EFFECT OF FEEDING PROTECTED LIPID ON THE UPTAKE OF PRECURSORS OF MILK FAT BY THE BOVINE MAMMARY GLAND

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Abstract

The feeding of protected lipid to lactating dairy cows resulted in a substantial increase in the proportion of fatty acid 18:2 and a decrease in fatty acids 4:0 to 16:0 in milk fat.

The triglyceride fraction of arterial blood was the only major source of long-chain fatty acid for milk-fat synthesis during the feeding of protected casein or protected lipid. There was a significant increase in the arterial concentration of triglyceride and in the quantity taken up by the mammary gland during the feeding of protected lipid. Under both dietary regimes most if not all of the triglyceride fatty acids removed by the mammary gland were carried in the blood in the form of very-low-density lipoproteins and to a lesser extent chylomicrons.

The arteriovenous difference for fatty acid 18:2 was very small during the feeding of protected casein but 18:2 became the major contributor of long-chain fatty acid to the mammary gland during the feeding of protected lipid. The latter diet resulted in a substantial decrease in the arterial concentration and arteriovenous difference of acetate.

I. INTRODUCTION

It is well established that approximately 60% of milk-fat fatty acids of ruminants are derived preformed from the blood and that the remainder are derived by *de novo* synthesis within the glandular epithelium from acetate or β-hydroxybutyrate. Arteriovenous difference studies in cows (Hartmann and Lascelles 1964) have shown that most of the preformed fatty acid removed by the mammary gland is carried in the blood as β-lipoprotein or chylomicron triglyceride. Chylomicrons are derived exclusively from the intestine following absorption of dietary and endogenous long-chain fatty acids (Hartmann and Lascelles 1966), and it seems probable that a considerable quantity of these acids are subsequently recycled as β-lipoprotein triglyceride (Lascelles *et al.* 1964).

Following the earlier discovery that formalin treatment prevents microbial degradation of protein in the rumen (Ferguson *et al.* 1967), it has been demonstrated recently that the feeding of large quantities of polyunsaturated oil particles coated with formalin-treated casein (protected lipid) gives rise to marked increases in the polyunsaturated composition of milk (Scott *et al.* 1971). These particles appear to be

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inert in the rumen and therefore relatively large amounts can be ingested without risk of inducing the ruminal stasis commonly observed when large quantities of unprotected lipid are fed to cows (Hartmann *et al.* 1966).

In the present study the above feeding technique has been used in an attempt to increase the availability of the preformed fatty acids in the blood for milk-fat synthesis with a view to studying the competition between uptake by the bovine mammary gland of the two types of precursors, namely β-lipoprotein and chylomicron triglyceride on the one hand, and acetate and β-hydroxybutyrate on the other (cf. Lascelles 1970).

II. Materials and Methods

(*a*) *Surgery*

Anaesthesia was induced in three cows by intravenous administration of sodium pentothal and subsequently maintained with a mixture of Fluothane (ICI Pty. Ltd., Melbourne) and oxygen in a closed circuit. The carotid artery was dissected free of fascia over a distance of 15 cm. The freed portion of the artery was brought to the surface of the wound and the musculature sutured so as to hold the artery in a subcutaneous position. The skin wound was sutured and a period of at least 3 weeks was allowed before blood sampling commenced.

(*b*) *Feeding Procedure*

Each cow was fed a control diet of protected casein (formaldehyde-treated casein, CSIRO, Prospect, N.S.W.) at the rate of 340 g/day for a period of 5–7 days. Protected lipid comprising safflower oil particles coated with formaldehyde-treated casein (CSIRO, Prospect, N.S.W.) was then fed for 5 days starting at 400 g/day and increasing to 1 kg/day on the 4th and 5th days. The ratio of safflower oil to casein in the above material was 2 : 1 (w/w). The control diet of protected casein was again fed for another 5–7 days. It should be noted that the quantity of casein fed per day in both control periods was equivalent to the calculated amount of casein present in 1 kg of protected lipid. The protected casein or lipid was mixed with 4 kg of lucerne chaff and crushed oats (3 : 1 w/w), half the ration being fed after the morning milking and the other half following the evening milking. Cows took between ½ and 2 hr to consume the ration, after which they were allowed to graze a mixed pasture of subterranean clover, white clover, and rye grass.

