Hunter, F. E. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 298.
Hunter, F. E., Davis, J. & Corlat, L. (1956). *Biochim. biophys. Acta*, **20**, 237.
Keilin, D. & Hartree, E. F. (1947). *Biochem. J.* **41**, 500.
Kielley, W. W. & Kielley, R. K. (1951). *J. biol. Chem.* **191**, 485.
Kielley, W. W. & Kielley, R. K. (1953). *J. biol. Chem.* **200**, 213.
Lardy, H. A. & Wellman, H. (1953). *J. biol. Chem.* **201**, 357.
Loomis, W. F. & Lipmann, F. (1949). *J. biol. Chem.* **179**, 503.
Luchsinger, W. W., Falcone, A. B. & Reynard, A. M. (1955). *Fed. Proc.* **14**, 247.
Martell, A. E. & Schwarzenbach, G. (1956). *Helv. chim. acta*, **39**, 653.
Martius, C. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 1.
Maruyama, K. (1954). *Arch. Biochem. Biophys.* **52**, 485.
Maruyama, K. (1956). *Arch. Biochem. Biophys.* **60**, 74.
Meyerhof, O. & Ohlmeyer, P. (1952). *J. biol. Chem.* **195**, 11.
Middlebrook, M. & Szent-Györgyi, A. (1955). *Biochim. biophys. Acta*, **18**, 407.
Myers, D. K. & Slater, E. C. (1957*a*). *Biochem. J.* **67**, 558.
Myers, D. K. & Slater, E. C. (1957*b*). *Nature, Lond.*, **179**, 363.
Nason, A. & Lehmann, I. R. (1956). *J. biol. Chem.* **222**, 511.
Novikoff, A. B., Hecht, L., Podber, E. & Ryan, J. (1952). *J. biol. Chem.* **194**, 153.
Polis, B. D. & Shmukler, H. W. (1954). *Abstr. 126th Meeting Amer. Chem. Soc.* p. 72c.
Potter, V. R. & Reif, A. E. (1952). *J. biol. Chem.* **194**, 287.
Potter, V. R., Siekevitz, P. & Simonson, H. C. (1953). *J. biol. Chem.* **205**, 893.
Robertson, H. E. & Boyer, P. D. (1955). *J. biol. Chem.* **214**, 295.
Sacktor, B. (1953). *J. gen. Physiol.* **36**, 371.
Schneider, W. C., Claude, A. & Hogeboom, G. H. (1948) *J. biol. Chem.* **172**, 451.
Shmukler, H. W. & Polis, B. D. (1953). *Abstr. 124th Meeting Amer. Chem. Soc.* p. 13c.
Siekevitz, P. & Potter, V. R. (1953). *J. biol. Chem.* **200**, 187.
Slater, E. C. (1949). *Biochem. J.* **45**, 14.
Slater, E. C. (1953*a*). *Nature, Lond.*, **172**, 975.
Slater, E. C. (1953*b*). *Biochem. J.* **53**, 157.
Slater, E. C. & Lewis, S. E. (1954). *Biochem. J.* **58**, 337.
Swanson, M. A. (1952). *J. biol. Chem.* **194**, 685.
Swanson, M. A. (1956). *Fed. Proc.* **15**, 367.
Tsou, C. L. (1951). *Biochem. J.* **49**, 512.

A Comparison of the Properties of Mitochondria Isolated from Liver and Heart

By F. A. HOLTON,
Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W. 12

W. C. HÜLSMANN, D. K. MYERS* and E. C. SLATER
Laboratory of Physiological Chemistry, University of Amsterdam, Netherlands†

(*Received* 24 *January* 1957)

It is now well established that in all animal cells which have been examined the mitochondria are the site of the main intracellular respiratory enzyme systems which are responsible for the synthesis of adenosine triphosphate (ATP). The uniformity of structure, composition and properties of mitochondria isolated from a wide variety of cells is most striking. Nevertheless, some differences might be expected between mitochondria of tissues which differ so much in form and function as the liver and heart muscle. A careful study and correlation of the differences between the mitochondria from the two tissues might possibly throw light on the biochemical mechanisms in the two granules.

