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Changes in the Water, Sodium and Potassium Content of Rat-liver Mitochondria during Metabolism

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Observations on the osmotic properties of mitochondria and their appearance in the phase-contrast and electron microscope suggest that these particles are surrounded by a membrane (Lazarow, 1943; Claude, 1944; Opie, 1948; Claude & Fullam, 1945; Zollinger, 1948; Dalton, Kahler, Kelly, Lloyd & Striebich, 1949). The existence of a selectively permeable membrane is borne out by the relative inaccessibility of the acid phosphatase of intact liver mitochondria to β-glycerophosphate, though the enzyme is readily demonstrable after lysis (Berthet, Berthet, Appelmans & Duve, 1951) and the fact that this phosphatase also becomes accessible after treatment with *Clostridium welchii* toxin suggests that the permeability is connected with the presence of lecithin (Datta & Macfarlane, unpublished).

Harman (1950) cast doubt on the existence of a semi-permeable membrane, since he found no selective penetration nor any concentration of cations in mitochondria present in cyclophorase preparation which had been oxidizing α-ketoglutarate. The demonstration of a concentration gradient, however, necessitates the separation of the mitochondria by centrifugation, and if during this time the metabolic process which is presumably necessary to maintain the gradient diminishes or stops it is probable that the gradient would be rapidly abolished. The conditions used by Harman were not optimal for the maintenance of metabolism; for example, centrifugation was carried out at 0°. We have carried out experiments with rat-liver mitochondria suspended in different media and subsequently centrifuged at room temperature, and have obtained evidence of the ability of the mitochondria to maintain a concentration of sodium and potassium ions above that of the surrounding medium; this ability appears to be linked with their capacity for oxidative phosphorylation and with the prevention of swelling.

After our experimental work was concluded, Bartley & Davies (1952) reported experiments demonstrating that mitochondria from sheep-kidney cortex could maintain concentration differences of inorganic and organic ions with the external fluid, in conditions similar to our own.

METHODS

Preparation of mitochondria. This was carried out in a room at 2–4° with chilled materials. Liver tissue, excised from rats immediately after death by bleeding out, was blotted with filter paper and weighed. Portions up to 5 g. at a time were ground in a precision-worked steel pressure mincer (Craigie, 1949), whose essential feature is a finely grooved plunger which operates in a closed cylinder; as the plunger is moved down the tissue is forced upwards through the grooves and efficiently pulped by a combination of cutting and shearing action. Much of the fibrous matter is caught in the grooves but almost all the pulp is forced to the top of the plunger and readily recovered by washing out with an appropriate liquid.

The liver pulp was suspended in 0·25 M-sucrose solution (Schneider, 1948) (10 ml./g. tissue), strained through fine muslin and centrifuged in 15 ml. pots at 2–4° for 30 min. at approximately 300 *g* to remove nuclei, red cells and unruptured liver cells. The supernatant was removed with a pipette and spun in a Servall angle centrifuge at approx. 7000 rev./min. for 30 min.; the supernatant and free-flowing layer of the deposit were removed, and the packed mitochondria resuspended in 0·25 M-sucrose or other liquids for washing or use as desired. The preparations were examined microscopically to check the absence of cells and nuclei. The separation of cell constituents by this technique, without washing, was fairly sharp judged by the succinoxidase activity, which is predominantly localized in the mitochondria (Hogeboom, Schneider & Pallade, 1948); about 10% of the original activity was found in the nuclear fraction, 65% in the mitochondrial suspension, and 5–10% in the supernatant containing the soluble constituents and microsomes.

Separation and analysis of mitochondria. After incubation of the mitochondria in various conditions described below, the mixtures (usually 24 ml.) were transferred to 50 ml. pots and spun in the Servall angle centrifuge at room temperature for 20 min. Longer spinning did not appreciably affect the degree of packing judged by the water content. The supernatant was removed, the last drops by draining and wiping with filter paper, and samples of the deposit immediately removed to tared silica crucibles with lids. After weighing and drying to constant weight at 105° to obtain the water content of the deposit (regarded as the mitochondrial water) the material was ashed overnight in an electric furnace at approx. 500°, and the ash dissolved in 10 ml. 0·3 N-H_2SO_4. Measured samples of the supernatant fluid were dried and ashed similarly. Glass-distilled water was used for the preparation of reagents and rinsing of apparatus.

