4. Swelling did not occur in the presence of AMP plus dinitrophenol, although oxidative phosphorylation was prevented.

5. It is concluded that the secretory action of mitochondria is dependent on active metabolism in the presence of an external adenine nucleotide and is linked with the capacity for oxidative phosphorylation in some way at present unknown.

We are indebted to Messrs Roche Products Ltd., for the gift of crystalline synthetic adenylic acid.

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Variations in the Ionic and Lactose Concentrations of Milk

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The ionic composition of cows' milk differs markedly from that of the blood serum; in milk the concentrations of sodium and chloride are lower than in blood, and the concentration of potassium is higher. This difference is greatest at the beginning of lactation, but later the ionic composition of milk tends towards that of blood: the sodium and chloride concentrations rise, while the potassium concentration falls. The lactose concentration of milk also falls as lactation advances. Milk samples from cows with mastitis show changes in composition similar to those found in advanced lactation. It has been shown that there is a relationship with a high correlation coefficient between the sodium and chloride concentrations in milk samples taken at any time in lactation, which can be expressed by a linear regression equation (Jones & Davies, 1935). There is a similar relationship between the lactose and chloride concentrations (Mathieu & Ferre, 1914).

In this paper the results are presented of a more detailed study than has been made previously of these changes in milk composition. First, the sodium, potassium and chloride concentrations in milk samples from thirty-eight cows in different stages of lactation were determined together with the sodium, potassium and chloride concentrations in blood serum samples from some of the same cows. From the results it is shown that there are relationships with high correlation coefficients between the potassium concentrations of these milk samples and their sodium and chloride concentrations which can be expressed by linear regression equations. These milk samples were all taken from separate cows with blood sera of slightly differing composition. The relation between the changes in milk composition and the composition of blood serum can be more clearly shown by analysing milk from the separate quarters of individual cows, since all four samples are formed from a common blood supply. Milk samples from the separate quarters of a cow are normally almost uniform in composition, but when a quarter is infected with mastitis organisms, the composition of its milk changes to an extent depending on the severity of infection. Secondly, therefore, the changes in milk composition were studied by analysing milk samples from the separate quarters of cows with mastitis, and blood samples from the same cows.

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EXPERIMENTAL

Sampling of blood and milk

The first series of milk samples were from thirty-eight healthy cows and were taken from the total milk yielded by each cow at a single milking. Since the concentrations of sodium, potassium and chloride in the blood serum vary little from cow to cow, blood samples were taken from only a few of the cows. They were drawn from the jugular vein immediately after collecting the milk, kept at 37° for 1 hr., and the serum then separated by centrifugation.

The milk samples (150 ml.) from the separate quarters were drawn at the beginning of normal milking. For this study it was necessary to find cows giving milk of different composition from each quarter, so a rapid survey was made of the quarter-samples from a large number of cows with mastitis by determining the chloride concentration of each. Seven of these cows were then selected for detailed analysis of their milk and blood. Immediately after milking, blood samples were taken and the serum separated as before.

Analytical methods

Sodium in milk was determined gravimetrically by the zinc uranyl acetate method (Barber & Kolthoff, 1928). Before precipitating the triple salt, the phosphate of milk, which would form a precipitate of uranyl phosphate with the reagent, and the protein and fat must be removed. Normally these are removed in two stages: protein and fat by wet ashing or precipitation, and phosphate by precipitation as zinc or calcium phosphate. For this work a more rapid and satisfactory procedure was devised in which the protein, fat and phosphate are removed in a single precipitation with uranyl acetate and acetic acid. Uranyl acetate has been previously used to remove phosphate from urine before sodium determination (Jendrassik & Halász, 1938), and also to remove protein and fat from milk before determining chloride (Kopatschek, 1922). Sodium in blood serum was also determined gravimetrically by the zinc uranyl acetate method, after removing proteins by precipitation with trichloroacetic acid.

Potassium in milk was determined gravimetrically by a sodium cobaltinitrite method (Piper, 1934), on the filtrate obtained after removing proteins and fat by precipitation with trichloroacetic acid. Potassium in blood serum was determined by an improved absorptiometric sodium cobaltinitrite method which has been published recently (Barry & Rowland, 1953).

Chloride in milk was determined by the method of Davies (1932), and chloride in blood serum by Whitehorn's application of the Volhard titration (Peters & Van Slyke, 1932).

Lactose was determined by a modification of the chloramine-T method of Hinton & Macara (1927).