* Present address: Suffield Experimental Station, Ralston, Alberta, Canada.
† Postal address: Jonas Daniël Meijerplein 3, Amsterdam-C, Netherlands.

Chance (1956) has recently drawn attention to some differences between the properties of liver mitochondria, as isolated in his laboratory, and heart-muscle mitochondria (sarcosomes) isolated by ourselves. Chance does not consider the possibility that these differences reflect a true difference between the mitochondria in the two tissues. On the contrary, he concludes that the differences are due to the fact that the sarcosomes which we used have been damaged in the course of their preparation.

It is the purpose of the present paper to compare in some detail the properties of the mitochondria isolated from the two tissues. The heart sarcosomes are indeed qualitatively similar to the liver mitochondria, but quantitative differences were observed. An attempt has been made to determine which of these differences can be ascribed to damage

of the sarcosomes and which represent a true difference between the two types of mitochondria *in vivo*.

Part of this work has already appeared in preliminary form (Holton, 1956).

METHODS

Mitochondrial preparations

Liver mitochondria. These were prepared as described in a previous paper (Myers & Slater, 1957).

Heart sarcosomes. Unless otherwise stated, these were prepared from rat heart, according to the procedure described by Cleland & Slater (1953), with 0·21 M-sucrose, 0·01 M-ethylenediaminetetra-acetate (EDTA), pH 7·4, as the isolation medium. The EDTA was the disodium salt brought to pH 7·4 with KOH. We are indebted to Miss A. Searle for most of these preparations.

In an attempt to prepare sarcosomes with a lower ATPase activity in the absence of 2:4-dinitrophenol (DNP), we have tested other isolation media and prepared the heart homogenates with the Potter–Elvehjem homogenizer in place of grinding by hand with sand in a mortar. For these experiments, the heart was minced as finely as possible in cold isotonic medium with a pair of small scissors. It was necessary to operate the homogenizer at a moderately high speed for 1–2 min. in order to obtain a homogeneous suspension of the heart tissue. More gentle grinding of the heart muscle decreased the yield of sarcosomes considerably and did not appear to improve their quality.

In the initial experiments the heart homogenates were centrifuged for 3 min. at 750–800 *g* and the supernatant fluid centrifuged for 7–10 min. at 6000–8000 *g* in order to sediment the sarcosomes. Later experiments showed that better results were obtained when the homogenate was centrifuged for 3 min. at about 600 *g*, the lowest acceleration required to sediment all the erythrocytes and debris. A considerable portion of the sarcosomes in the supernatant fluid could be recovered by a second centrifuging for 10–15 min. at 600 *g*; however, the yield was improved by centrifuging for 10 min. at about 6000 *g*. In contrast with liver homogenates, the preparations obtained from rat heart contain little microsomal material (cf. Cleland & Slater, 1953) and show no evidence of a fluffy layer under the above conditions. In order to minimize the amount of soluble protein associated with the sedimented sarcosomes, we have used large volumes of medium (20–40 ml./rat heart) in the isolation procedure and decanted the supernatant fluid as completely as possible after sedimenting the sarcosomes. The sediment was resuspended in cold isotonic medium with the aid of the homogenizer.

Enzymic tests

Respiratory rates and P:O *ratios.* The rate of O_2 uptake at 25°, expressed as μl. of O_2/mg. of protein/hr. (Q_{O_2}), and the P:O ratios were determined as described by Slater & Holton (1954).

ATPase activity. This was measured at different pH values as described by Myers & Slater (1957).

Analyses

Protein content. This was determined by the biuret method, as described by Cleland & Slater (1953).

Calcium. This was determined as described by Slater & Cleland (1953).

Total iron. This was determined by the phenanthroline method (Mahler & Elowe, 1954) after wet ashing with HNO_3.

Both the calcium and iron determinations were carried out by Mrs A. J. Haarbrink-Haitsma.

