de la teneur en fer de la rate. *C. R. Acad. Sci. Paris, sér. D*, **252**, 2762-2764.
- FOURNIER P., PIETTE M., 1961c. Etude chez le rat des propriétés hémolytiques du lactose : apparition d'hémosidérine et accroissement de la sidérémie. *C. R. Acad. Sci. Paris, sér. D*, **253**, 2765-2767.
- HEMMELE G., 1951. Les méthodes du dosage du fer 9-46. In *Métabolisme du fer*. Masson et Cie (Paris).
- HERNDON J. F., RICE E. G., TUCKER R. G., VAN LOON E. J., GREENBERG S. M., 1958. Iron absorption and metabolism. The enhancement of iron absorption in rats by D-sorbitol. *J. Nutr.*, **64**, 615-623.
- HUBBEL R. B., MENDEL L. B., WAKEMAN A. G., 1937. A new salt mixture for use in experimental diets. *J. Nutr.*, **14**, 273-285.
- SALTMAN P., 1965. The role of chelation in iron metabolism. *J. chem. Educ.*, **42**, 682-687.
- SCHWARTZ D., 1972. *Méthodes statistiques à l'usage des médecins et des biologistes*. Flammarion (Paris).