2. Enzymic activities of the particles were compared. Whereas milk microsomes have high alkaline phosphatase and xanthine dehydrogenase, together with low reduced diphosphopyridine nucleotide-diaphorase and reduced diphosphopyridine nucleotide-cytochrome *c* reductase activities, gland microsomes have about the same alkaline phosphatase, much less xanthine dehydrogenase, and much more diaphorase and reduced diphosphopyridine nucleotide-cytochrome *c* reductase activities.

3. It was found that incubation of gland microsomes in milk serum or in buffer at pH 6.7 caused changes in enzymic activities to a pattern similar to that of milk microsomes. The considerable increase in xanthine dehydrogenase activity is probably due to changes in the physical state of the enzyme.

4. Cytochrome *b₅* occurs in milk microsomes, although most of the haemprotein is denatured. Since this cytochrome is restricted to the microsome fraction of mammary gland, milk microsomes must arise mainly from this source. Changes occurring during storage of the extracellular milk in the gland could account for the different pattern of enzymes in milk and gland microsomes.

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Comparative Properties of Microsomes from Cow's Milk and from Mammary Gland

2. CHEMICAL COMPOSITION*

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In the preceding paper (Bailie & Morton, 1958) it was shown that the differences in the enzymic activities of microsomes isolated from milk and from mammary gland are probably caused by changes which occur after secretion of the milk.

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Adsorption of the microsomes by the fat globules, and the action of enzymes of the milk microsomes themselves as well as of enzymes of the milk serum, probably account for the changes in enzymic activities.

The chemical compositions of the particles from the two sources were compared at the same time. The milk and mammary-gland microsomes differed considerably in their content of nucleic acid. However, it was found that this difference could be due

to loss of nucleic acid from the gland microsomes after secretion into the milk. This paper gives the chemical compositions of microsomes from milk and from mammary gland of the cow, and from rat liver, as well as of a preparation of disrupted sarcosomes of pig heart, made by the method of Keilin & Hartree (1947).

MATERIALS AND METHODS

Chemicals. The nucleic acids, lipid solvents and perchloric acid used were the same as those described by Martin & Morton (1956a).

Tissue preparations

Milk and mammary-gland microsomes. The procedures for isolation of these have already been described (Baillie & Morton, 1958).

For chemical analyses, the final preparations were washed twice by dispersion in 0.15M-NaCl soln. and centrifuging at 50 000 *g* for 20 min. The washed material was dispersed in water and frozen and dried over conc. H_2SO_4 *in vacuo* at room temperature (about 18°).

Heart-muscle preparation. This was the same as that used by Martin & Morton (1956a).

Rat-liver microsomes. The livers from eight rats were removed, chilled in cold 0.15M-KCl soln. and dispersed in about 9 vol. of 0.25M-sucrose with a Potter-Elvehjem glass homogenizer. The dispersion was centrifuged at 600 *g* for 10 min. at 0–4° and the precipitate was washed twice by dispersing it in 0.25M-sucrose and centrifuging at 600 *g* for 10 min. The supernatant and washings were combined and centrifuged at 8500 *g* for 10 min. at 0–4°. The precipitate was washed twice by dispersion in 0.25M-sucrose and centrifuging at 8500 *g* for 10 min., and the combined supernatant and washings were centrifuged at 50 000 *g* for 30 min. at about 5°. The precipitate was washed twice by dispersing it in 0.15M-NaCl soln. and centrifuging at 50 000 *g* for 30 min.; it was then dispersed in water, frozen and dried to constant weight over conc. H_2SO_4 *in vacuo* at room temperature (about 18°).

Analytical procedures

Dry weight. The material was dried to constant weight over conc. H_2SO_4 *in vacuo* at room temperature.

Deoxyribose. The method of Ceriotti (1952) was used. Commercial chloroform, or, in later studies, chloroform purified as described by Martin & Morton (1956a), was used in the reaction.

Phosphorus. The methods of Allen (1940) and of Weil-Malherbe & Green (1951) were used.