Measurement of the difference spectrum of sarcosomes

(*a*) *Optical arrangement.* The sarcosome suspension, containing 6–10 mg. of protein/ml., was contained in a 1 mm. silica cuvette mounted directly in front of the entry slit of the monochromator of the Hilger Uvispek spectrophotometer, model H700/306. It was illuminated by light from the hydrogen lamp supplied with this instrument. The quartz prism was mounted in the monochromator, and wavelength calibration was carried out with the hydrogen-emission lines at 486·1 mμ and 656·3 mμ. Light emerging from the exit slit was measured by a photomultiplier (E.M.I. Type 6255B with quartz window). In order to measure at high sensitivity the small changes of extinction due to respiratory catalysts, most of the photomultiplier current was opposed by a current variable at will, and the balance was amplified. The amplified current was recorded by an Evershed and Vignoles Ltd. quick-response pen-writing recording milliammeter. Responses of the recorder were calibrated in terms of changes of extinction by using a mechanical shutter interposed between the light source and the cuvette. This shutter could be moved into the light beam into a series of reproducible positions by means of a micrometer head. During the course of each experiment, while the sarcosome suspension was contained in the cuvette and was in its anaerobic steady state, the response of the recorder was measured for a series of shutter positions. This series was then repeated, and the photomultiplier current measured at each position. From these data the extinction changes corresponding to the recorder responses were readily calculated. The calibration was repeated at several different values of opposing current so that the sensitivity of the recording arrangement at any given value of opposing current was calculable. Full details of this apparatus will be described elsewhere (F. A. Holton, in preparation).

(*b*) *Aeration of suspensions.* The sarcosome suspension was withdrawn from the bottom of the cuvette by means of a fine plastic tube. After passing through a roller pump this tube terminated about 5 mm. above the meniscus of the suspension contained in the cuvette, which was only partly full. During operation of the pump, the suspension circulated at a rate of 1–3 ml./min. and became aerated as it flowed into the cuvette. To assist the establishment and maintenance of an aerobic steady state controlled by the oxidase activity of the suspension, the air above the meniscus was replaced by O_2 during circulation of the suspension, and the circulating tube was kept at 5–10° by a water jacket. Fig. 9 illustrates that this arrangement resulted in an aerobic steady state, which persisted for 15 sec. after circulation had ceased, after which the record returned to the value characteristic of the anaerobic steady state. This illustrates that the aerobic steady state observed was characteristic of the activity of the respiratory enzymes. If the steady response during circulation had been due to a degree of oxygenation controlled by the rate of aeration, the record would have returned to the level of the anaerobic steady state immediately after the flow was stopped.

RESULTS

Respiratory rates and P:O *ratios*

The rates of respiration and the P:O ratios with liver and heart mitochondria oxidizing α-oxoglutarate, succinate and β-hydroxybutyrate are compared in Table 1. Two rates of respiration are given: (*a*) the actual initial rates measured in the determination of the P:O ratios; (*b*) rates which more closely represent the capacity of the mitochondria to oxidize the substrate. The latter rates were measured in the absence of fluoride, which inhibits all the oxidase systems, especially succinic dehydrogenase and the oxidation of β-hydroxybutyrate by heart sarcosomes. A high concentration of succinate (0·06 M) was used in both measurements with the heart sarcosomes, since the rate of respiration is much greater in the presence of high concentrations and the P:O ratio is not affected. With liver mitochondria, the high concentration was also used in method (*b*), in order to measure the true activity of the system oxidizing succinate; however, a lower concentration was used for the measurement of the P:O ratio, since the ratio was thereby increased (K. Minnaert, unpublished observations), and because most other workers studying liver mitochondria have used low succinate concentrations.