The Na and K contents were estimated by the flame photometer (Spencer, 1950).

Other methods. Phosphorus estimations were made by a colorimetric method (Martland & Robison, 1926) to determine the following fractions: total P after ashing; apparent orthophosphate by direct estimation; acid-labile P by hydrolysis in N-HCl at 100° for 7·5 min. In several experiments the orthophosphate was also determined by estimation of the amount precipitable with $CaCl_2$ in 10% ethanol, to detect any phosphocreatine-like ester, but none was found.

Oxygen uptake was measured in a Warburg manometric apparatus, usually at 28° in air with 3·0 ml. reaction mixture, 0·5 ml. 20% (w/v) trichloroacetic acid (TCA) in the side arm and 0·3 ml. 2 N-NaOH in the centre cup. Readings were begun after 10 min. equilibration.

Materials. Cytochrome *c* was prepared according to Keilin & Hartree (1945). Crystalline synthetic adenosine 5′-phosphate (AMP) neutralized with NaOH was used.

RESULTS

Distribution of sodium and potassium in liver fractions

Preliminary experiments were made to establish the reproducibility of results with the methods used, and the range of values in different fractions. The results of two experiments are shown in Table 1. Two points are of interest. Firstly, only about 60% of the sodium but about 90% of the potassium in whole liver tissue (dried and ashed without grinding) was found in the liver pulp, indicating that a high proportion of the sodium is held in the connective tissue fibres. Secondly, the concentrations of sodium and potassium in the mitochondrial water were higher than in the supernatant liquid, i.e. a gradient had been maintained during the preparation. The supernatant is of course not a natural fluid, but a mixture of the extracellular and intracellular fluids diluted in sucrose solution.

Changes in the water, sodium and potassium content of mitochondria during oxidation of glutamate

It is well known that isolated mitochondria in presence of magnesium ions, inorganic phosphate and an adenine nucleotide such as adenosine triphosphoric acid (ATP) can oxidize metabolites of the Krebs tricarboxylic acid cycle with a coupled synthesis of high-energy phosphate compounds, the phenomenon being known as oxidative phosphorylation. It was expected that such a medium, which can support other synthetic activities, e.g. synthesis of *p*-aminohippuric acid (Kielley & Schneider, 1950), would be necessary for secretory activity. Kielley & Kielley (1951) showed that AMP can act as an external phosphate acceptor for the esterifica-

Table 1. *Distribution of sodium and potassium in rat-liver fractions*

(For details see text.)

	Na				K			
	mg./g. fresh tissue		m-equiv./l. water in fraction		mg./g. fresh tissue		m-equiv./l. water in fraction	
Sample	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
A, Fresh liver	1·46	1·36	90·6	84·5	4·21	4·47	154	164
B, Liver pulp	0·87	0·85	—	—	4·0	3·94	—	—
C, Fractions of *B*: nuclei and cells	0·036	0·052	5·8	—	0·15	0·27	14·4	—
Mitochondria	0·034	0·045	11·3	14·7	0·20	0·23	39·8	44·8
Supernatant	0·74	0·58	3·2	2·7	3·18	2·91	8·2	8·0

tion of inorganic phosphate to ATP and adenosine diphosphoric acid (ADP), and it was decided to use this simple system in the first place, with L-glutamate as substrate, and to consider the concentration of acid-labile P as a measure of the capacity of the system for oxidative phosphorylation.

The design of these experiments was limited by the time required for separating the mitochondria by centrifugation; with the Servall angle centrifuge used, holding 3 × 50 ml. pots, 20 min. was required for good packing. It was therefore not possible to follow the rapid initial changes on mixing the mitochondria with the medium, nor to take samples at less than 20 min. intervals. It is obvious that the oxygenation conditions are not optimal during centrifuging, and reversal may occur. Generally the experiments were arranged so that comparison could be made after a given time between the composition of mitochondria incubated in media identical save for the presence or absence of AMP, as well as between the mitochondria and the external medium.

