were quite inactive until treated with alkaline phosphatase. Only partial activity is, however, restored. This confirms the earlier work of Atkins & Ward (1945).

Investigation (Table 2) as to whether the action of the quinone derivatives was bacteriostatic or bactericidal showed that, as expected, lower concentrations of the active compounds reduced growth but left bacteria viable, while higher concentrations caused complete destruction of the organism.

As the pH of the cultures varied from 6.0 to 7.3 the quinones tended to become somewhat less active.

There seems to be no obvious correlation in this series between antibiotic and antimutagenic action.

SUMMARY

1. Certain unsaturated cyclic imides have been shown to have strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

2. The corresponding unsaturated dicarboxylic acids are inactive in this respect.

3. Earlier conclusions as to the antibiotic activity of quinones have been confirmed, and it has been shown that addition of sulphhydryl compounds to the quinone destroys the activity. Increasing pH somewhat decreases the activity of the quinones.

Our thanks are due to Dr F. Bergel for his interest and helpful discussions. One of us (E.F.) is indebted to Messrs May and Baker Ltd. for financial support.

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The Effect of Mastitis on the Carotenoids, Vitamin A and Phosphorus Compounds of Milk

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(Received 28 August 1952)

Of the various infections to which cattle are exposed, mastitis is one of the most prevalent and is the cause of great economic losses in all branches of milk production and manufacture (Davis & McClellmont, 1939). Observations dating back many years have shown that mastitis is accompanied by definite changes in the chemical composition of milk. Amburger (1912) showed that protein, fat, lactose, ash, chloride and specific gravity were affected by mastitis, the chloride being affected in the opposite way to the lactose. About the same time other investigators showed similar changes in mastitic milk from sheep and goats (Grimmer, 1926). In the present paper studies of the effects of mastitis on phosphatase and on the partition of phosphorus, carotenoids and vitamin A are reported. At the same time results of analysis for other milk consti-

tuents are included, in order to show that the milk from the cows which were studied had its composition changed by the disease in the same way as had been found by earlier workers. Chanda & Owen (1949, 1951) found the changes in the partition of phosphorus produced by thyroxine and thiouracil treatment could be related to simultaneous changes of phosphatase. Accordingly, phosphatase was also studied in the present experiments.

Grimmer (1926) cites the observations of many earlier workers who studied the effects of mastitis caused by a variety of pathogens and of others who studied 'salty milk'. These early observers, among whom were Bögold & Stein (1890), noted that phosphate in the ash of milk became less as the proportion of sodium increased; and Grimmer remarks: 'Die Zusammensetzung der Asche nähert

sich somit derjenigen der Blutasche, die Milchdrüse hat scheinbar teilweise ihre Fähigkeit verloren, die ihr zugeführten Salze in passender Weise aufzunehmen, durch die erhöhten Chlornatriummengen wird der Salzgeschmack der Milch bedingt.' Accordingly, the blood of some normal and some mastitic cows was also studied in the present experiments.

EXPERIMENTAL

Eight Ayrshire cows were used, four controls (no. 1-4) and four suffering from mastitis (no. 5-8). In the controls there was no evidence of the presence of *Streptococcus agalactiae* or of micrococcal or staphylococcal mastitis, but in the four infected animals *Strep. agalactiae* was present in most of the quarters. The tests for the organisms were made by Mr A. L. Wilson, of the West of Scotland Agricultural College, Ayr. The degrees of infection recorded in the tables were assigned on the basis of chloride titres after it was known from bacteriological tests that *Strep. agalactiae* was present.

Methods of analysis. The total N of the milk was determined by the Kjeldahl method, and non-casein N by the procedure of Rowland (1938). The difference between them gave the casein N. Fat was estimated by the Gerber method (British Standards Institution, 1936), and total solids by drying a weighed sample of milk on a water bath followed by 3 hr. in an oven at 100°. The content of non-fatty solids was obtained by difference, but was calculated and recorded on the basis of fat-free milk. For estimating chloride the method of Davies (1938) and for phosphatase and the partition of phosphorus the procedures used by Chanda & Owen (1951) were adopted. Total thiamine and the partition of thiamine were determined by a slight modification (Chanda, McNaught & Owen, 1952) of the method described by Houston, Kon & Thompson (1940). The reading for each fraction was made in a Spekker fluorimeter in *isobutanol* as thiochrome (Jansen, 1936). For the determination of the partition of carotenoids and vitamin A in milk, the fat was extracted by the method of Olson, Hegsted & Peterson (1939). The chromatographic separation of β -carotene from other carotenoids and of vitamin A alcohol from vitamin A ester were made in the extracted fat by a modification (Chanda, Owen & Cramond, 1951; Chanda & Owen, 1952a) of Ganguly, Kon & Thompson's (1947) modification of Müller's (1944) method. Both the total carotenoids and the β -carotene were determined by measuring their extinctions at 451 m μ . in a Unicam spectrophotometer. In calculating the potency it was assumed that $E_{1\%}^{1\text{cm}}$ in *n*-hexane for both the mixed carotenoids and β -carotene was 2500. All the *E* readings for the vitamin A fractions were corrected by the three-point method of Morton & Stubbs (1946), 315, 328 and 337 m μ . being used as fixation points. The corrected *E* value at 328 m μ . was multiplied by the conversion factor 1900 to calculate the potency in international units (World Health Organization: Expert Committee on Biological Standardization, 1949). When calculating the potency on a weight basis, 1 i.u. was taken as equivalent to 0.3 μ g. vitamin A alcohol.