(*c*) *Collection of Samples*

Blood samples were collected simultaneously from the carotid artery and subcutaneous abdominal vein of each cow on the last day of each feeding period. Extreme care was taken with sampling and in all cases cows were sampled in the standing position without disturbance (cf. Hartmann and Lascelles 1964).

Cows were milked twice daily by machine and samples of milk were stored at 5°C for subsequent analysis of milk fat.

(*d*) *Analytical Techniques*

Plasma free fatty acids (FFA) were determined by the method of Dole (1956) and plasma glucose by the glucose oxidase method (Huggett and Nixon 1957). Blood acetate concentrations were determined as described by Cook and Miller (1965) and β-hydroxybutyrate by the specific enzyme method of Williamson *et al.* (1962) as modified by Koundakjian and Snoswell (1970). β-Hydroxybutyrate dehydrogenase was obtained from Boehringer Pty. Ltd., Germany. Determinations of serum phospholipid were carried out according to the method of Allen (1940) and milk fat by the Babcock method (Davis and MacDonald 1953).

Extraction of total lipid from milk and serum was carried out by the method of Folch *et al.* (1957) and triglyceride and cholesterol ester were subsequently isolated by thin-layer chromatography (Hartmann and Lascelles 1965*a*). The concentration of esterified fatty acid in these fractions was determined by the method of Stern and Shapiro (1953).

Separation of chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein, and high-density lipoprotein in blood serum from the carotid artery and subcutaneous abdominal vein was carried out by ultracentrifugation as described by Lascelles and Wadsworth (1971). The esterified fatty acid content of each fraction was determined by the method of Stern and Shapiro (1953).

Separation of triglyceride from other lipids in chylomicron and VLDL fractions was carried out on pooled ethanol–ether extracts (representing 60 ml of blood) by thin-layer chromatography. The triglyceride fatty acids from chylomicron, VLDL, and milk were methylated (Wadsworth 1970) or butylated (Sampugna *et al.* 1966) and analysed by gas–liquid chromatography using 12% diethylene glycol succinate on Gas Chrom P (Applied Sciences Laboratories).

III. Results

(*a*) *Milk and Fat Production*

The results for milk and fat production computed from data collected over the last 2 days of feeding protected casein and protected lipid to lactating cows were as follows (values are means ± standard errors for three cows):

Order of feeding	1	2	3
Diet	Protected casein	Protected lipid	Protected casein
No. of days on diet	7	5	7
Milk yield (kg/24 hr)	11·3±3·7	12·5±4·0	11·2±3·5
Milk fat (g/day)	551·6±183·1	599·6±109·2	502·2±151·9

It can be seen that mean values for both milk and fat production were greater during the feeding of protected lipid than during the feeding of protected casein. However, analysis of variance of these results revealed that the differences were not significant.

TABLE 1

FATTY ACID COMPOSITION OF MILK DURING THE FEEDING OF PROTECTED CASEIN AND PROTECTED LIPID TO THREE LACTATING COWS

Values (expressed as percentage by weight of total fatty acids ≤18:3) are means ± standard errors for three cows

Diet	Protected casein	Protected lipid	Diet	Protected casein	Protected lipid
No. of samples	4	3	No. of samples	4	3
Fatty acid			Fatty acid		
4:0	3·6±0·6	1·6±0·2	15:0	1·4±0·2	1·2±0·1
6:0	3·3±0·1	2·2±0·4	16:0	30·4±1·9	15·6±2·6
8:0	1·3±0·1	0·9±0·1	16:1	1·9±0·5	1·1±0·3
10:0	2·5±0·2	2·4±0·3	18:0	12·1±0·8	11·5±0·5
12:0	2·9±0·3	2·7±0·2	18:1	24·2±0·5	24·2±1·1
14:0	10·4±1·2	8·3±0·7	18:2	2·6±0·5	24·6±1·6
14:1	1·8±0·1	1·5±0·2	18:3	1·7±0·1	2·4±0·2

(*b*) *Fatty Acid Composition of Milk*

The fatty acid composition of milk samples collected under the two dietary regimes are shown in Table 1. The feeding of protected lipid was associated with a ninefold increase in the proportion of fatty acid 18:2 in milk fat with little change in the proportions of fatty acids 18:0 and 18:1. There was a concomitant decrease in the proportion of each of the fatty acids between 4:0 and 16:0.