Sucrose-low salt medium. Exp. 74. Unwashed mitochondria (10 ml., 17 mg. N) in 0·25 M-sucrose were added last to 20 ml. medium in each of five 250 ml. conical flasks (*A–E*), the final composition being: sodium L-glutamate, 0·02 M; $MgSO_4$, 0·002 M; potassium phosphate, pH 7·4, 0·01 M; cytochrome *c*, 2×10^{-5} M; calc. m-equiv./l.: Na, 20; K, 18. The flasks *A*, *B* and *C* also contained 0·001 M-AMP. Samples (3·0 ml.) were removed for estimation of O_2 uptake, and the open flasks rocked at 28°. After the desired time, a sample (3·0 ml.) was removed into 0·5 ml. cold 20% (w/v) TCA, for estimation of acid-soluble phosphates. The remainder (24 ml.) was centrifuged for 20 min.: the supernatant was removed and measured, samples being taken for analysis, and the deposit of mitochondria weighed, dried and ashed.

The results are shown in Table 2. In the mixtures containing AMP there was a rapid esterification of orthophosphate to acid-labile P, and a decrease in the water content of the mitochondria followed by an increase in the sodium and potassium concentration. The acid-labile P, however, decreased as the incubation proceeded, and after 60 min. the water content was increasing and the sodium and potassium concentrations approaching that of the external fluid. The samples containing AMP in the manometric flasks were at this time still taking up oxygen at the initial rate (41 μl./mg. N/10 min.), but the uptake in the conditions of the experimental flasks may have been different. After 10 min. incubation there was an appreciable difference between the acid-labile P of the whole mixture and that of the supernatant subsequently separated by centrifuging, but at later stages the two values were practically the same. This suggested that, though some decrease in acid-labile P might take place during centrifuging, part of the initially formed acid-labile P was attached to the mitochondria, and that this part decreased during incubation more rapidly than that in the medium.

This was also indicated in another similar experiment, in which the acid-labile P was determined in the trichloroacetic acid filtrates from the whole mixture, and from the supernatant and deposited mitochondria. After incubation with AMP and L-glutamate for 10 min. at 28°, the acid-labile P in the whole mixture, the supernatant and deposit was 2·2, 1·8 and 0·11 mg., respectively; after 30 min. incubation the corresponding figures were 2·3, 2·2 and 0·03 mg.

Variation in potassium and sodium concentration of medium. Exp. 66. Unwashed mitochondria (10 ml., 19 mg. N) in 0·25 M-sucrose were added last to 20 ml. medium containing L-glutamate, $MgSO_4$, cytochrome *c* and AMP as before and varying amounts of Na and K as phosphate buffer, pH 7·4. Samples (3 ml.) were taken for the manometric flasks and the remainder incubated for 30 min. and the mitochondria separated and analysed as before.

The results, shown in Table 3, are similar to the previous experiment, but it may be noted that the rate of oxygen uptake was lower with the higher potassium concentration in the external medium. In other experiments in which the concentrations of sodium and potassium were varied by the addition of sodium chloride or potassium chloride, the rate of oxygen uptake was also lower when the potassium/sodium ratio of the suspending fluid was very high.

Table 2. *Sodium and potassium concentration in mitochondria oxidizing* L-*glutamate in a sucrose-low salt medium*

(The basal medium was the same in all flasks; flasks *A*, *B* and *C* contained also 0·001 M-AMP. The calculated concentrations were checked by analysis of supernatants from *A* and *D*. For further details see text.)

Flask	AMP	Incubation (min.)	Acid-labile P (mg./30 ml.) Suspension	Acid-labile P (mg./30 ml.) Supernatant	Mitochondria Dry wt. (%)	Mitochondria m-equiv./l. water Na	Mitochondria m-equiv./l. water K	Supernatant m-equiv./l. water Na	Supernatant m-equiv./l. water K
A	+	10	1·32	0·93	20·2	29	29	26	18
B	+	30	0·78	0·75	20·5	52	68	—	—
C	+	60	0·39	0·40	19·0	24	31	—	—
D	0	10	0·06	0·06	12·5	26	30	22	16
E	0	30	0·06	0·06	11·6	22	22	—	—

Table 3. *Variation of sodium and potassium concentrations in external medium*

(For details see text.)