Blood. The partition of carotenoids and vitamin A in blood serum was made by the same chromatographic procedure as for milk fat (Chanda & Owen, 1952a). The extraction of blood serum lipids, together with carotenoids and vitamin A, was as described by Yudkin (1941) but *n*-hexane was used as the solvent. The correction procedure of

Morton & Stubbs (1946) was applied for the final estimation of vitamin A ester or vitamin A alcohol, and here again the conversion factor 1900 was used to calculate the potency. Cama & Goodwin (1949) have shown that the estimation of serum vitamin A by the three-point correction of the ultra-violet extinctions agrees with that obtained by the SbCl₃ method.

RESULTS

The fat, non-fatty solids, chloride and phosphatase contents of the milk. Table 1 shows that, as found by most previous observers, the fat content of the milk was decreased by mastitis, while at the same time, and again in accord with previous observers, the non-fatty solids were decreased and chloride increased. As reported by previous investigators, mastitic infection was accompanied by an enhancement of the phosphatase activity of the milk (Table 2).

The ratio of nitrogen to phosphorus (N/P) in the milk. During the course of the experiment, cow 1 was in its 6th-10th week of lactation, whereas cow 2 was between its 16th and 20th weeks. The fact that the N/P ratio was higher for cow 2 (6.3 in Table 1) than for cow 1 (5.2) is consistent with the observation of Chanda & Owen (1952b) that the N/P ratio attains a minimum between the 2nd and 4th weeks post-partum and that thereafter it increases throughout lactation. For the milk from the individual quarters of the two control cows (Table 1) the N/P ratios were remarkably similar, but in the milk from the infected quarters of the other cows the ratios were much higher than in the uninfected quarters. Thus for the severely infected quarter of cow 6 the ratio was as high as 9.4 compared with 5.3 for the normal quarter. The maximum value previously obtained by Chanda & Owen (1952b) was 7.2 in the milk of cows in their 40th week of lactation. Somewhat higher values were obtained also for the milk of cows treated with thiouracil (Chanda & Owen, 1951) but no value as large as 9.4 was observed. It will be seen from Table 1 that the abnormal ratios were caused by both an increase in the nitrogen and a decrease in the P of the milk.

The partition of phosphorus. Table 1 shows that where the degree of infection was severe, as judged by the chloride values, the phosphatase content of the milk was high. Thus in cow 6, the phosphatase in the milk of the right hind quarter was about 4 times that of the right fore quarter, and the values for the milk of the left fore and left hind quarters were somewhat greater than for the milk of the healthy right fore quarter. The differences between the phosphatase titres of the milk of the four quarters of each of the two control cows were relatively small.

Chanda & Owen (1952b) observed that the changes in phosphatase as lactation progressed were inversely correlated with the ester P, lipid P and casein P. The partition of P recorded in Table 2

Table 1. *The effect of mastitis on the content of some of the principal constituents of the milk*