The values of Q_{O_2} in columns (*b*), Table 1, represent approximately the activity of the one-step oxidations. With α-oxoglutarate, malonate was added in an amount (0·01 M) which nearly completely suppresses the oxidation of succinate (Slater & Holton, 1954). With succinate, no inhibitor of malate oxidation could be used, but it is probable that under the conditions of high succinate concentration the activity of the succinic oxidase system is so high that the malic oxidase system would be unable to compete successfully for common carriers in the respiratory chain. However, under the conditions in which the P:O ratio of liver mitochondria oxidizing succinate was measured, when the succinic dehydrogenase was operating at much below its full activity (presumably because of the presence of inhibitory oxaloacetate), a considerable proportion of the O_2 uptake probably represents oxidation beyond the malate stage. The Q_{O_2} for liver mitochondria oxidizing β-hydroxybutyrate undoubtedly represents the activity of the single-step oxidation, since acetoacetate is not oxidized by these preparations (Lehninger & Smith, 1949; Walker, 1954). It seems that heart sarcosomes oxidize β-hydroxybutyrate beyond the stage of acetoacetate (Beechey, 1957), but the activity compared with the other substrates is very low.

Table 1 also includes two calculations for each substrate of Q_P, the rate of phosphorylation (μl. of P/mg. of protein/hr.), calculated from each Q_{O_2} by the expression $Q_P = Q_{O_2} \times (P:O) \times 2$. The same value of the P:O ratio was used in the two calculations. Thus, the values of Q_P in calculation (*b*) represent the theoretical rate of phosphorylation, assuming that the P:O ratios were maintained under the conditions in which the maximum Q_{O_2} values were observed. This assumption is probably not valid for liver mitochondria oxidizing succinate.

The other components of the reaction mixture and the procedure used in the determinations of the P:O ratios were the same as previously described (Slater & Holton, 1954), except that a lower concentration of fluoride was used in some measurements. The values reported in Table 1 for liver mitochondria are similar to those obtained by Copenhaver & Lardy (1952), but the P:O ratio with α-oxoglutarate is somewhat lower, and that with succinate appreciably higher.

Table 1. *Rates of respiration and* P:O *values of rat-liver mitochondria and heart sarcosomes*

Mitochondria were isolated from rat liver in 0·25 M-sucrose. Sarcosomes were isolated from rat heart in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4, by the procedure of Cleland & Slater (1953).

In all cases the reaction mixture contained potassium phosphate, pH 7·4 (0·03 M); glucose (0·02 M); cytochrome *c* ($2–5 \times 10^{-5}$ M); adenosine monophosphate (6×10^{-4} M); adenosine diphosphate (6×10^{-4} M); $MgCl_2$ (0·005 M); EDTA (0·001–0·002 M); hexokinase. Substrate concentrations were: α-oxoglutarate (0·01 M); succinate [0·06 M, except under condition (*a*) for liver mitochondria, when it was 0·006 M]; β-hydroxybutyrate (DL) (0·015 M). Malonate (0·01 M) was used only with α-oxoglutarate. Fluoride concentrations: condition (*a*): with α-oxoglutarate and β-hydroxybutyrate, 0·04 M for heart sarcosomes and 0·02 M for liver mitochondria, with succinate, 0·02 M in both cases; condition (*b*): no fluoride with any substrate.

Values given are the means. Q_{O_2} = μl. of O_2/mg. of protein/hr. Figures in parentheses after P:O are the no. of expts.

$$Q_P = 2 \times Q_{O_2} \times [P:O]$$

	Heart sarcosomes		Liver mitochondria	
	(*a*)	(*b*)	(*a*)	(*b*)
α-Oxoglutarate				
Q_{O_2}	91	148	22	30
P:O	2·86 (19)	—	3·06 (9)	—
Q_P	520	850	135	183
Succinate				
Q_{O_2}	188	327	25·7	162
P:O	0·98 (17)	—	2·21 (8)	—
Q_P	367	650	113	(716)*
β-Hydroxybutyrate				
Q_{O_2}	11·6	25	32	32
P:O	1·97 (4)	—	2·58 (6)	—
Q_P	46	98	165	165

* See text for discussion of this value.

The most important differences between the heart sarcosomes and liver mitochondria shown in Table 1 are:

(1) The much greater respiratory activity of heart sarcosomes, which oxidize α-oxoglutarate five times and succinate twice as fast as liver mitochondria. The activities towards β-hydroxybutyrate do not differ appreciably.