Flask	Phosphate concn. (M × 10^{-2})	O_2 uptake (μl./10 min./ mg. N)	Mitochondria (*M*) Dry wt. (%)	Mitochondria (*M*) m-equiv./l. water Na	Mitochondria (*M*) m-equiv./l. water K	Supernatant (*S*) m-equiv./l. Na	Supernatant (*S*) m-equiv./l. K	Ratio of concn. *M*/*S* Na	Ratio of concn. *M*/*S* K
A	1	62	19·3	39	37	24	16	1·6	2·3
B	2·5	41	21·8	*	96	2	60	—	1·6
C	2·5	52	21·2	82	12	64	6	1·3	2·0

* Too low for estimation.

Table 4. *Sodium and potassium concentrations in mitochondria oxidizing* L-*glutamate in a potassium chloride medium*

(The basal medium was the same in all flasks; flasks *A*, *B*, *C* and *D* contained also 0·0015M-AMP. The calculated concentrations were checked by analysis of supernatants from *A* and *E*. For further details see text.)

Flask	AMP	Time (min.)	Acid-labile P in supernatant (mg./30 ml.)	Mitochondria Dry wt. (%)	Mitochondria m-equiv./l. water Na	Mitochondria m-equiv./l. water K	Supernatant m-equiv./l. Na	Supernatant m-equiv./l. K
A	+	10	2·0	19·1	46	72	35	53
B	+	30	1·8	18·8	50	75	—	—
C	+	60	1·6	18·1	51	82	—	—
D	+	90	1·5	17·7	46	65	—	—
E	0	10	Nil	12·0	35	61	35	53
F	0	30	Nil	10·6	37	59	—	—

KCl *medium*. In other experiments, similar in design but using a medium like that of Kennedy & Lehninger (1949), the mitochondria were washed once in 0·25M-sucrose and resuspended in 0·15M-KCl. The final concentrations were: KCl, 0·05M; sodium L-glutamate, 0·02M; $MgSO_4$, 0·005M; Na_2HPO_4-KH_2PO_4 (pH 7·4), 0·01M; cytochrome *c*, 3×10^{-5}M; AMP (when present) 0·0015M. Calc. Na, 39; K, 52 m-equiv./l.

The results of one such experiment are shown in Table 4, and are similar to those of Exp. 74.

The initial water content of mitochondria deposited from the sucrose solution was about 83%; on incubation with glutamate, but without AMP, there was usually no oxygen uptake, and the water content increased to 88–90% while the sodium and potassium approached that of the medium; occasionally with unwashed mitochondria there was a slight oxygen uptake, the concentration gradient was maintained and the water content was lower than usual in these controls, although no acid-labile P was detectable. This may have been due to traces of adenine nucleotides in the suspension.

Generally speaking, the concentrations of sodium and potassium in the mitochondria in presence of glutamate and AMP were greater than those in the medium or in mitochondria incubated without AMP, and the maintenance of this higher level appeared to be linked with a decrease in the water content and with the concentration of acid-labile P in the system, which in these experiments generally decreased as the incubation time was prolonged.

In Fig. 1, the water content of the separated mitochondria has been plotted against the amount of acid-labile P present in the mixture before

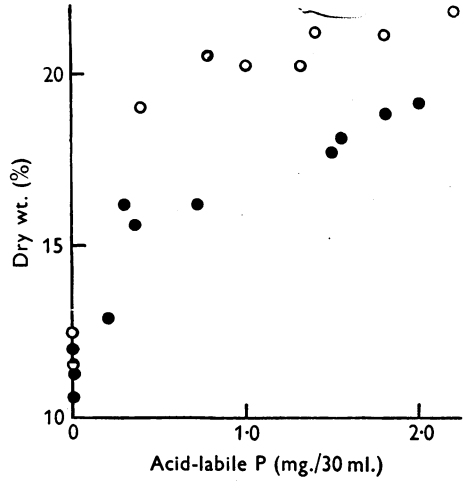

Fig. 1. Correlation between water content of mitochondria and the concentration of acid-labile P in the system. O—O, Exps. 74 and 75, sucrose-low salt medium; ●—●, Exps. 85 and 88, KCl medium.

centrifuging; the points are taken from samples obtained during the course of two experiments with each of two media. For either medium, there is clearly an association between the water content of

the mitochondria and the concentration of the acid-labile P in the system; the experiments illustrate the necessity of having an internal check, such as the level of esterified P, on the state of the mitochondrial sample analysed for electrolytes, even though the conditions are ostensibly suitable. It appears from the following experiments with dinitrophenol (DNP), however, that this association does not reside directly in the amount of external high-energy phosphate formed, but in some at present undefinable property of the mitochondria which is linked with their capacity (or potential capacity) for oxidative phosphorylation.