Nitrogen. A micro-Kjeldahl procedure (Morton, 1955a) was used.

Extraction of lipids, acid-soluble phosphates, nucleic acids and proteins

The general procedures were similar to those already described by Martin & Morton (1956a). The dried material was weighed into centrifuge tubes (ground internally), and dispersed by homogenization in the solvent as described by Martin & Morton (1956a).

Lipids. Successive extraction with methanol, chloroform-methanol, butan-1-ol and diethyl ether was carried out as described by Martin & Morton (1956a), except that each extraction was for 15 min. instead of 30 min. at room temperature.

Acid-soluble phosphates. Except with the Schneider (1945) method these were extracted by suspending the lipid-free material in 0.2N- $HClO_4$ at 0° and centrifuging. The supernatant was removed and the residue extracted rapidly as before. The combined supernatants were used.

Nucleic acids and proteins. The methods described by Schneider (1945) and Ogur & Rosen (1950), and a number of modifications of the latter method, were investigated with different preparations. Analyses were mostly carried out with the procedure of Martin & Morton (1956a), modified by using unbuffered NaCl (10%, w/v) instead of buffered NaCl soln. Evidence for the suitability of this procedure for extraction of nucleic acids from cytoplasmic particles from animal tissues is considered in the Experimental section.

EXPERIMENTAL AND RESULTS

Extraction of nucleic acids

Martin & Morton (1956a, b) compared several published methods for extraction of nucleic acids from plant materials, and developed a procedure which was reasonably satisfactory for analysis of microsomes and mitochondria of plant tissues. Comparable studies of different procedures for estimation of nucleic acids of animal materials were carried out at the same time by the present authors.

Studies with pig-heart sarcosomes. A Keilin & Hartree (1947) preparation from pig hearts was used for initial studies. Analyses were carried out with the Ogur & Rosen (1950) procedure (Fig. 1). The residue remaining after extraction of lipids and acid-soluble phosphates was treated for two successive 18 hr. periods with N- $HClO_4$ at 0–4°, since Martin & Morton (1956a) had found that a single extraction for 18 hr. (Ogur & Rosen, 1950) was insufficient for extraction of ribonucleic acids (RNA) from beet petioles.

A modification (Fig. 1, Procedure A) was also investigated. Ogur & Rosen (1950) used treatment with 0.5N- $HClO_4$ at 70° to extract deoxyribonucleic acid (DNA). However, it was considered that treatment with 0.3N-KOH soln. (cf. Davidson & Smellie, 1952) at 38° for 16 hr. would extract DNA and that this would be precipitated on acidification, according to the procedure of Schmidt & Thannhauser (1945). The acidified supernatant would contain protein phosphorus and any RNA phosphorus not extracted by N- $HClO_4$. The RNA phosphorus could be adequately determined by measurements at 260 m μ .

The results are shown in Table 1. It is seen that Procedure A gave little improvement on the results obtained with the Ogur & Rosen (1950)

procedure. With both methods, there was reasonable agreement between the values of RNA phosphorus as determined directly and as estimated from absorption measurements. However, clearly the treatment with $N-HClO_4$ at 0° failed to extract all the RNA in the preparation. With Procedure A, the fraction containing protein phosphorus extracted with $0.3N-KOH$ soln. also contained some nucleic acid phosphorus, since the ultraviolet-absorption curve of this fraction showed a maximum at $260 m\mu$. However, the characteristics of the absorption spectrum indicated that the extract contained other compounds in addition to nucleic acid. Neither procedure gave adequate differentiation of RNA and DNA, since in both the estimate of DNA by the specific Ceriotti (1952) colori-

metric method for deoxyribose was very much less than were the estimates based on organic phosphorus or on absorption.

In earlier studies with the same preparation, Martin & Morton (1956*a*) had shown that the method of Schmidt & Thannhauser (1945) was unsuitable for determination of the nucleic acids in that organic phosphorus compounds other than RNA occurred in the RNA fraction. The results in Table 1 may be directly compared with those of Martin & Morton (1956*a*, Table 1). The latter workers, however, extracted the lipids with butanol only. This solvent extracts almost all of the phospholipids but little of the neutral fats, so that the lipid figures of Martin & Morton (1956*a*) for the pig-heart preparation are lower than the true values.