(2) The much lower P:O ratios obtained with heart sarcosomes oxidizing succinate and β-hydroxybutyrate.

Because of the higher rate of respiration, the heart sarcosomes are able to synthesize ATP at a much greater rate (Q_P) than liver mitochondria.

Hydrolysis of adenosine triphosphate

Fig. 1 shows the pH–activity curves for the hydrolysis of ATP by liver mitochondria and heart sarcosomes (isolated in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4, by the procedure of Cleland & Slater, 1953), both in the absence and presence of DNP (10^{-4} M) and in the absence and presence of Mg^{2+} ions (0·003 M).

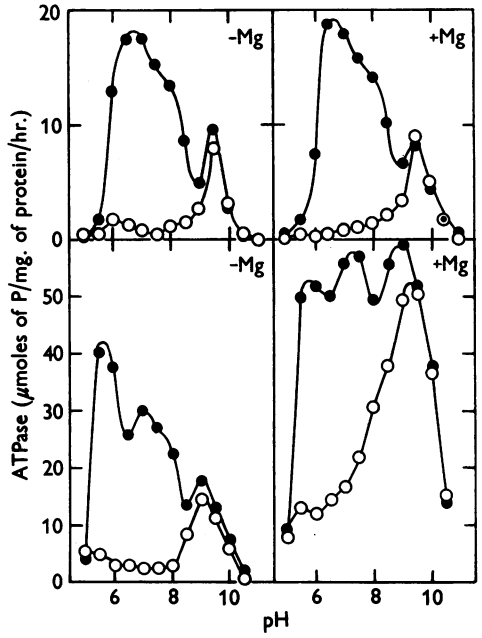

Fig. 1. Effect of DNP and Mg^{2+} ions on the hydrolysis of ATP by (upper curves) rat-liver mitochondria (isolated in 0·25 M-sucrose) and (lower curves) heart sarcosomes (isolated in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4). The reaction mixture contained 0·075 M-KCl, 0·05 M-sucrose, 0·05 M-tris buffer, 0·001 M-EDTA, 0·002 M-ATP, 0·1 mg. (liver) or 0·05 mg. (heart) of mitochondrial protein/ml., with or without 0·003 M-$MgCl_2$ as shown. ○, Absence of DNP; ●, addition of 10^{-4} M-DNP.

In the absence of Mg^{2+} ions, the shapes of the curves with the two types of preparation are similar both in the absence and in the presence of DNP. In both cases there is little activity under acid or neutral conditions in the absence of DNP, but considerable activity was found in alkaline solution, with optima at pH 9·4 (liver) and 9·2 (heart). DNP markedly activates the ATPase in both preparations below pH 9, but has no appreciable effect above pH 9. The degree of stimulation of the ATPase brought about by DNP is similar in the two preparations.

The two preparations differ in their response to Mg^{2+} ions, which have little effect on liver mitochondria, but markedly activate the ATPase of the heart sarcosomes, especially in the absence of DNP. Consequently, in the presence of Mg^{2+} ions, the degree of stimulation of the ATPase brought about by the addition of DNP at neutral pH is much less with heart sarcosomes than with liver mitochondria. Average rates of hydrolysis of ATP at pH 7·4 for the two types of mitochondria are compared in Table 2.

In a previous paper (Myers & Slater, 1957), it was shown that four peaks (at pH 6·3, 7·4, 8·5 and 9·4) could be obtained in the pH–activity curves of the ATPase of liver mitochondria, under various conditions. The first three peaks could be demonstrated by the addition of different concentrations of DNP to freshly prepared mitochondria, and the fourth was already present in the absence of DNP. In the presence of DNP (10^{-4} M), the pH–activity curve with heart sarcosomes is similar to that given by liver mitochondria, except that the alkaline system is much more dominant in the sarcosomes, in the

Table 2. *Comparison of the ATPase activities of rat-liver mitochondria and heart sarcosomes at* pH 7·4.