Cow		No. of samples	Quarter of udder*	Degree of infection	Fat (%)	Non-fatty solids in fat-free milk (%)	Chloride (mg./100 g.)	Nitrogen (mg./100 g.)	Phos-phorus (mg./100 g.)	N/P	
State	No.					(%)	(mg./100 g.)	(mg./100 g.)	(mg./100 g.)		
Normal	1	5	RF	—	3.6	8.76	78	592	115	5.1	
		5	RH	—	4.2	8.81	85	578	111	5.2	
		5	LF	—	3.5	8.79	81	595	114	5.2	
		5	LH	—	4.0	8.72	75	575	112	5.1	
	2	5	RF	—	3.6	8.45	117	581	94	6.2	
		5	RH	—	3.5	8.39	115	596	95	6.3	
		5	LF	—	3.9	8.54	124	567	90	6.3	
		5	LH	—	3.2	8.48	113	591	92	6.4	
	Mastitis	5	1	RF	—	4.3	8.86	69	611	127	4.8
			1	RH	+	4.4	8.63	111	629	121	5.2
			1	LF	+++	2.9	7.94	180	716	102	7.0
			1	LH	+++	3.2	8.29	144	709	108	6.6
6		4	RF	—	3.5	8.96	76	569	108	5.3	
		4	RH	+++	1.7	7.58	185	694	74	9.4	
		4	LF	+	2.8	8.54	112	611	98	6.2	
		4	LH	+	2.9	8.65	117	625	95	6.6	
7		4	RF	—	3.3	8.81	85	591	109	5.4	
		4	RH	++	2.6	8.32	122	654	93	7.0	
		4	LF	+++	2.1	7.46	194	679	78	8.7	
		4	LH	++	2.3	8.23	129	645	88	7.3	
8		4	RH	+	3.1	8.27	117	657	112	5.9	
		4	LH	+++	1.8	7.54	198	725	77	9.4	

* RF=right fore; RH=right hind. LF=left fore; LH=left hind.

Table 2. *The effect of mastitis on the partition of phosphorus in the milk*

Cow		No. of samples	Quarter of udder*	Degree of infection	Total P (mg./100 g.)	Total P as				Phosphatase (phenol unit†)
State	No.					Inorganic (%)	Ester (%)	Lipid (%)	Casein (%)	
Normal	1	3	RF	—	112	58.4	11.7	11.2	18.7	64
		3	RH	—	107	59.8	11.2	10.9	18.1	75
		3	LF	—	110	59.2	11.6	11.4	17.8	69
		3	LH	—	108	60.3	11.3	10.7	17.7	78
	2	3	RF	—	96	66.7	7.2	10.2	15.9	129
		3	RH	—	98	65.5	7.6	10.5	16.4	121
		3	LF	—	93	66.9	7.0	10.0	16.1	134
		3	LH	—	95	66.2	7.2	10.3	16.3	127
	Mastitis	5	RF	—	127	53.7	13.9	13.1	19.3	48
			RH	+	121	60.1	11.0	10.7	18.2	78
			LF	+++	102	74.5	4.6	7.6	13.2	189
			LH	+++	108	71.8	5.4	8.5	14.4	166
		6	RF	—	109	54.9	13.5	12.8	18.8	54
			RH	+++	72	75.3	4.2	7.7	12.8	202
			LF	+	99	61.5	10.8	10.5	17.2	80
			LH	+	95	62.5	10.5	10.7	16.3	92
		7	RF	—	107	61.7	10.7	10.9	16.7	87
			RH	++	91	65.9	7.5	10.4	16.2	115
			LF	+++	79	75.6	4.2	7.5	12.7	207
			LH	++	89	66.3	7.3	10.4	16.0	121
		8	RH	+	114	65.8	7.8	10.3	16.1	114
			LH	+++	76	75.7	4.3	7.1	12.9	229

* See Table 1 for explanation of symbols.

† See Chanda & Owen (1951).

shows that in mastitis there is a similar relationship; the changes in phosphatase were accompanied by changes in the partition of P. Thus in the milk of the severely infected right hind quarter of cow 6 the inorganic P formed 75 % of the total P compared with 55 % in the sound right fore quarter. The two left quarters which were only mildly infected also gave milk with more of the P in the inorganic form. It will be observed that the milk of control cow 2 was richer in phosphatase than that of cow 1, and that it had also a larger proportion of P in the inorganic form. In fact, as shown in Fig. 1, the inorganic P was positively correlated with phosphatase.

Fig. 1. The relationship between inorganic P, expressed as % of total P and phosphatase in normal and mastitic milk. Curve fitted by the method of least squares. ○, normal cow no. 1; ●, normal cow no. 2; △, mastitic cow no. 5; ▲, mastitic cow no. 6; □, mastitic cow no. 7; ■, mastitic cow no. 8.

Ester, casein and lipid P were greater in the normal quarters than in the infected quarters and all these fractions of organic P were negatively correlated with phosphatase. These correlations held whether the fractions were expressed as concentrations of P in the milk or as percentages of the total P. The negative correlation between phosphatase and ester P is recorded in Fig. 2, and that between lipid P and phosphatase in Fig. 3. Ester P, like phosphatase, and some other constituents, may be used to compare the severity of infection in different quarters of the same cow. Even the lowest ester P values corresponding to the highest

Fig. 2. The correlation between phosphatase and ester P in normal and mastitic milk. Symbols and cows are the same as those in Fig. 1.