(c) *Uptake of Esterified Fatty Acid by the Mammary Gland*

The concentration of fatty acids in serum triglyceride, phospholipid, and cholesterol ester from the carotid artery, together with the arteriovenous difference for each of these fractions, is shown in Table 2. The feeding of protected lipid resulted

TABLE 2

ARTERIAL CONCENTRATION AND ARTERIOVENOUS DIFFERENCE OF ESTERIFIED FATTY ACID (EFA) IN SERUM TRIGLYCERIDE, PHOSPHOLIPID, AND CHOLESTEROL ESTER DURING THE FEEDING OF PROTECTED CASEIN AND PROTECTED LIPID TO LACTATING COWS

Values (expressed as mg EFA/100 ml serum) are means ± standard errors for three cows. The average molecular weight of fatty acids was taken as 280. Minus signs indicate that the mean concentration in the venous serum was higher than that in the arterial serum

Order of feeding	1	2	3
Diet	Protected casein	Protected lipid	Protected casein
EFA in triglyceride			
Artery	8·2±0·3	16·1±1·8	8·9±0·3
Arteriovenous difference	3·2±0·5	9·0±1·2	3·5±0·3
EFA in phospholipid			
Artery	118·2±3·8	172·1±21·3	124·2±12·0
Arteriovenous difference	1·0±2·4	1·6±3·8	−0·4±1·2
EFA in cholesterol ester			
Artery	58·5±3·5	73·0±4·9	55·2±3·0
Arteriovenous difference	−0·5±1·1	0·7±0·6	−0·7±0·6

in a sharp increase in the arterial concentration of all three fractions but uptake by the mammary gland on both diets was confined to the triglyceride fraction. A striking feature of the results in Table 2 was the substantial increase in uptake of triglyceride fatty acid during feeding of protected lipid. Analysis of variance of the results (Table 3),

TABLE 3

SUMMARY OF ANALYSIS OF VARIANCE FOR ARTERIOVENOUS DIFFERENCE OF ESTERIFIED FATTY ACID IN SERUM TRIGLYCERIDE, PHOSPHOLIPID, AND CHOLESTEROL ESTER

"Treatments" refers to comparisons between one period of protected lipid feeding and two periods of protected casein feeding. To calculate the variance ratios for treatments and cows sources of variation, the mean square for treatments × cows has been used as the denominator. In the case of the linear and quadratic components of treatments source of variation, the mean square for linear × cows and quadratic × cows, respectively, has been used as the denominator

Source of variation	D.F.	Mean squares for arteriovenous difference of EFA in:		
		Triglyceride	Phospholipid	Cholesterol ester
Treatments	2	32·2*	3·3	1·7
Linear	1	0·1	2·8	1·2
Quadratic	1	64·2*	4·0	1·8
Cows	2	1·0	13·2	0·3
Treatments × cows	4	2·1	25·4	2·8
Linear × cows	2	1·0	19·2	2·7
Quadratic × cows	2	3·2	30·8	2·5

* $P<0·05$.

in which data for one period of protected lipid feeding were compared with two periods of protected casein feeding, revealed significant ($P<0\cdot05$) treatment effects for the triglyceride fraction, the variation of which was almost entirely attributable to the quadratic component. The latter reflected the significance ($P<0\cdot05$) of the increase in mammary uptake of esterified fatty acid from this fraction during protected lipid feeding.

TABLE 4

ARTERIAL CONCENTRATION AND ARTERIOVENOUS DIFFERENCE OF ESTERIFIED FATTY ACID IN SERUM LIPOPROTEIN FRACTIONS DURING THE FEEDING OF PROTECTED CASEIN AND PROTECTED LIPID TO LACTATING COWS

Values (expressed as mg EFA/100 ml serum) are means ± standard errors for three cows. Other details as for Table 2