Mitochondria and sarcosomes were isolated as in Table 1. The reaction mixture contained 0·075 M-KCl, 0·108 M-sucrose, 0·001 M-EDTA and 0·002 M-ATP with or without further additions of $MgCl_2$ and DNP as noted below. The mixture was incubated for 15 min. at 20° and pH 7·4 after addition of 0·08–0·3 mg. of mitochondrial protein/ml. or 0·05–0·1 mg. of sarcosomal protein/ml. The average rates of hydrolysis of ATP observed with eleven preparations of liver mitochondria and three preparations of heart sarcosomes are given.

DNP (M)	$MgCl_2$ (M)	Average rates of hydrolysis of ATP (μmoles of P/mg. of protein/hr.)	
		Liver mitochondria	Heart sarcosomes
0	0	1·1	1·2
10^{-4}	0	13·1	31·2
0	0·003	0·75	27·7
10^{-4}	0·003	11·9	54·0

presence of Mg^{2+} ions. When DNP was added, three clear peaks were obtained with the heart sarcosomes at pH 5·7, 7·1–7·3 and 9–9·2 respectively, both in the absence and presence of Mg^{2+} ions. A peak at pH 8·2 is shown by plotting the difference curve ($+Mg^{2+}$ ions *minus* $-Mg^{2+}$ ions) in the absence of DNP (Fig. 2).

The specific activity of the heart sarcosomes at neutral pH in the presence of Mg^{2+} ions and DNP is about five times that of liver mitochondria. In the absence of DNP, liver mitochondria can be activated by ageing, and especially by freezing and thawing after ageing. The activity after this treatment is, in fact, much greater than obtained with unaged mitochondria in the presence of DNP (Myers & Slater, 1957). Fig. 3 shows that heart sarcosomes (isolated in 0·25 M-sucrose, without EDTA) behave in the same way, and that the pH–activity curves of the two preparations after ageing, freezing and thawing are very similar, both being dominated by the peaks at pH 9, but also showing inflexions at about pH 7. The specific activity of the heart sarcosomes treated in this way was double that of the liver mitochondria.

It is clear that there are striking resemblances between the systems hydrolysing ATP in the two types of mitochondria. The chief difference is the response of the system in the sarcosomes to Mg^{2+} ions, particularly at alkaline pH values.

In the absence of DNP but presence of Mg^{2+} ions, the hydrolysis of ATP by heart sarcosomes was not inhibited by 0·06 M-NaF. In this respect this ATPase behaves like that in aged mitochondria, frozen mitochondria or in the Keilin & Hartree heart-muscle preparation (Myers & Slater, 1957). However, the increment of the ATPase activity of heart sarcosomes brought about by the addition of 10^{-4} M-DNP was almost completely inhibited by 0·06 M-NaF. The DNP-stimulated ATPase activity of liver mitochondria is likewise inhibited by 0·06 M-NaF.

Attempts were made to isolate heart sarcosomes more closely resembling liver mitochondria by varying the procedure of isolation. Similar results were obtained by grinding the heart in a mortar with sand, as in the earlier procedure (Cleland & Slater, 1953), or by using a Potter–Elvehjem homogenizer (Fig. 4). In experiments in which various isolation media were tried, the Potter–Elvehjem homogenizer was used. A series of glass homogenizers with different clearances between the tube and pestle was tested, but none of these provided better preparations than those obtained with the standard