Fig. 3. The correlation between phosphatase and lipid P in normal and mastitic milk. Symbols and cows are the same as those in Fig. 1.

phosphatase titres observed in the most severely infected quarters are thus of limited diagnostic value, similar figures having been noted in the milk of cows treated with thiouracil (Chanda & Owen, 1951) and in cows towards the end of lactation (Chanda & Owen, 1952b). Nevertheless, large differences between the milks of different quarters of any one cow may well indicate the presence of some form of mastitis.

The ratio of N/P in casein. Both the casein N and the casein P of the milk were decreased by mastitis. The decreases in casein P were, however, much greater than those in casein N so that the ratio N/P for the casein was greatly increased in the milk from infected quarters (Table 3). For example, in cow 7 the ratio casein N/casein P was as large as 38 in the infected quarter (left fore) as compared with only 24 in the normal quarter (right fore). The ratio of

Table 3. *The ratio of nitrogen to phosphorus in the casein of milk affected by mastitis*

Cow		Quarter of udder*	Degree of infection	Total N	Casein N	Casein N	Casein P	N/P in
State	No.			(mg./100 g.)	(mg./100 g.)	as % of total N		
Normal	3	RF	—	581	412	71	20.4	20.2
		RH	—	577	414	72	20.1	20.6
		LF	—	584	418	72	19.9	21.0
		LH	—	572	423	74	20.5	20.6
	4	RF	—	567	407	72	16.4	24.8
		RH	—	586	434	74	17.6	24.7
		LF	—	574	427	74	17.9	23.9
		LH	—	579	422	73	17.5	24.1
Mastitis	5	RF	—	611	453	74	24.5	18.5
		RH	+	629	445	71	22.0	20.2
		LF	+++	716	422	59	13.5	31.3
		LH	+++	709	429	61	15.5	27.7
	7	RF	—	598	435	73	17.8	24.4
		RH	++	646	417	65	14.5	28.9
		LF	+++	694	388	56	10.2	38.0
		LH	++	656	421	64	14.2	29.6

* Only one sample from each quarter of each cow was analysed. Symbols as in Table 1.

Table 4. *The effect of mastitis on the partition of thiamine in the milk*

Cow		Quarter of udder*	Degree of infection	Thiamine (µg./100 ml. fat-free milk)		Total thiamine present as			Phosphatase (phenol units†)
State	No.			Free	Total	Free (%)	Cocarcboxylase (%)	Protein-bound (%)	
Normal	3	RF	-	23.2	39.7	58.5	28.7	10.8	58
		RH	-	23.9	40.4	59.2	29.5	9.5	56
		LF	-	23.3	38.6	60.4	27.4	10.2	61
		LH	-	22.6	39.2	57.7	29.9	10.6	53
	4	RF	-	23.6	32.5	72.6	16.9	8.7	134
		RH	-	24.5	34.6	70.7	18.8	8.9	131
		LF	-	24.8	33.9	73.1	17.1	8.3	136
		LH	-	22.7	32.1	70.8	18.5	9.2	129
Mastitis	5	RF	-	24.3	44.6	54.4	33.4	11.5	48
		RH	+	23.0	37.3	61.7	27.2	9.5	78
		LF	+++	23.9	28.6	83.6	8.6	5.6	189
		LH	+++	25.3	30.5	82.9	9.4	5.9	166
	6	RF	-	22.9	41.8	54.8	32.5	10.9	52
		RH	+++	22.9	27.2	84.2	8.8	5.5	198
		LF	+	21.9	35.5	61.8	26.6	9.4	83
		LH	+	21.4	34.4	62.2	26.1	9.7	89
	7	RF	-	23.7	38.4	61.6	26.7	9.3	82
		RH	++	22.6	32.9	68.7	21.9	7.5	118
		LF	+++	22.3	26.5	84.0	8.2	5.3	203
		LH	++	22.2	31.6	70.3	20.7	7.6	124
	8	RH	+	23.8	34.9	68.1	22.3	7.4	119
		LH	+++	22.0	25.7	85.4	7.9	5.1	221

* Two samples of milk were analysed from each quarter, except for the quarters of cow 5 for which only one sample was analysed. Symbols as in Table 1.

† See Chanda & Owen (1951).

N/P in the casein was remarkably constant in the different quarters of each of the two control cows, but cow 4, which was at a later stage of lactation than cow 3, showed a bigger ratio.