Effect of DNP on oxygen uptake and water content of mitochondria oxidizing L-*glutamate*

The effect on the swelling of mitochondria of DNP, which in general in the systems used abolishes the coupled phosphorylation and increases the rate of oxygen uptake, was examined in two experiments, in conditions similar to those in Table 2. Table 5 shows that in both experiments the rate of oxygen uptake was increased by DNP; in the first experiments the concentration used did not suppress the phosphorylation completely, but in the second experiment the acid-labile P was no greater than in the control without AMP. The water content of the mitochondria, however, was substantially the same in presence or absence of DNP, when AMP was also present; in absence of AMP the water content rose as usual.

Effect of adenylic acid on the agglutination and water content of mitochondria oxidizing succinate

It was noticed in the foregoing experiments that in the absence of AMP the mitochondria became agglutinated during incubation. This point was investigated further, using succinate as a substrate, as the oxidation of this substance *per se* does not require AMP.

Exp. 87. 1·0 ml. mitochondrial suspension in 0·25 M-sucrose (1·6 mg. N) was added last to 2·0 ml. medium in manometric flasks, the final concentrations being 0·02 M-sodium succinate; 0·005 M-$MgSO_4$; 2×10^{-5} M-cytochrome *c*; 0·001 M-AMP (when present); Na_2HPO_4-KH_2PO_4, pH 7·4 as indicated. The O_2 uptake at 28° was measured. Duplicate mixtures were kept at room temperature and smears made at intervals for microscopic examination.

The results for the lowest and highest phosphate concentrations are shown in Table 6. The oxygen uptake was initially considerably higher in the flasks without AMP, but decreased steadily and had almost ceased after 1 hr.; the oxygen uptake in presence of AMP decreased much more slowly and was proceeding at about 40% the initial rate after 80 min. Agglutination occurred within 10 min. in the mixtures with the lowest salt concentration and no AMP, and at each salt concentration was delayed in presence of AMP. These mixtures were not shaken, and it is possible that agglutination occurred more rapidly in the manometric flasks.

In another experiment the effect of AMP on the water content was determined.

Table 5. *Effect of dinitrophenol on water content of mitochondria*

(General conditions as in Exp. 74, see text.)

	AMP ($M \times 10^{-3}$)	DNP ($M \times 10^{-5}$)	Incubation time (min.)	O_2 uptake (μl./mg. N/10 min.)	Acid-labile P (mg.)	Mitochondrial dry wt. (%)
Exp. 75	1	0	10	39	1·8	21·1
	1	0	30	37	2·2	21·8
	1	2	10	50	1·0	20·2
	1	2	30	49	1·4	21·2
	0	0	10	0	0·2	12·9
	0	0	30	0	0	11·5
Exp. 76	2	0	15	41	2·8	20·9
	2	5	15	70	0·4	20·2
	0	0	15	0	0·4	13·9

Table 6. *Effect of AMP on oxidation of succinate and on agglutination in mitochondrial suspensions*

(Exp. 87, for details see text.)

Flask	Phosphate (M)	AMP ($M \times 10^{-3}$)	Rate of O_2 uptake (μl./mg. N/10 min.) at 10 min.	30 min.	60 min.	Agglutination at 10 min.	30 min.	60 min.
A	0·01	0	144	101	24	+	+ +	+ +
B	0·01	1	118	103	55	0	0	+
C	0·075	0	195	65	13	0	+	+ +
D	0·075	1	114	95	58	0	0	0

+, Microscopic; + +, macroscopic agglutination.

Mixtures were set up in two 250 ml. flasks *A* and *B* containing, in 30 ml. total vol.: 0·01 M-potassium phosphate, 0·01 M-$MgSO_4$, 2×10^{-5} M-cytochrome *c*, 10 ml. mitochondria in 0·25 M-sucrose, 0·01 M-sodium succinate. Flask *B* also contained 0·002 M-AMP. The flasks were rocked at 28° for 20 min., at which time O_2 was still being taken up in both the manometric samples, and the mitochondria separated and analysed as usual.