RESULTS

Composition of the milk of individual cows

The relationships between the sodium and chloride, sodium and potassium, and potassium and chloride concentrations in thirty-eight milk samples from cows at various stages of lactation are shown graphically in Fig. 1, and the analyses which were made on blood serum from these cows are also plotted. The straight lines were drawn from linear regression equations, calculated from the milk analyses by the method of least squares. The regression equations, the standard errors of the regression coefficients, and the correlation coefficients, are shown in Table 1. For the components of a biological system the correlation coefficients are surprisingly high. A similar relationship between sodium and chloride has been shown before by Jones & Davies (1935), although our equation for the regression of sodium on chloride differs slightly from their equation of $Cl = 1{\cdot}24\,Na + 18{\cdot}09$. This equation has often been used for calculating the approximate sodium concentration in a milk sample from the more easily determined chloride. If the sodium, potassium or chloride concentration of a milk sample is known it is now possible, using the equations in Table 1, to calculate the approximate concentrations of the two other elements. These relationships, which were derived for normal lactation, also apply to mastitis milk as is shown below.

Composition of milk from the separate quarters of the cow

The relationships between the sodium, potassium and chloride concentrations in the quarter samples from a typical one of the seven cows which were selected because they gave milk of different composition from each quarter, are shown in Fig. 2, and the blood-serum analyses are also plotted. The correlations are similar to those found in the milk samples from individual cows but are much more precise, the points lying almost exactly on straight lines. The relationships between the lactose and the sodium, potassium and chloride concentrations in the same milk samples are also shown. The blood-serum analyses have been plotted assuming that the reducing-sugar concentration of the serum corresponds to 0·10% lactose (equivalent to 0·05% glucose which is approximately the normal value). Similar precise correlations were found in the quarter samples of the remaining six cows studied in this way, although the regression equations were slightly different from cow to cow; the points always fell amost exactly on straight lines, and the blood analyses lay in similar positions relative to the lines. Therefore, each cow has apparently its own regression equation depending on its blood composition, but since the blood composition varies only slightly from cow to cow, milk samples from a large number of cows give the almost linear relationships shown in Fig. 1.

DISCUSSION

Figs. 1 and 2 show that the composition of normal milk early in lactation lies near the point A, and that late in lactation or in mastitis it approaches B, thus

Table 1. *The correlation of sodium, potassium and chloride in the milk of cows at various stages of lactation*

Correlation between	Regression equations	Standard error of regression coefficients	Correlation coefficients
Na and Cl	Cl = 0·924 Na + 54·6	$5·50 \times 10^{-3}$	0·98
	Na = 1·032 Cl − 52·6	$6·17 \times 10^{-3}$	
Na and K	K = −0·570 Na + 183·5	$5·00 \times 10^{-3}$	−0·95
	Na = −1·59 K + 298·8	$1·39 \times 10^{-2}$	
K and Cl	Cl = −1·53 K + 339·5	$1·067 \times 10^{-2}$	−0·97
	K = −0·613 Cl + 216·6	$4·37 \times 10^{-3}$	

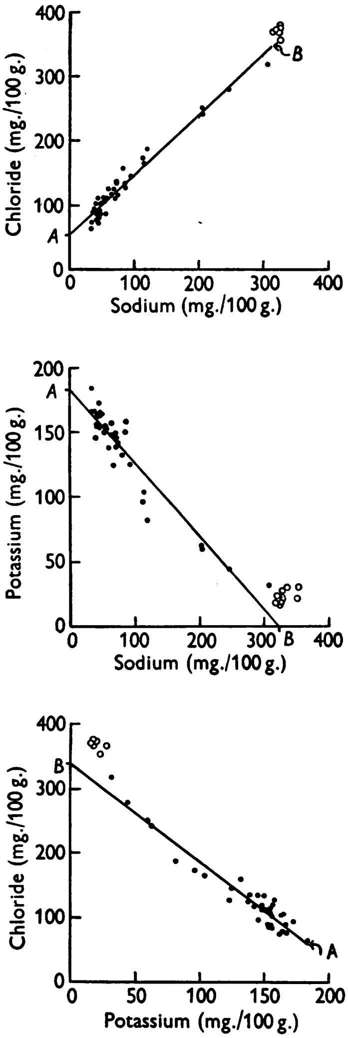

Fig. 1.