Phosphatase and the partition of thiamine. The results for the partition of thiamine are shown in Table 4 where the phosphatase titres for the same samples of milk are also recorded. The values for free thiamine were very similar for the milks from all the quarters of all six cows, in spite of considerable differences in the total thiamine. For example, in the milk of the two control cows there was a large difference in phosphatase titre, the larger titre in cow 4 corresponding to a smaller figure for total thiamine, but the absolute amount of free thiamine was the same for the milk of both cows, the decrease in the total thiamine occurring in the cocarboxylase and protein-bound forms. These relationships were even more apparent in the milks from individual quarters of the infected cows. Where the phosphatase was high in the milk of a particular infected quarter, the amount of free thiamine was the same as in the milk of the non-infected quarter of the same cow, but the total thiamine and the cocarboxylase and protein-bound forms were all markedly reduced. In the milks from the individual quarters of each of the two control cows there were no significant differences.

The fact that the percentage of the total thiamine which is present in the free form increases in the milk of infected quarters was noted earlier by Thompson (1945), but the present work goes further in showing the constancy of the values for free thiamine and the large decreases which can occur in the cocarboxylase and protein-bound forms. In the severely infected quarters of cows 5-7, the cocarboxylase was only 8 or 9% of the total, whereas for the normal quarters the corresponding figures were around 30%. There was thus an inverse relationship between cocarboxylase and phosphatase. The close but curvilinear inverse correlation between these two substances is shown in Fig. 4. The values for protein-bound thiamine in the milk of normal and infected quarters varied in much the same way as the values for cocarboxylase.

The positive correlation between the phosphatase titre and the percentage of the total thiamine present in the free form (Table 4) appears to be general. It was observed by Houston *et al.* (1940) for the milk of cows and goats, and by Braude *et al.* (1947) for sow's milk. Chanda *et al.* (1952) found it to exist in the milk of cows treated with thyroxine and thiouracil. The inverse relationship between phosphatase and phosphorylated forms of thiamine were also shown to hold for cow's milk throughout lactation (Chanda & Owen, 1952*b*), and also in the milk of cows treated with thyroxine and thiouracil (Chanda, McNaught & Owen, 1952). It appears

therefore that there is a persistent relationship between phosphorylated compounds and phosphomonoesterase whether the changes are produced by mastitis (Table 4 and Fig. 4) or are induced artificially by hormones (Chanda *et al.* 1952) or are produced naturally during declining lactation (Chanda & Owen, 1952*b*). The inverse relationship between phosphatase and phosphorylated thiamine has also been demonstrated in human milk (Chanda *et al.* 1951) and in goat milk (Chanda, 1951).

Fig. 4. The relation between phosphatase and cocarboxylase in normal and mastitic milk. O, normal cow no. 3; ●, normal cow no. 4; △, mastitic cow no. 5; ▲, mastitic cow no. 6; □, mastitic cow no. 7; ■, mastitic cow no. 8.

The partition of carotenoids and vitamin A. Large increases in the total-carotenoid content of milk during clinical mastitis were reported by Thompson (1945). The results recorded in Table 5 show that, though the fat content of the milk was decreased by mastitis, the carotenoids were considerably increased in the milk from infected quarters. The percentage of the total carotenoids which was present as β -carotene was surprisingly constant and was not influenced by mastitis, but the content of β -carotene/g. fat was very markedly increased for the milk of infected quarters. Where the infection was greatest the amount of β -carotene/g. fat was 6 or 10 times as large as the corresponding figure for the milk of a normal quarter (Table 5), and even for

Table 5. *The effect of mastitis on the partition of the carotenoids and vitamin A in cows' milk*

Cow		Quarter of udder*	Degree of infection	Fat (%)	Total caro- tenoids ($\mu\text{g./}$ 100 ml.)	β -Carotene		Partition of vitamin A				Ester (% of total)	A/C†	
						($\mu\text{g./g.}$ fat)	(As % of the total caro- tenoids)	Total		Alcohol				
State	No.							(i.u./ 100 ml. milk)	(i.u./ g. fat)	(i.u./ g. fat)	(% of total)			
Normal	3	RF	—	3.4	28.9	7.5	88	115	33.8	1.5	4.3	93.9	1.51	
		RH	—	3.9	30.2	6.8	88	120	30.8	1.8	5.8	92.2	1.51	
		LF	—	3.7	29.7	7.4	92	117	31.6	1.6	5.1	93.0	1.42	
		LH	—	4.1	28.4	6.3	91	124	30.2	1.7	5.6	92.6	1.60	
	4	RF	—	3.5	27.8	7.3	91	97	27.7	1.5	5.4	92.5	1.26	
		RH	—	3.7	29.4	7.2	90	108	29.2	1.3	4.5	93.3	1.35	
		LF	—	3.8	29.7	7.0	90	109	28.7	1.4	4.9	93.0	1.37	
		LH	—	3.3	27.2	7.3	89	92	27.9	1.4	5.1	92.9	1.27	
	Mastitis	6	RF	—	3.6	29.5	7.4	90	118	32.8	1.9	5.7	92.3	1.47
			RH	+++	1.6	130.6	74.4	91	49	30.6	6.0	19.6	78.6	0.14
			LF	+	2.7	52.7	17.2	91	86	31.9	3.0	9.5	88.3	0.60
			LH	+	2.9	54.8	17.2	91	91	31.4	2.8	8.9	88.9	0.61
7		RF	—	3.2	28.4	8.0	90	95	29.7	1.7	5.7	92.3	1.24	
		RH	++	2.5	58.7	20.9	89	76	30.4	3.5	11.6	86.5	0.48	
		LF	+++	2.1	125.2	53.5	90	66	31.4	6.7	21.2	77.2	0.20	
		LH	++	2.4	63.6	24.1	91	74	30.8	3.9	12.5	85.6	0.43	
8		RH	+	3.0	55.9	16.6	89	92	30.7	3.3	10.8	87.3	0.61	
		LH	+++	1.9	123.4	58.7	90	59	31.1	5.4	17.6	80.6	0.18	