Fig. 1. Methods used for extraction of nucleic acids and proteins from pig-heart sarcosomes (Table 1).

Studies with milk microsomes. Preliminary investigations indicated that the nucleic acid content of the milk microsomes was very low. Several different methods for extraction of the nucleic acids were therefore compared to ensure that all the nucleic acid was estimated. The extraction of RNA and DNA with trichloroacetic acid, as used by Schneider (1945), was compared with the method of Ogur & Rosen (1950). In addition, the extraction of the total nucleic acid with hot 10% NaCl soln. (see Ada & Perry, 1954; Martin & Morton, 1956*a*) was investigated (Fig. 2, Procedure *B*). The method described by Martin & Morton (1956*a*) in which DNA (and some RNA) is extracted with hot 10% NaCl soln. after extraction of most of the RNA with $N-HClO_4$ at 0–4° (see Fig. 2, Procedure *C*) was also used. Fig. 2 shows the modifications of the Ogur & Rosen (1950) procedure used in these analyses.

Table 2 shows the distribution of the organic

Table 1. *Chemical composition of disrupted sarcosomes from pig heart*

Results are the means of duplicate (Ogur & Rosen method) or quadruplicate (Procedure *A*) determinations on a preparation of disrupted sarcosomes from pig heart [Keilin & Hartree (1947) preparation]. All results are given as percentages of dry wt.

Determination	Results	
	Ogur & Rosen (1950) method (see Fig. 1)	Procedure <i>A</i> (see Fig. 1)
Total lipid	35.1	36.7
Lipid P	1.04	1.08
Lipid N	0.54	0.56
Phospholipid (P × 25)	26.0	27.0
Acid-soluble P	0.30	0.28
RNA phosphorus		
Direct	0.055	0.042
From absorption	0.049	0.035
'DNA' phosphorus		
Direct	—	0.046
From absorption	0.093	0.047
From deoxyribose	0.032	0.018
Total nucleic acid P*		
Direct	—	0.088
From absorption	0.142	0.082
Protein P	0.110	0.150,† 0.083‡
Total P (by determination)	1.78	1.78
Total P (by addition)	1.60§	1.68

* By addition of RNA phosphorus and DNA phosphorus.

† Supernatant after acidification of 0.3*N*-KOH extract.

‡ In *N*-NaOH extract.

§ 'DNA' phosphorus from absorption measurement was used in calculating this figure.

phosphorus of the microsomes. It is seen that most of the phosphorus occurs in the lipid and acid-soluble fractions. The total nucleic acid phosphorus, by all four methods, is very low. The results show that 0.5*N*- $HClO_4$ at 80° [Ogur & Rosen (1950) method] and 10% NaCl soln. at 100° (as used in Procedure *B*) extract phosphorus compounds which are not DNA, since the absorption measurements give values which are very much less than the direct determinations of phosphorus. The method of Schneider (1945) gave better results. However, the good agreement between the estimates obtained by absorption and by direct determination suggests that Procedure *C* is the most satisfactory analytical procedure. Although differential extraction of RNA and DNA was attempted, the material shown as 'DNA phosphorus' in Table 2 is almost certainly RNA phosphorus, since, as shown by Table 4, milk microsomes have no deoxyribose as estimated by the Ceriotti (1952) method.

Studies with liver microsomes. Since the milk microsomes were found to contain very little nucleic acid, before proceeding to a comparison of milk and gland microsomes it seemed desirable to investigate the suitability of the procedures for extraction of nucleic acid from microsomes rich in RNA. Liver microsomes were therefore used. In this study, Procedure *C*, which had been found to be most satisfactory with the milk microsomes (Table 2) and with plant microsomes (Martin & Morton, 1956*a*), was compared with the Schneider (1945) method. The results are shown in Table 3. It is seen that Procedure *C* gave very good agreement between the values for nucleic acid phosphorus as obtained from direct determination and from absorption measurements.

