Significance of Lactose in the Diet in Aminoaciduria caused by Maleic Acid

In studies with rachitic rats Harrison and Harrison[1] established aminoaciduria as one of the signs of maleic acid intoxication. Angielski *et al.*[2] produced, by intraperitoneal administration of maleic acid, aminoaciduria in rats receiving a diet containing 50 per cent skim milk. We have studied the effect of dietary lactose on the production of aminoaciduria by maleic acid. Three groups of rats received the three diets described in Table 1.

Table 1. Composition of Diets (gm./kgm.)

Component	Diet		
	Milk	With lactose	Without lactose
	I	II	III
Dried skim milk ..	480	—	—
Casein	—	170	170
Wheat starch	270	270	480
Sucrose	130	130	230
Lactose	—	310	—
Rape-seed oil	88	88	88
Wesson's[3] salt mixture	32	32	32

To 1 kgm. of diet were added: 331 mgm. of a vitamin mixture[4]; 1,000 mgm. choline hydrochloride; 5 mgm. menapthone; 150 mgm. vitamin E; 25,000 I.U. vitamin A 2,500 I.U. vitamin; D.

The rats were kept in metabolic cages allowing quantitative collection of urine uncontaminated by faeces or diet. They received unlimited food and water. Neutralized maleic acid was given intraperitoneally as a molar solution, in one dose of 400 mgm./kgm. body-weight. α-amino nitrogen was estimated in urine by the method of Yemm and Cocking[5]. The rats received their respective diets for 7–14 days before injection of maleic acid. The results are given in Table 2.

Table 2. Adult Rats, Males and Females weighing 120–370 gm. Mean values per Rat for 24 hours for Groups of 5 Rats. Figures in parentheses show the range.

	α-Amino nitrogen (mgm.)		
	Diet I	Diet II	Diet III
Before administration of maleic acid .. .. (mean over 4 days) ..	3·3 (1·65–5·7)	6·2 (4·0–8·1)	4·6 (2·5–7·5)
After administration of maleic acid			
Day 1	7·5 (5·0–9·3)	22·5 (14·8–31·6)	4·1 (3·4–5·8)
Day 2	10·9 (5·4–15·3)	18·0 (15·0–22·0)	1·7 (1·1–2·2)
Day 3	20·5 (11·5–27·5)	37·0 (16·0–57·0)	3·2 (1·1–7·7)
Day 4	9·5 (6·5–12·2)	25·5 (17·3–30·5)	7·1 (3·1–12·0)
Day 5	4·6 (2·6–9·5)	11·8 (7·0–15·9)	6·8 (4·0–9·4)

400 mgm. maleic acid per kgm. body weight produced no aminoaciduria in rats on a diet devoid of lactose. Rats receiving lactose, whether from milk or as such, responded by marked aminoaciduria to the same dose of maleic acid. Maximum excretion of α-amino nitrogen was generally observed on the second or third day after administration, the values reached being five to ten times those before maleic acid was injected. After a week the excretion returned to normal again.

Maleic acid is the causative agent of the aminoaciduria but lactose seems to be necessary for its appearance.

A full report of these findings will be published in *Acta Biochimica Polonica*.

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Extraction of an Actomyosin-like Protein from Human Thrombocytes

Continuing the work of Lüscher[1], we have studied the metabolism of blood platelets in relation to clot retraction, as the latter seems to be one of the most important thrombocytic functions. Lüscher[2] and Bounameaux[3] have pointed out that in the presence of a buffered medium containing divalent cations (Mg^{++} or Ca^{++}), glucose is a factor which improves retraction. On the other hand the existence of mitochondria has been observed with electron microscopic techniques and it has been known for years that blood platelets are able to consume oxygen.

In our experiments[4] we noticed a constant relationship between an active glycolytic system and maximal retraction capacity. This relationship does not exist for oxygen consumption. Using isolated and washed thrombocytes we were able to confirm the results of Born[5] obtained with platelet-rich plasma. This author observed that the adenosine triphosphate level, which is very high in thrombocytes, shows a rapid fall during clotting. Also, fresh thrombocytes exhibiting maximal retraction have a high adenosine triphosphate content (about 5×10^{-2} μ moles/10^9 platelets for isolated and washed cells), whereas platelets, even if preserved at 0° C., hydrolyse their adenosine triphosphate and at the same time lose their ability to retract.

Lüscher[6] has suggested that viscous metamorphosis is linked to the appearance of a viscous and retractile protein of complex composition, which was obtained from platelets and named 'protein *S*'.

In view of these facts and the results obtained by Hoffmann-Berling[7] on undifferentiated cells, from which he isolated a contractile protein, we tried to extract a contractile protein from thrombocytes, in a way analogous to the extraction of actomyosin from muscle.

Thrombocytes from normal human blood were isolated by means of centrifugal fractionation; they were washed twice in 0·9 per cent sodium chloride containing 1‰ of the disodium salt of ethylendiamine tetra-acetic acid; they were washed once more with a Weber-Edsall solution (potassium chloride, 0·6 *M*; sodium carbonate, 0·01 *M*; and sodium bicarbonate 0·04 *M*) and after discarding the supernatant they