* Three samples of milk were analysed for each quarter. Symbols as in Table 1.

† A/C is the ratio of vitamin A to β -carotene, both expressed as $\mu\text{g./g. fat.}$

mildly infected quarters the increase was considerable. There was, however, little variation from quarter to quarter in the two control cows.

Table 5 shows that the total vitamin A content, unlike the carotene content of the milk, was decreased by mastitis. The decreases in vitamin A were, however, proportional to the decreases in the fat content so that the vitamin A content of the milk fat remained practically unchanged. In cow 6 the vitamin A content was only 49 i.u./100 ml. milk in the most infected (right hind) quarter as compared with 118 i.u./100 ml. in the sound (right fore) quarter, but the corresponding values expressed as i.u./g. fat were 31 and 33 respectively. This small difference in the vitamin A content of the fat cannot be considered of any significance since, in cow 7, one of the infected quarters had 1.7 i.u. more vitamin A/g. fat than its own control quarter, and there were differences of the same order in the values for the different quarters of the control cows. The decreases in the total vitamin A content of the milk from infected quarters which accompany the simultaneous decreases in the fat content are consistent with the observation of Thompson (1945), but are in contrast to that of Miller, Lease & Anderson (1940) who reported an increase in the vitamin A content in the milk from the infected quarter of a cow in which mastitis was artificially produced.

The vitamin A content of the milk fat of control cow 3 was higher than for that of control cow 4.

This agrees with the earlier finding of the present author (Chanda, 1953) that the vitamin A content of cow and goat milk fat, in contrast to that of the carotenoids, continues to change throughout lactation. In these earlier experiments Chanda (1953) showed that variations in the ratio of vitamin A/g. fat to β -carotene/g. fat (A/C) for the milk fat of different cows was determined by the vitamin A content, since the carotene content of the fat was constant as long as the diet remained unchanged. In the present experiments the A/C ratios recorded in Table 5 show that control cow 4 which was at about the 25th week of lactation gave a slightly smaller A/C ratio than control cow 3 which was at an earlier stage of lactation, and that the difference was caused by differences in the vitamin A contents of the fat. When, however, as in mastitis, the A/C ratio in any one cow varied widely from quarter to quarter it was the carotene and not the vitamin A which determined the variation in the A/C ratio. The smallest ratio of 0.14 was recorded in the very severely infected quarter of cow 6 corresponding to the largest β -carotene figure. As in normal milk there was no correlation between the vitamin A and the β -carotene contents of the fat of mastitic milk, but a close negative correlation was found between β -carotene and the A/C ratio.

In normal milk only a small percentage of vitamin A is present as alcohol (Parrish, Wise & Hughes, 1947; Ganguly *et al.* 1947; Chanda & Owen, 1952a). This was confirmed in the present experiments for

Table 6. *The partition of carotenoids and vitamin A in the blood serum of normal and mastitic cows*

Cow		Total lipid (mg./ 100 ml.)	Total carotenoids (μ g./ 100 ml.)	β -Carotene		Vitamin A			A/C* $\times 10^3$
				(% of total carotenoids)	(μ g./g. lipid)	Alcohol (i.u./ 100 ml.)	Ester (i.u./ 100 ml.)	Total (μ g./g. lipid)	
Normal	1	367	1329	91	3621	105	39	131	36.1
	2	349	1213	88	3476	98	32	123	25.4
	3	356	1465	91	4115	96	43	129	31.4
	4	334	1396	90	4180	102	30	132	31.5
Mastitis	6	388	1152	89	2969	97	37	116	39.1
	7	375	1097	88	2925	95	30	112	38.3
	8	392	1194	88	3046	103	36	117	38.5