Order of feeding	1	2	3
Diet	Protected casein	Protected lipid	Protected casein
EFA in chylomicron			
Artery	2·6±0·3	4·5±0·9	2·9±0·3
Arteriovenous difference	0·4±0·1	1·8±0·3	0·5±0·1
EFA in very-low-density lipoprotein			
Artery	7·9±0·5	14·6±0·6	7·8±0·5
Arteriovenous difference	3·2±0·3	8·3±0·4	3·5±0·4
EFA in low-density lipoprotein			
Artery	97·5±16·5	135·8±16·3	115·8±9·9
Arteriovenous difference	−2·1±4·2	−0·9±1·7	−0·7±3·4
EFA in high-density lipoprotein			
Artery	35·8±1·8	58·9±13·4	38·0±3·9
Arteriovenous difference	1·6±0·9	1·6±2·6	0·1±1·9

TABLE 5

SUMMARY OF ANALYSIS OF VARIANCE FOR ARTERIOVENOUS DIFFERENCE OF ESTERIFIED FATTY ACID IN SERUM LIPOPROTEIN FRACTIONS

Details as for Table 3

Source of variation	D.F.	Mean squares for arteriovenous difference of EFA in:			
		Chylomicron	VLDL†	LDL	HDL
Treatments	2	1·83**	24·25**	1·72	2·21
Linear	1	0·01	0·14	2·94	3·23
Quadratic	1	3·64*	48·35*	0·50	1·18
Cows	2	0·11	0·22	66·61	10·14
Treatments × cows	4	0·08	0·55	15·40	11·27
Linear × cows	2	0·01	0·29	2·80	5·73
Quadratic × cows	2	0·16	0·83	28·01	16·81

* $P<0\cdot05$. ** $P<0\cdot01$.

† VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Details of the arterial concentration and arteriovenous difference of esterified fatty acid in the various lipoprotein fractions in serum during the feeding of protected casein and protected lipid are shown in Table 4. It is evident that the feeding of protected lipid caused substantial increases in the esterified fatty acid content of all

lipoprotein fractions. However, only the chylomicron and VLDL fractions exhibited consistent arteriovenous differences of esterified fatty acid, the differences being much greater during protected lipid feeding. Analysis of variance of the results for arteriovenous differences (Table 5) revealed significant ($P<0\cdot01$) treatment effects for chylomicron and VLDL fractions. It can be seen that the variation was due to the quadratic components which reflected the significance of increases in mammary uptake of esterified fatty acid from these fractions during the feeding of protected lipid.

Reference to the results in Tables 2 and 4 reveals that virtually all the triglyceride fatty acid removed by the gland was accounted for by the arteriovenous difference of esterified fatty acid in chylomicron and VLDL fractions.

(*d*) *Fatty Acid Composition of Triglyceride in Chylomicron and VLDL Fractions*

The fatty acid composition of triglyceride in VLDL and in chylomicrons from samples of blood serum collected from the carotid artery and subcutaneous abdominal vein are shown in Table 6. In order to obtain sufficient fatty acids for analysis, blood

TABLE 6

FATTY ACID COMPOSITION OF TRIGLYCERIDE IN VLDL AND CHYLOMICRON FRACTIONS FROM ARTERIAL AND VENOUS SERUM COLLECTED DURING THE FEEDING OF PROTECTED CASEIN AND PROTECTED LIPID TO LACTATING COWS

Values are presented as percentage by weight of total fatty acids ≤18:3. Serum samples from three cows were pooled to obtain sufficient triglyceride for analysis

Diet	Protected casein		Protected lipid		Protected lipid	
Triglyceride	VLDL		VLDL		Chylomicron	
Serum	Artery	Vein	Artery	Vein	Artery	Vein
Fatty acid:						
12:0	1·0	1·5	1·3	1·7	0·5	0·5
14:0	1·9	3·5	1·3	0·8	1·7	1·2
14:1	1·3	1·3	0·5	tr	0·4	tr
15:0	2·9	2·9	1·4	2·1	1·3	3·2
16:0	28·7	26·9	16·6	32·1	16·5	32·8
16:1	3·3	6·9	3·1	9·0	1·7	7·6
18:0	30·6	24·2	23·1	17·9	30·4	18·0
18:1	19·1	18·8	17·3	23·8	18·9	21·9
18:2	3·5	3·3	29·6	7·1	22·8	9·0
18:3	1·8	1·0	0·9	1·0	2·4	1·9
Others	5·9	9·7	4·3	4·5	3·4	3·9

samples from the three cows were pooled. Unfortunately, an insufficient quantity of chylomicrons for fatty acid analysis could be obtained from blood collected during the period of protected casein feeding. It can be seen from Table 6 that there was an eightfold increase in the proportion of fatty acid 18:2 in the VLDL triglyceride of arterial blood collected during the feeding of protected lipid. The high proportion of fatty acid 18:2 was also apparent in the chylomicron triglyceride fraction obtained from arterial blood during the feeding of protected lipid.