The Schneider (1945) method of analysis was less satisfactory than Procedure *C*. The material extracted by 5% trichloroacetic acid at 90° clearly contained phosphoprotein phosphorus in addition to nucleic acid phosphorus. The results indicated that Procedure *C* was satisfactory for a comparison of milk and mammary-gland microsomes. It appeared that the measurement of the deoxyribose content of the extracts by the Ceriotti (1952) method would give the best estimate of the DNA content of the microsomes.

Comparative chemical composition of milk and mammary-gland microsomes

Microsomes from the milk and mammary gland of the same cow were isolated from two different animals (cows nos. 1 and 2) as already described (Bailie & Morton, 1958), and the analyses were carried out by Procedure *C* (see Fig. 2). The results given in Table 4 show that the microsomes from the two sources differ considerably in that the

Table 3. *Analyses of microsomes from rat liver*

Microsomes were isolated as described in Methods. Suitable portions (about 100 mg.) of dried material were used for each analysis. All results are the means of duplicate determinations and are expressed as percentages of dry wt.

Determination	Results with various procedures	
	Schneider (1945) method	Procedure C (see Fig. 2)
Lipid P	0.81	0.77
Phospholipid (P × 25)	20.6	19.3
Acid-soluble P	0.027	0.053
RNA phosphorus		
Direct	—	0.31
From absorption	—	0.32
'DNA' phosphorus		
Direct	—	0.13
From absorption	—	0.12
Total nucleic acid P*		
Direct	0.528	0.44
From absorption	0.447	0.44
Protein P	0.087	0.24
Total P (by direct determination)	1.52	1.52
Total P (by addition)	1.45	1.50

* By addition of RNA phosphorus and DNA phosphorus.

gland microsomes contain considerably more lipid and nucleic acid phosphorus than do the milk microsomes from the same cow. The microsomes from mammary gland resemble those from liver (Table 3) more than those from milk (Tables 2 and 4).

It seemed possible that such a difference might arise by loss of components during the normal period of incubation of the milk in the lumina and ducts of the mammary gland after secretion from the cells. Therefore a portion of the microsomes from the gland of cow no. 2 was suspended in milk serum from the same cow (see Baillie & Morton, 1958, Fig. 3), and incubated at 38° in stoppered tubes containing chloroform vapour to restrict bacterial growth. After incubation for 12 hr., the particles were sedimented from the serum by centrifuging, washed with 0.15M-NaCl soln., and dried. The chemical composition of the particles was again determined as before. The results in Table 4 show that this treatment caused some loss of lipid and a considerable loss of nucleic acid. The gland microsomes, after incubation, very closely resemble the milk microsomes in chemical composition.

DISCUSSION

Extraction of nucleic acids from cytoplasmic particles

Although a large number of different methods have been used for estimation of nucleic acids of animal tissues, in few cases is there good evidence that the procedures give accurate values. Especially when the nucleic acid content of the material is low, as in some plant materials (Martin & Morton, 1956*a*) and in milk microsomes (Tables 2 and 4), it is necessary to avoid extraction of non-nucleic acid material if the nucleic acid is to be estimated accurately, either by determination of phosphorus or from absorption measurements.

Agreement between estimates of nucleic acids based on absorption at or near 260 m μ (due to purines and pyrimidines), nucleic acid sugar, and organic phosphorus was sought, and this criterion was used to assess the suitability of the various procedures for estimation of RNA and DNA. Estimation of RNA by measurement of ribose by the orcinol reaction (Schneider, 1945) was not used since Martin & Morton (1956*a*) and Ada & Perry (1954) had found this unsatisfactory with plant and virus materials respectively. Preliminary observations confirmed this finding with animal materials. However, the Ceriotti (1952) reaction for deoxyribose was found to be suitable for estimation of DNA.