The water content was 88 % in *A* and 80 % in *B*, indicating that swelling had occurred in the absence of AMP, although oxidation was proceeding.

DISCUSSION

The experiments reported here show that, in contrast to the findings of Harman (1950), isolated mitochondria can maintain concentration differences of sodium and potassium ions between themselves and the suspending medium in certain conditions, i.e. they can do secretory work. This was demonstrated firstly by using a medium containing L-glutamate, inorganic phosphate, magnesium ions and adenosine 5′-phosphate (AMP), in which, as has been shown by other workers, the mitochondria can oxidize the substrate with a coupled esterification of inorganic phosphate to high-energy phosphate compounds such as ATP; and secondly, by maintaining this state of active metabolism as much as possible during the separation of the mitochondria for analysis by centrifuging at room temperature, instead of at 0°. The method has obvious limitations, particularly in assessing the absolute values of the concentrations which can be maintained, since reversal may occur during centrifuging, but it seems practicable for the direct study of the effect of specific conditions, such as adrenalectomy, or of specific substances upon the osmotic activity of the isolated mitochondria.

These preliminary experiments, which were made with rat-liver mitochondria, show that the maintenance of a concentration gradient and the prevention of swelling in hypotonic media are dependent upon the metabolic activity. When mitochondria were incubated with glutamate but without AMP, no oxidation occurred, the sodium and potassium concentrations of the mitochondria approached that of the medium and the water content rose to about 90 %; in these conditions the mitochondria agglutinated rather rapidly. In the presence of AMP, there was a concentration gradient, the water content decreased slightly from the initial value and was maintained at about 80 % and agglutination was deferred; in these experiments the rate of oxygen uptake often decreased on prolonged incubation and the acid-labile P also often decreased from an initial rapidly achieved maximum so the conditions were clearly not ideal.

In any particular system, as is shown in Fig. 1, there is some association between the level of acid-labile P and the degree of swelling, and the experiments using succinate as a substrate in the presence of AMP also indicate that the prevention of swelling is associated with the capacity of the system for oxidative phosphorylation, or more accurately, with the presence of an adenine nucleotide. On the other hand, in the presence of dinitrophenol, swelling was prevented although the level of external acid-labile P was practically nil. It is conceivable that the maintenance of selective permeability is dependent on the phosphorylation of some constituent of the membrane, that this constituent may be inactivated by incubation in unfavourable conditions and that it is in some way concerned in the phenomenon of oxidative phosphorylation. The facts observed may be related to the protective effect of adenine nucleotides on the 'ageing' of mitochondria incubated without substrate, noted by Kielley & Kielley (1951); on the other hand, 'ageing' may be due to the loss by diffusion of essential coenzymes from the mitochondria as our experiments show that swelling and equilibration with the medium occur rapidly in absence of AMP.

The ratios of internal/external concentration observed in the present experiments with rat-liver mitochondria, about 1·5–2 for sodium ions and 2–3 for potassium ions, are not high, compared, for instance, with the ratios for potassium in muscle and red blood cells. Bartley & Davies (1952) found in similar experiments with sheep-kidney mitochondria a high ratio (26) for sodium ions and also a marked concentration of fumarate. Apart from any function in the transport of ions peculiar to a particular tissue, it may well be an important consequence of the maintenance of a selective permeability that intermediary metabolites are kept in relatively high concentration in proximity to corresponding enzyme systems localized in the mitochondria.

SUMMARY

1. The ability of rat-liver mitochondria to do secretory work has been demonstrated by incubating them in a medium which would support oxidative phosphorylation and maintaining them, during separation for analysis, in a state of active metabolism by centrifuging at room temperature.

2. On incubation in a medium containing L-glutamate, magnesium ions, inorganic phosphate and adenosine 5′-phosphate (AMP), mitochondria maintained a higher concentration of sodium and potassium ions than that of the external fluid, and swelling was prevented.

3. In absence of AMP there was no concentration gradient, the water content rose from 80 to 90 % and agglutination readily occurred.

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.

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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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