* A/C is the ratio of vitamin A to β -carotene, both expressed as μ g./g. lipid.

individual quarter samples (Table 5). For the two control cows the vitamin A alcohol ranged between 4 and 6 % of the total vitamin A, whereas in mastitis, when the total vitamin A content of the milk was considerably decreased, both the percentage present as alcohol and the actual amount of the alcohol form were increased (see also Ganguly *et al.* 1947). For example, in the normal (right fore) quarter of cow 7 the amount of vitamin A alcohol was found to be 5.4 i.u./100 ml. milk, while the value for the severely infected quarter of the same cow was 14 i.u. The alcohol form of vitamin A in the milk increased therefore by 159 % while the total vitamin A (Table 5) was decreased by 30 %. Similar results were obtained for cow 6. It will be noted also from Table 5 that the content of vitamin A ester in the milk of mastitic quarters was considerably less than in the milk of normal quarters.

The partition of carotenoids and vitamin A in the blood serum. The results for the partition of carotenoids and vitamin A determined in four normal cows and three infected cows are recorded in Table 6. They suggest that the lipid content of the serum of mastitic cows was slightly greater than that of the normal cows. This is in contrast to the corresponding results observed for the fat content of the milk. Total carotenoids were consistently less in the serum of the mastitic cows than in that of the normal cows, but there were no significant differences in the percentages of total carotenoids present as β -carotene. The contents of β -carotene in the blood-serum lipids of mastitic cows were much smaller than those for the normal cows. The partition of vitamin A showed that the alcohol form of vitamin A in the serum varied within the range of 96 and 105 i.u./100 ml. (Table 6), and that there was no difference in this respect between the normal and the infected cows. In confirmation of earlier findings (Chanda, 1953) it was observed that the blood serum of grazing cattle contained vitamin A ester, but again there was no difference between the vitamin A ester values of the normal and of the infected cows. Since the β -carotene content of the serum of mastitic

cows was smaller than for the normal animals, the ratio of vitamin A to β -carotene (A/C) tended to be greater in the mastitic cows than in the normal cows. The A/C ratio for the blood serum, was, however, much smaller than in the milk fat, and the A/C ratio in the milk fat of mastitic quarters lay between the ratio for the serum lipid and for the normal milk fat from the same cow. Thus a typical value for normal milk fat was about 1.4 (Table 5); for milk from a mastitic quarter it was on one occasion as low as 0.14 (Table 5), and for blood serum it was about 0.036 (Table 6).

DISCUSSION

Variations in the composition of milk

The composition of the blood and milk taken at the same time from the four normal cows used in the present work is recorded in Table 7, from which it can be seen that those constituents like nitrogen and chloride, which are present in the blood serum in greater amounts than in the milk, were increased by mastitis. Fat, phosphorus and thiamine, which are present in smaller amounts in the blood than in the milk, showed large decreases in mastitis. This generalization does not, however, apply to phosphatase which was increased by mastitis in spite of the fact that it was present in lesser amount in the blood serum of the cow. It is known that the mammary gland itself is strikingly rich in phosphatase (Folley & Kay, 1935) and it is probable that the milk phosphatase originates in the mammary gland itself.

Chanda & Owen (1951) showed that the variation in the ratio of N/P is an index of the activity of the mammary gland, for when the activity of the gland was artificially enhanced by thyroxine treatment, the ratio was increased. By contrast, thiouracil had the opposite effect. The decreased activity of the mammary gland in mastitis is also reflected here by the larger N/P ratio in the milk of infected quarters (Table 1). Chanda & Owen (1951) showed that the phosphorus content of casein can vary considerably

Table 7. *The average concentration of some constituents in the milk and blood serum of four normal cows*

Constituent (per 100 ml.)	Blood serum	Milk	Increased (+) or decreased (-) in the milk from quarters infected with mastitis
Fat (mg.)	350	3685	-
Nitrogen (mg.)	1227	587	+
Chloride (mg.)	333	98	+
Phosphorus (mg.)	17	102	-
Ester P (mg.)	2	10	-
Phosphatase (phenol units)*	21	98	+
Thiamine (μ g.)	14	36	-
β -Carotene (μ g.)	1206	27	+
Vitamin A (i.u.)	134	108	-

* See Chanda & Owen (1951).