As found by Martin & Morton (1956*a, b*) with plant materials, extraction of a portion of the nucleic acid with N-HClO₄ at 0–4°, and of the remainder with 10% NaCl soln. is generally more satisfactory than extraction by the Ogur & Rosen (1950) method (Tables 1 and 2), or extraction with trichloroacetic acid (Tables 2 and 3) as introduced by Schneider (1945). Martin & Morton (1956*a*) had earlier confirmed that the Schmidt & Thannhauser (1945) method is unreliable for estimation of RNA (see also Davidson, Frazer & Hutchison, 1951; Mitchell & Moyle, 1951; Davidson & Smellie, 1952; Drasher, 1953). Both the extracts obtained with N-HClO₄ and with 10% NaCl soln. (Fig. 2, Procedure C) had absorption spectra characteristic of nucleic acids, with well-defined maxima at about 259 m μ . Table 5 compares the characteristics of the absorption spectra of the extracts from mammary-gland microsomes (Table 4, cow no. 2) with those of nucleic acid from yeast. It is seen that the agreement is as good as could be expected for nucleic acids from different sources. Although the examination of the methods was not extended to estimation of the purine and pyrimidine bases (cf. Martin & Morton, 1956*a*), it is considered that Procedure C gives a reliable estimation of the distribution of organic phosphorus among the lipid, nucleic acid and protein components of microsomes from animal tissues.

Table 4. *Comparative chemical composition of microsomes from cow's milk and mammary gland*

Microsomes were isolated from the milk and the mammary gland of the same cow (see Bailie & Morton, 1958, Figs. 1 and 3). About 100 mg. portions of the dried material were used for each analysis. The results for the gland microsomes from cow no. 1 are the means of quadruplicate determinations. Results are also given for microsomes of cow no. 2 after incubation in milk serum for 12 hr. at 38° as described in the text. All results are expressed as percentages of dry wt., determined by Procedure C (Fig. 2).

Determination	Results				
	Cow no. 1		Cow no. 2		
	Gland microsomes	Milk microsomes	Gland microsomes	Milk microsomes	Gland microsomes after incubation
Total lipid	40.4	24.6	36.5	29.7	32.9
Lipid P	0.673	0.520	0.690	0.619	0.672
Phospholipid (P × 25)	16.9	13.0	17.3	15.5	16.8
Acid-soluble P					
Total P	0.48	0.12	0.107	0.157	0.284
Inorganic P	0.34	0.09	—	—	—
Organic P	0.14	0.03	—	—	—
Nucleic acid P					
(a) In N-HClO ₄ extract (RNA)					
Direct	0.627	0.017	0.533	0.171	0.102
From absorption	0.570	0.013	0.520	0.154	0.087
From deoxyribose	0.001	0	—	—	—
(b) In 10% NaCl extract (RNA and DNA)					
Direct	0.103	0.015	0.111	0.054	0.053
From absorption	0.059	0.017	0.102	0.050	0.045
From deoxyribose	0.014	0	—	—	—
RNA phosphorus*	0.045	0.017	—	—	—
Total RNA phosphorus†	0.615	0.030	—	—	—
Total nucleic acid P‡	0.629	0.030	0.622	0.204	0.132
Protein					
Nitrogen	4.8	7.4	—	—	—
Total P	0.058	0.110	0.153	0.073	0.154
Protein (N × 6.25)	30.0	46.5	—	—	—
Total P (by determination)	2.02	0.80	1.68	1.17	1.34
Total P (by addition)	1.94	0.78	1.59	1.07	1.27

* Difference between nucleic acid phosphorus (from absorption) and DNA phosphorus (from deoxyribose).

† Sum of RNA phosphorus in N-HClO₄ and 10% NaCl soln. extracts (from absorption measurements).

‡ From absorption.

Table 5. *Optical properties of nucleic acid extracted from mammary-gland microsomes with N-HClO₄*

The extract was obtained by Procedure C (Fig. 2) from microsomes from cow no. 2 (see Table 4).

