a lower yield is obtained, and the product crystallizes less readily). The crude product was filtered off and treated with water in order to remove any dibromo acid left. The yield of acid thus obtained was 18 g (calc. 20.2 g), and the melting point was about 130°. After five recrystallizations from formic acid the product was pure and showed the m.p. 135—136°. (Found: Eq.wt. 199.6; S 31.93; Calc. for $C_{13}H_{20}O_6S_4$ (400.4): Equiv. wt. 200.2; S 32.02).

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On the Growth-Inhibition of *Lactobacillus bifidus* by Certain Fatty Acids

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Recent investigations [1,2] have shown that free long-chain (C>6) saturated fatty acids as well as unsaturated oleic and linoleic acids inhibit the growth of *Lactobacillus bifidus in vitro*. According to Barbero *et al.*[3] the same is true *in vivo*; in their experiments the number of *L. bifidus* in the intestinal tract of infants increased when the inhibitory saturated acids were removed from the cow's milk used in the feeding. A comparison of the composition of human milk and cow's milk shows that their content of the most strongly inhibiting saturated acids (lauric and myristic acids) is approximately equal [4]. Since, however, it is known that in practice human milk promotes the growth of *L. bifidus* in the intestinal tract of infants, it has been concluded that human milk must contain substances which can reverse the effect of the inhibitory acids. Some evidence has been cited [1,5] to show that proteins, and lactalbumin in particular, act as the detoxifying agents. The stronger detoxifying effect of human milk compared with cow's milk could, accordingly, be explained by the relatively greater content of whey proteins in human milk.

The *L. bifidus* strains used in our investigation were isolated from the faeces of breast-fed infants. The basal medium was that proposed by Hassinen *et al.*[6], supplemented with tryptic digest of casein as a source of strepogenin, found by us [7,8] to be essential for *L. bifidus*. The growth was measured titrimetrically after incubation at 37° C for 72 hours. The fatty acids investigated were added to the media as potassium salts, obtained by saponification of the corresponding acids with KOH in ethanol.

Our results on the inhibition of *L. bifidus* by different fatty acids agree in general with the data given by Tomarelli *et al.*[1] and Hassinen *et al.*[2] Propionic and butyric acids were found to have no effect, caproic acid was slightly inhibitory, and with increasing length of the carbon chain the inhibitory effect increased until lauric acid, which had the strongest effect. With the chain-length increasing further the toxicity decreased, the effect of myristic acid being nearly identical with that of capric acid. According to Hassinen *et al.*[2] palmitic, stearic and oleic acids do not inhibit *L. bifidus;* in our experiments, however, these acids also showed inhibiting, although distinctly weaker, effects than those of linoleic, lauric, capric and myristic acids. It is interesting to note in this connection that ricinoleic acid had an effect that was about 5 times as strong as that of oleic acid.

In order to overcome the inhibitory effect of fatty acids we added different proteins and yeast extract to the media containing lauric or linoleic acids. The results are given in Table 1. It will be seen that the additions of egg albumin, casein and blood albumin have permitted a fairly good growth of the bacteria, even in the presence of the inhibiting acids. Gelatin had a somewhat weaker effect whereas

Table 1. Effect of certain proteins and yeast extract on the growth-inhibition of L. b i f i d u s by lauric and linoleic acids. (Consumption of 0.1 N NaOH, ml/10 ml medium.)

Addition to the medium, 5 mg/ml	Medium		
	Basal	Basal + 200 γ lauric acid/ml	Basal + 200 γ linoleic acid/ml
None	7.5	0.0	0.0
Egg albumin	6.9	2.5	7.1
Casein	6.7	4.0	5.5
Blood albumin	7.1	3.2	4.9
Gelatin	7.1	1.6	3.3
Trypsin	8.1	1.3	1.3
Pepsin	7.4	1.0	0.2
Yeast extract	8.3	0.3	0.4

trypsin, pepsin and yeast extract had a very weak or practically no effect. In general the inhibitory effect of linoleic acid was easier to overcome than that of lauric acid. It is noticeable that casein was as effective as the albumins tested, indicating that the detoxifying ability *in vivo* may not be limited specifically to the albumin of milk.

Results of experiments conducted to show the detoxifying effects of skimmed human and cow's milk are given in Table 2. It can be seen that both milks possess the detoxifying ability, increasing amounts of milk leading to a more complete effect. It is also evident that skimmed cow's milk has a stronger detoxifying potency than skimmed human milk. The results with tryptic digests of milk show that in both cases detoxification is due to the proteins and not to their degradation products.

The results arrived at by earlier authors investigating the inhibitory effects of certain saturated and unsaturated fatty acids on the growth of *L. bifidus* thus were confirmed. The inhibitory effect could be overcome by certain pure proteins and by skimmed cow's or human milk. Since human milk and cow's milk are very similar in their content of inhibitory fatty acids and since the proteins of human milk have a weaker detoxifying potency than those of cow's milk, it can be concluded that the different effects of human and cow's milk on *L. bifidus in vivo* must be ascribed to other factors than an inhibition of this bacterium by certain fatty acids present in milk fat.

Table 2. Effect of skimmed human milk and skimmed cow's milk on the growth-inhibition of L. b i f i d u s by lauric, linoleic, and oleic acids. (Consumption of 0.1 N NaOH, ml/10 ml medium).

Additions to 10 ml medium	Medium			
	Basal	Basal + 200 γ lauric acid/ml	Basal + 200 γ linoleic acid/ml	Basal + 200 γ lauric + 500 γ oleic acid/ml
None	6.8	0.5	0.4	0.6
Skimmed human milk				
0.2 ml		1.0	0.5	
0.5 ml		2.5	5.0	2.6
1.0 ml		4.2	5.8	
Tryptic digest of skimmed human milk				
0.5 ml		1.4		0.7
Skimmed cow's milk				
0.2 ml		3.8	5.0	
0.5 ml		4.9	6.5	5.2
1.0 ml		6.4	6.9	
Tryptic digest of skimmed cow's milk				
0,5 ml		2.8		1.9

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