and that it tends to increase during thyroxine treatment and decrease with thiouracil. In the present experiments this variation in the phosphorus content of casein was very evident in mastitic milk (Table 3). The casein fraction of the milk from infected quarters was poorer in phosphorus than that from normal quarters. This difference in composition is in accord with the well-known fact that it is difficult to prepare casein of constant phosphorus content (Rogers, Associates of, 1935). Even in the two normal cows which were at different stages of lactation, the ratio of N/P in the casein was significantly different (Table 3). The theory of Synge (1950) on the existence of families of nearly identical proteins is compatible with the present observations on the differences in the composition of casein. The present results are also consistent with the recent observations of Warner (1944), of Hipp, Groves, Custer & McMeekin (1952) and of Dovey & Campbell (1952) who confirmed the earlier observation of Mellander (1939) and found three distinct components in casein. Hipp *et al.* (1952) showed further that the phosphorus content of the three components differed, but that their nitrogen contents were remarkably similar. The larger differences shown in Table 3 for the ratios of N/P in casein may therefore reasonably be attributed to changes in the proportion of the three components of casein when a quarter is infected.

Carotenoids and vitamin A in the blood serum and the milk

Normally the passage of carotenoids into the milk, unlike that of vitamin A, is very limited (Deuel, Hallman, Johnston & Mattson, 1942). It is also interesting to note that the vitamin A content continues to change throughout lactation whilst that of carotenoids does not (Chanda, 1953). In mastitis, however, the ability of the mammary gland to hold back carotenoids is so impaired that much greater amounts of β -carotene and other carotenoids find their way into the milk. The phenomenon is probably associated with a decrease in the fat

content of the milk (Table 5) and a decrease in the average size of the fat micelles with a consequent increase of the area of micellar surface per unit weight of micelles (Kon & Mawson, 1950). It has been observed that the concentration of carotenoids in milk fat varies inversely with the size of the fat globules (Kon, Mawson & Thompson, 1944). The increase in the carotenoid content of mastitic-milk fat could well be due to an enhanced leakage of blood-plasma lipids into the milk through the damaged glandular epithelium, for, as is known and as Table 7 illustrates, blood serum is very much richer in carotenoids than is milk, so that it would not require much leakage to account for the difference observed in the milk of infected quarters. Vitamin A alcohol, like the carotenoids, would be expected to be more surface-active than vitamin A ester and therefore to be like the carotenoids preferentially concentrated in the chylomicrons, which have a greater ratio of surface area to mass than do normal milk globules. It can be seen from Table 5 that the amount of vitamin A alcohol/g. fat was increased by mastitis in much the same ratio as carotene and carotenoids were increased.

SUMMARY

1. Eight lactating cows were used to study the effect of mastitis on the concentration and partition of carotenoids, vitamin A and certain phosphorus compounds in milk. Mastitis was judged to be present when infection with *Streptococcus agalactiae* was detected. The increases of the chloride of the milk were taken as indicators of the intensity of the infection.
2. Phosphorus was considerably reduced and, since nitrogen increased, the ratio of nitrogen to phosphorus was greater in mastitic milk than in normal milk.
3. The ratio of nitrogen to phosphorus in the casein of the milk was increased by mastitis.
4. The concentrations of total phosphorus, ester phosphorus, lipid phosphorus, casein phosphorus of

fat and of non-fatty solids were decreased in mastitic milk. The values of the phosphorus fractions were negatively correlated with phosphatase, but inorganic phosphorus was positively correlated with phosphatase.

5. Mastitis caused a large decrease in the thiamine content of the milk and in the proportion of thiamine present in the cocarboxylase and protein-bound forms. The proportion of thiamine present in the free form was increased. There was a positive correlation between free thiamine and phosphatase, but between phosphatase and the cocarboxylase and protein-bound forms of thiamine there were negative correlations.

6. The carotenoid content of the milk fat was increased by mastitis, but the percentage of total carotenoids present in the form of β -carotene remained unchanged. The vitamin A content of the milk fat remained unchanged. The percentage of

the vitamin A present in the milk in the alcohol form was increased by mastitis.

7. The blood serum of the mastitic cows was found to contain more lipid than that of the normal cows. The β -carotene content was, however, less in the mastitic than in the normal cows. Neither the vitamin A contents of the serum lipids nor the partition of vitamin A between ester and alcohol in the serum was affected.

The author wishes to thank Dr E. C. Owen for suggesting the investigation, for collaborating in it and for many valuable discussions during its course. He is much indebted to Mr A. L. Wilson and his staff of the West of Scotland Agricultural College for obtaining the pathological samples. Thanks are due to Miss M. Millar and Miss P. McGuigan for technical assistance. Grateful acknowledgement is made to the Agricultural Research Council for a grant which the author received during the course of the work.

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