Ratio of wavelengths (mμ)	Ratio of extinction values	
	Mammary-gland extract	Yeast nucleic acid
260/290	2.30	2.68
260/280	1.40	1.59
260/270	1.10	1.15
260/250	1.29	1.24
260/240	2.40	2.16

As found by Martin & Morton (1956a) with microsomes from plant tissues, microsomes from milk and mammary gland contain RNA which is not extracted by prolonged treatment with

N-HClO₄ at 0–4° but which is extracted with 10% NaCl soln. at 100°. This is probably true of liver microsomes also, but the deoxyribose contents of the extracts from these were not estimated. Thus animal tissues appear to contain two types of RNA, as already found for plant tissues (Stafford, 1951; Martin & Morton, 1956a). The proportion of RNA extracted by N-HClO₄ varies in different microsomes, being about 90% for gland (Table 4), about 60% for milk (Table 4) and wheat root (Martin & Morton, 1956b, Table 3), and about 50% for beet-petiole microsomes (Martin & Morton, 1956a, Table 4).

Chemical composition of microsomes from animal tissues

The results in Tables 3 and 4 show that microsomes from liver and from mammary gland are rather similar in chemical composition. Both have

a high proportion of phospholipid and of nucleic acid. Assuming that the phospholipids contain 4 % of phosphorus, and the nucleic acids 9.8 % of phosphorus, liver microsomes contain about 19 % of phospholipids and about 4.3 % of nucleic acid, and gland microsomes about 17 % of phospholipids and about 6.1 % of nucleic acids (all figures as percentage dry wt.; see Tables 3 and 4). The protein phosphorus of liver microsomes is rather higher (0.24 %, Table 3) than that of the gland microsomes (0.058 and 0.153 % for cows nos. 1 and 2 respectively; Table 4). Table 9 of Martin & Morton (1956*a*) shows that microsomes from lactating mammary gland have a higher nucleic acid content than microsomes from most other tissues so far examined.

Much of the rather high content of 'acid-soluble phosphates' in mammary-gland microsomes (0.48 and 0.107 % for cows nos. 1 and 2 respectively, as compared with 0.053 % in liver microsomes; see Tables 3 and 4) appears as 'inorganic phosphorus' as estimated by the Weil-Malherbe & Green (1951) procedure. Since the particles were washed well before drying, this 'inorganic phosphorus' must be firmly bound by the lipoprotein particles. The acid-soluble phosphates of washed beet-petiole microsomes also contain a large amount of inorganic phosphorus (Martin & Morton, 1956*a*, Table 4). It is possible that the inorganic phosphorus is derived from very acid-labile phosphorylated compounds. Since the 0.2*N*-HClO₄ extracts of the gland microsomes had very little absorption at or near 260 mμ, nucleotides represent only a small proportion of the acid-soluble phosphates. The nature of the organic compounds in this fraction from microsomes is not yet known, and these may be worthy of further study particularly in relation to the incorporation of amino acids by microsomes (see, for example, Littlefield, Keller, Gross & Zamecnik, 1955).

As shown by the results in Table 1, disrupted mitochondria (sarcosomes) may resemble microsomes in chemical composition, although containing much less nucleic acid.

Relationship between microsomes of milk and mammary gland

Tables 2 and 4 show that the chemical composition of microsomes from milk differs considerably from the microsomes from mammary gland. Compared with the microsomes from the glands, as shown in Table 4, those from the milk of the same cow have much less lipid [40.4 and 24.6 % (cow no. 1); 36.5 and 29.7 % (cow no. 2)], rather less lipid phosphorus [0.673 and 0.520 % (cow no. 1); 0.690 and 0.619 % (cow no. 2)], and considerably less nucleic acid phosphorus [0.629 and 0.030 % (cow no. 1); 0.622 and 0.204 % (cow no. 2)]. The microsomes isolated from mixed herd milk (Table 2)

have even less lipid phosphorus (0.34 %) and nucleic acid phosphorus (0.022 %).