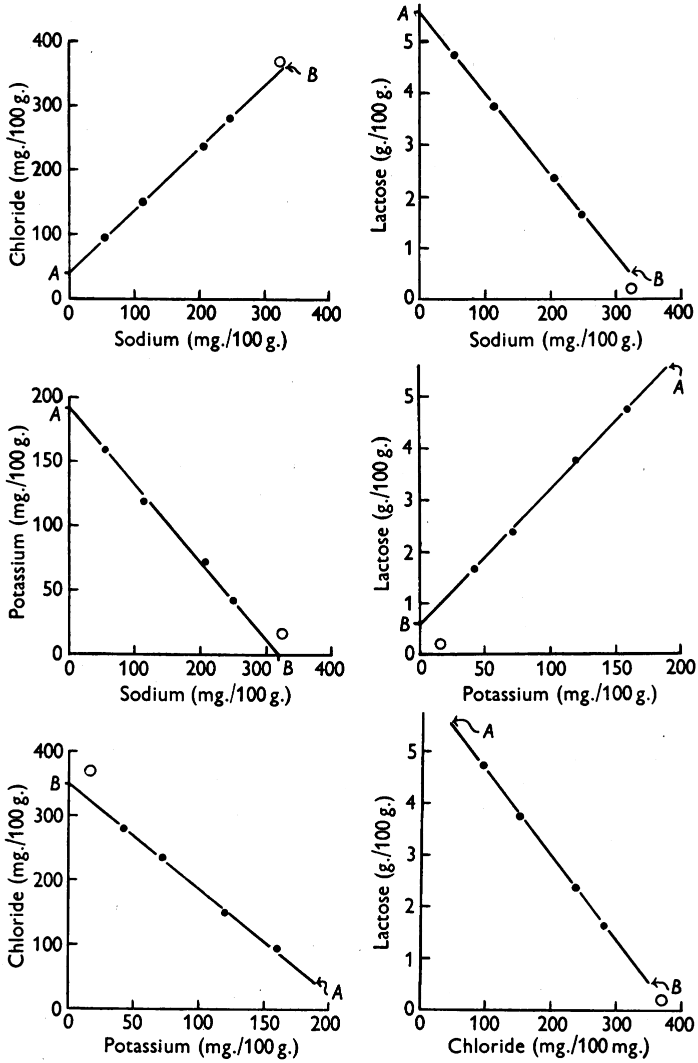

Fig. 2.

Fig. 1. Relationships between the Na, K and Cl concentrations in milk samples from individual cows and in blood plasma samples from some of the same cows. Milk composition is represented by solid circles and plasma composition by hollow circles.

Fig. 2. Relationships between the Na, K, Cl and lactose concentrations in milk samples from the separate quarters of a single cow and in the blood plasma of the same cow. Milk composition is represented by solid circles and plasma composition by hollow circles.

tending approximately towards the composition of blood serum. These changes can be considered as follows. Suppose two solutions whose compositions are represented by the points A and B (Figs. 1 and 2) are mixed in varying proportions; then the composition of any of these mixtures will also lie on the straight lines AB. Therefore, the composition of all milk samples could result from the mixing of a 'true milk' whose composition is constant and lies on AB near A, with a diluent whose composition is also constant and lies on AB near B; mixtures of 'true milk' and diluent in all proportions will lie on the straight lines AB. It can be seen that the diluent would differ slightly in composition from blood serum, since the serum analyses do not lie on the lines AB. The composition of the diluent cannot be calculated without knowing the concentration of one of its constituents. But since its potassium concentration cannot be less than zero, Figs. 1 and 2 show that its sodium and chloride concentrations during normal lactation and mastitis could not be greater than about 320 and 340 mg./100 g. respectively. The concentrations of sodium and chloride in the diluent would thus be slightly lower than in normal transudates.

There are two distinct mechanisms by which these variations in milk composition could be produced within the mammary gland. First, in advanced lactation and during mastitis, the milk secreted by the alveolar epithelial cells may change in composition; that is, the hypothetical mixing of 'true milk' and diluent occurs within the epithelial cells. This is the generally accepted mechanism (Turner, 1946). Secondly, throughout lactation and during mastitis, the epithelial cells may secrete a milk in which the concentrations of sodium, potassium, chloride and lactose are constant, and which, after secretion, is mixed with a diluent in which the concentration of these components is approximately the same as in blood serum. A similar mechanism has been suggested previously from other evidence (Davies, 1933; Peskett & Folley, 1933). The diluent, for example, might be a transudate from blood serum which continually filters into the lumen of the alveolus without passing through the epithelial cells; when the volume of milk secreted by the epithelial cells is depressed during late lactation or mastitis or certain artificial treatments (Turner, 1946), the proportion of transudate in the milk withdrawn from the mammary gland will rise.

There is too little evidence to decide which of these is the correct mechanism, but the following fact can be explained better by the second. The sodium, potassium and chloride concentrations in milk early in lactation are similar to those in most body cells: the potassium concentration is much higher than in blood serum and the sodium and chloride concentrations are lower (Shohl, 1939). The reason for this may be that the alveolar epithelial cells must secrete a fluid whose ionic composition is similar to that of their normal cell contents; if this is true, it would be expected that the ionic composition of this fluid would be maintained throughout the life of the cell.

SUMMARY

1. A study has been made of the variations in the sodium, potassium, chloride and lactose concentrations of milk and their relation to the composition of blood serum.

2. The linear regression equation relating the sodium and chloride concentrations in any milk sample is confirmed, and it is shown that the potassium and sodium, and the potassium and chloride concentrations are related by similar equations.

3. From analyses of blood and milk samples it is shown that the composition of all milk samples studied could result from the mixing, in varying proportions, of a milk similar to that produced early in lactation, with a fluid differing slightly in composition from blood serum.

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