(*e*) *Concentration of Individual Fatty Acids in VLDL Triglyceride*

The arterial concentration and arteriovenous difference of the major fatty acids in the VLDL triglyceride fraction during the feeding of protected casein and lipid

computed from the results in Tables 4 and 6 were as follows (fatty acid concentrations are expressed as mg/100 ml serum):

Diet	Protected casein		Protected lipid	
Measurement	Artery	Arteriovenous difference	Artery	Arteriovenous difference
Fatty acid:				
16:0	2·27	1·06	2·42	0·40
18:0	2·42	1·33	3·37	2·24
18:1	1·51	0·66	2·53	1·03
18:2	0·28	0·13	4·32	3·87

Whereas the arteriovenous difference for fatty acid 18:2 during the feeding of protected casein was very small when compared with the other major fatty acids, 18:2 became the predominant contributor of long-chain fatty acid to the mammary gland during the feeding of protected lipid. There were also considerably smaller increases in the arteriovenous differences of fatty acids 18:0 and 18:1 during protected lipid feeding. In contrast, there was an almost threefold decrease in uptake of fatty acid 16:0 by the mammary gland during the feeding of protected lipid compared with protected casein feeding.

(*f*) *Concentration of Glucose, FFA, Acetate, and β-Hydroxybutyrate*

The arterial concentration and arteriovenous difference of glucose, FFA, acetate, and β-hydroxybutyrate during the feeding of protected casein and protected lipid are shown in Table 7. The mean arterial concentrations obtained for glucose and

TABLE 7

ARTERIAL CONCENTRATION AND ARTERIOVENOUS DIFFERENCE OF GLUCOSE, FFA, ACETATE, AND β-HYDROXYBUTYRATE DURING THE FEEDING OF PROTECTED CASEIN AND PROTECTED LIPID TO LACTATING COWS

Values are means ± standard errors for three cows

Order of feeding	1	2	3
Diet	Protected casein	Protected lipid	Protected casein
Glucose (mg/100 ml plasma)			
Artery	54·6±8·3	62·5±4·9	52·3±9·9
Arteriovenous difference	10·8±2·9	15·0±2·9	12·1±1·5
FFA (mg/100 ml plasma)			
Artery	11·4±5·6	11·6±6·0	10·9±5·5
Arteriovenous difference	2·2±1·4	2·8±1·5	2·2±0·5
Acetate (mg/100 ml blood)			
Artery	18·8±0·7	7·7±1·3	18·7±0·2
Arteriovenous difference	7·3±0·6	3·0±0·6	7·8±0·8
β-Hydroxybutyrate (mg/100 ml blood)			
Artery	14·7±10·5	13·0±9·9	16·8±8·9
Arteriovenous difference	1·3±0·5	3·0±2·4	4·3±1·4

FFA increased whereas the value for acetate decreased during the feeding of protected lipid. There was a wide variation in the concentration of β-hydroxybutyrate under both feeding regimes; in two of the three cows there was a decrease in the arterial

concentration during the feeding of protected lipid, while in the remaining cow the concentration increased. A summary of the analysis of variance for the arteriovenous differences given in Table 7 is presented in Table 8. There was a significant treatment difference for acetate, the quadratic component accounting for 99% of the total treatment variation. It is apparent from Table 3 that this significant quadratic component ($P<0{\cdot}05$) was due to the reduction in uptake of acetate by the mammary gland during the feeding of protected lipid. There were no significant differences for glucose, FFA, or β-hydroxybutyrate.