This marked difference in chemical composition may suggest that milk microsomes are unrelated to gland microsomes. However, Table 4 shows that incubation of gland microsomes in milk serum causes some loss of lipid and phospholipid and considerable loss of nucleic acid, so that the product has a composition very similar to that of the microsomes from the milk of the same cow. In the preceding paper (Baillie & Morton, 1958) it was shown that the same incubation in milk serum caused a change in enzymic activities to a pattern characteristic of milk microsomes. The retention of alkaline phosphatase, increase of xanthine dehydrogenase, decrease of DPNH-diaphorase and of DPNH-cytochrome *c* reductase, substantial denaturation of cytochrome *b₅*, decrease of phospholipid and substantial loss of nucleic acid which occur on incubation of microsomes from mammary gland give a product which closely resembles milk microsomes. Although, under the experimental conditions used, the gland microsomes were not converted into a product identical with the milk microsomes, nevertheless the trend of the change was so pronounced that it is concluded that the milk microsomes are formed from gland microsomes after secretion of the milk.

It is probable that the changes produced in the chemical composition of gland microsomes on incubation are due to the activities of enzymes of the microsomes themselves, possibly acting together with enzymes of the milk serum. Milk phosphodiesterase (Zittle & Della Monica, 1952), together with the phosphomonoesterase (Morton, 1953*a*, 1955*b*), could cause depolymerization and dephosphorylation of nucleic acids. The same enzymes could also cause dephosphorylation of phospholipids. It would not be surprising if gland microsomes also contained, in addition to xanthine dehydrogenase, other enzymes concerned with metabolism of nucleosides or free bases derived from nucleic acids.

Milk microsomes appear to vary considerably, both in enzymic and chemical properties. The milk microsomes isolated from cow no. 2 had greater alkaline phosphatase activity (see Baillie & Morton, 1958, Table 2) and higher nucleic acid content (this paper, Table 4) than those from cow no. 1, and about ten times the nucleic acid content of microsomes from mixed herd milk (see Tables 2 and 4). Zittle, Della Monica, Custer & Rudd (1956) also found that the nucleic acid of microsomes from commercial mixed milk was very low. In earlier studies, Morton (1954) had found that microsomes from Jersey milk had quite a high nucleic acid content. However, the analytical methods used by Morton (1954) were much less reliable than those

used in the present study. The lipids were extracted with butan-1-ol only, and since this solvent extracts mainly the phospholipids, but not neutral fats, this could account for the low total lipid content found by Morton (1954). The nucleic acids were extracted with N-HClO_4 at 80° for 15 min., a procedure now known to extract organic compounds (other than nucleic acids) which makes the value for nucleic acid phosphorus, as calculated from the absorption at $262 \text{ m}\mu$, higher than the true value. Nevertheless, the milk microsomes first isolated by Morton (1953b, 1954) probably contained more phospholipid and nucleic acid than those examined in the present investigation.

The variation in chemical composition and enzymic activities of milk microsomes probably reflect changes in the enzymic activities and chemical composition of the gland microsomes. These might be expected to alter with the stage of lactation. The variability of milk microsomes is consistent with their origin from gland microsomes.

Origin of microsomes from mammary gland and from milk

It is now established that microsomes are very largely derived from the endoplasmic reticulum of animal cells (Palade & Siekevitz, 1956; Hodge *et al.* 1956; Hodge & Morton, 1956) and of plant cells (Hodge & Morton, 1956; Hodge, Martin & Morton, 1957). Electron micrographs of thin sections of mouse mammary gland (Hodge & Morton, 1956; M. J. Bailie, A. J. Hodge & R. K. Morton, unpublished work) have shown that the endoplasmic reticulum of the secretory cells is rather similar to that of the secretory cells of the pancreas, as studied by Palade (1956). The 'rough membranes', consisting of lipoprotein with associated electron-dense nucleoprotein particles, are particularly prominent. However, areas of 'smooth membranes', 'Golgi zones' and secretory granules are also present (A. J. Hodge & R. K. Morton, unpublished work). It is probable that all of these portions of the cytoplasm contribute to the microsome fraction as studied here, which therefore cannot be homogeneous. It is very probable that the milk microsome fraction is also non-homogeneous.