TABLE 8

SUMMARY OF ANALYSIS OF VARIANCE FOR ARTERIOVENOUS DIFFERENCES OF GLUCOSE, FFA, ACETATE, AND β-HYDROXYBUTYRATE

Details as for Table 3

Source of variation	D.F.	Mean squares for arteriovenous difference of:			
		Glucose	FFA	Acetate	β-Hydroxy-butyrate
Treatments	2	13·5	0·45	21·4**	7·0
Linear	1	2·3	0·01	0·3	13·8
Quadratic	1	24·7	0·80	42·3*	0·1
Cows	2	46·8*	10·90*	2·7	14·8
Treatments × cows	4	6·0	1·20	0·8	4·0
Linear × cows	2	3·3	2·89	0·2	2·9
Quadratic × cows	2	8·7	1·94	1·5	5·1

* $P<0{\cdot}05$. ** $P<0{\cdot}01$.

IV. DISCUSSION

In agreement with an earlier report (Scott *et al.* 1970) the feeding of protected safflower oil particles to cows resulted in substantial increases in the proportion of fatty acid 18:2 and a decrease in fatty acid 16:0 in milk fat. In addition, the results in this paper have shown proportional reductions in the shorter-chain fatty acids.

The feeding of protected lipid to cows resulted in substantial increases in serum concentrations of phospholipid, cholesterol ester, and triglyceride. However, in accord with earlier reports for cows (Hartmann and Lascelles 1964) and goats (Linzell *et al.* 1967) fed forage diets, triglyceride was the only lipid fraction taken up in significant quantities by the mammary gland.

The results in Table 4 show that most of the long-chain fatty acid removed by the mammary gland was derived from VLDL and to a lesser extent from chylomicron. Increases in the concentration of esterified fatty acid in these fractions which occurred during the feeding of protected lipid were accompanied by an increase of comparable magnitude in uptake by the mammary gland, illustrating that the ability of the gland to utilize preformed fatty acids in the blood is at least partly governed by precursor availability.

Although Hartmann and Lascelles (1966) have demonstrated that large quantities of chylomicron triglyceride are transported to the blood stream via the thoracic duct

lymph in the pasture-fed lactating cow, it has been shown in the present study, in agreement with an earlier report (Glascock *et al.* 1966), that most of the triglyceride taken up by the mammary gland under normal feeding conditions is derived from VLDL. It is suggested that chylomicrons are rapidly removed from the circulation of the cow, and contribute fatty acids to the triglyceride of VLDL presumably formed in the liver (cf. Lascelles *et al.* 1964).

The results in Table 6 and Section III(*e*) demonstrate that the feeding of protected lipid resulted in a spectacular increase in the uptake of fatty acid 18:2 with a corresponding increase in the proportion of this acid in milk fat (Table 1). Although there were moderate increases in the uptake of fatty acids 18:1 and 18:0, there was a substantial reduction in uptake of 16:0, the remaining long-chain fatty acid of quantitative importance. The decreased uptake of the latter cannot be explained in terms of precursor availability, since the arterial concentration of this acid was almost identical on both diets. This raises the question of whether the mammary gland exercises preference for certain fatty acids under conditions of abundance of long-chain fatty acid.

Our attempts to examine the competition between long-chain fatty acid and acetate as precursors of milk-fat fatty acid under conditions where the availability of long-chain fatty acid was experimentally increased were frustrated by a marked decrease in the concentration of acetate in arterial blood during protected lipid feeding. The decrease in uptake of acetate in these circumstances was almost certainly due to the decreased availability of substrate (cf. Hartmann and Lascelles 1965*b*) rather than to any effect of competition at the level of the glandular epithelium. The most likely explanation for the decrease in acetate concentration during protected lipid feeding is that the lipid particles suppressed acetate production in the rumen, probably because they were not completely protected from ruminal hydrogenation (cf. Hogan *et al.* 1972). The increased uptake of fatty acids 18:1 and 18:0, which are known to be the main hydrogenation products of fatty acid 18:2, are in accord with this suggestion, although safflower oil is known to contain approximately 12 and 2% of fatty acids 18:1 and 18:0 respectively (Wadsworth 1968). The possibility remains that feeding of protected lipid results in more rapid utilization of blood acetate by tissues other than the mammary gland, thus reducing its availability for milk-fat synthesis. It would be interesting to know whether substantial increases in milk-fat production would occur if it were possible to maintain acetate availability during the feeding of protected lipid.

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