The evidence already presented strongly suggests that the milk microsomes are derived from the secretory cells of the gland. There are at least two possible ways in which the microsomes may be released into the milk. Degeneration of certain secretory cells would be accompanied by disorganization so that the cytoplasmic contents would pass through into the collecting ducts of the gland. In this case, the microsomes derived at any one time would arise from a comparatively small proportion of the total number of secretory cells. It would be expected that the number of secretory cells which

are degenerating would increase towards the end of lactation, so that the amount of microsomes in milk would be expected to be greater during late lactation, and less during early lactation. A limited number of observations suggest that this occurs. Alkaline phosphatase activity of milk (which is correlated with the number of microsomes in milk) increases markedly towards the end of lactation. It is also relatively high in colostrum. If the hormonal factors also influence the relative proportion of secretory cells undergoing degeneration, this could account for the finding of Chanda & Owen (1952) that thyroxine treatment decreases, and thiouracil treatment increases, the alkaline phosphatase activity of milk.

However, the microsomes may arise from all secretory cells, rather than from a small proportion. Evidence on this point is lacking. It is possible, however, that the so-called 'secretory granules' normally pass through the basement membrane of the cell together with particulate protein such as casein. Schneider & Hogeboom (1956) have already suggested this as the origin of the milk microsomes described by Morton (1953b, 1954).

Palade (1956) has recently described electron-dense particles which occur in the sacs of the endoplasmic reticulum of the exocrine cells of the pancreas. These appear and disappear with different stages of secretion. It is possible that milk microsomes are derived from such granules, since the fine structure of the secreting mammary gland is rather similar to that of the exocrine cells of the pancreas.

If the suggestions as set out above are correct, it would be expected then that microsomes isolated from the milk of animals other than the cow would differ little from the microsomes from the mammary gland of the same animal. Particularly when young are suckling, the milk will be withdrawn from the gland very soon after secretion. There will therefore be much less time for enzymic and other changes and the material in the milk should therefore closely resemble the mammary-gland microsomes. Since the present work was initiated in relation to the problem of the 'milk factor', which accelerates tumour development in mice (see Morton, 1953b; Bailie & Morton, 1958), this implies that the milk factor may be carried into the young almost unaltered from its state in the mammary gland. There is every possibility that the milk factor is a constituent normally associated with the endoplasmic reticulum of the gland cells.

SUMMARY

1. Several methods for estimation of the nucleic acid content of cytoplasmic particles from animal tissues were investigated. A procedure is described

which gave satisfactory results on the basis of agreement between estimates based on absorption at 260 m μ and organic phosphorus content.

2. The chemical composition of a preparation of disrupted sarcosomes from pig heart was determined.

3. Microsomes from rat liver and from mammary gland of the lactating cow were found to contain relatively high proportions of phospholipid and of nucleic acids, whereas microsomes from cow's milk contained much less lipid and considerably less nucleic acid.

4. Incubation of mammary-gland microsomes in milk serum caused some loss of lipid and considerable loss of nucleic acid, so that the product had a composition closely resembling that of microsomes from milk.

5. The significance of these results in relation to the origin of milk microsomes is discussed.

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Effect of Sulphanilamide on Formation of Sterols in *Saccharomyces cerevisiae* and *Neurospora crassa*

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Dietary administration of sulphonamides has been shown to produce deficiencies of various vitamins in experimental animals. In particular, sulphaguanidine and succinylsulphathiazole have been widely used for inducing a deficiency of folic acid in rats. In the course of an investigation on the relationship between folic acid and coenzyme A, Popp & Totter (1952) observed that the addition of succinylsulphathiazole to an otherwise normal diet resulted in a significant increase in the liver content

of coenzyme A in rats. The increase was taken to represent the response of the animal to an enhanced demand for coenzyme A required for the acetylation of the free sulphonamide released by the breakdown of absorbed succinylsulphathiazole.

Klein & Lipmann (1953a, b) have demonstrated the dependence on coenzyme A of sterol synthesis in both yeasts and rats and its sensitivity to changes in the tissue levels of the coenzyme. In the present studies it has been found that there is a significant