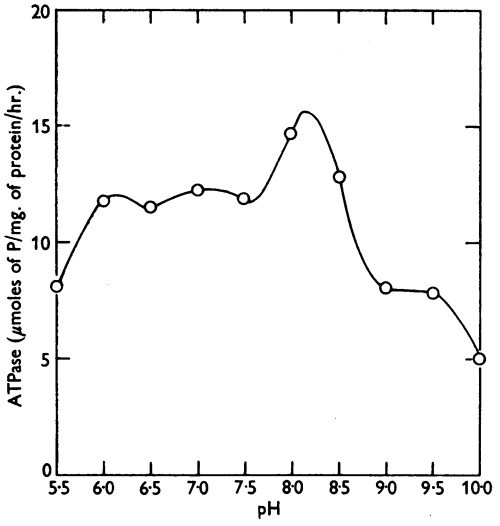

Fig. 2. Difference curve showing the effect of Mg^{2+} ions on the hydrolysis of ATP by rat-heart sarcosomes (isolated in 0·23 M-sucrose, 0·005 M-ATP, pH 7·4). Reaction mixture: KCl (0·075 M); EDTA (6×10^{-4} M); ATP (0·002 M); tris buffer (0·05 M); sucrose (0·05 M); sarcosomes (0·18 mg. of protein/ml.). The ATPase activity plotted is activity measured in the presence of 0·003 M-$MgCl_2$ *minus* activity measured in the absence of added Mg^{2+} ions.

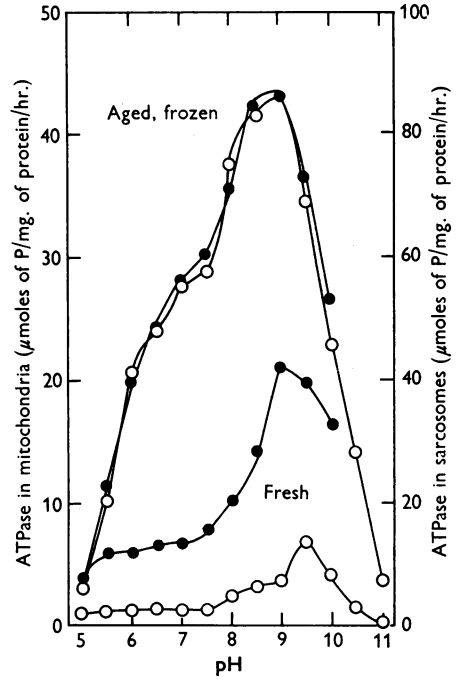

Fig. 3. Stimulation of the ATPase activity of liver mitochondria (○) and of heart sarcosomes (●) by ageing (2 hr. at 30°) followed by freezing and thawing (upper curves). The activities of the freshly prepared mitochondria (○) and sarcosomes (●) are shown in the lower curves. Both preparations were isolated in 0·25 M-sucrose. Reaction mixture: 0·075 M-KCl, 0·05 M-sucrose, 0·05 M-tris buffer, 0·002 M-ATP and 0·001 M-$MgCl_2$.

homogenizer with a polytetrafluoroethylene (Teflon) pestle. The degree of stimulation of the ATPase activity by DNP was not increased appreciably when the isolation was carried out under anaerobic conditions.

In a series of experiments with different isolation media, it was found that the following all gave similar results: (1) 0·25M-sucrose (cf. Plaut & Plaut, 1952; Maley & Plaut, 1953); (2) 0·21 or 0·225M-sucrose, 0·01M-EDTA, pH 7·4 (Cleland & Slater, 1953); (3) 0·24M-sucrose, 0·5% plasma albumin (Sacktor, 1954; Lewis & Slater, 1954); (4) 0·225M-sucrose, 0·01M-α-oxoglutarate, pH 7·4; (5) 0·225M-sucrose, 0·01M-citrate, pH 7·4, 6·8 or 6·2; (6) 0·23M-sucrose, 0·005M-adenosine diphosphate (ADP), pH 7·4; (7) 0·23M-sucrose, 0·005M-adenosine monophosphate, pH 7·4; (8) 0·15M-KCl; (9) 0·135M-KCl, 0·02M-phosphate, pH 7·4 (Cleland & Slater, 1953); (10) 0·121M-KCl, 0·02M-phosphate, 5×10^{-4}M-ATP, pH 7·4 (Montgomery & Webb, 1956). In all cases, the ATPase activity of the sarcosomes at pH 7·4 in the presence of Mg^{2+} ions was increased approximately twofold by the addition of 10^{-4}M-DNP. The degree of stimulation by DNP was significantly smaller when the concentration of sucrose in the isolation medium was increased to 0·88M or when the sarcosomes were isolated in 0·44M-sucrose, 0·01M-citrate, pH 6·2 as recommended by Witter, Watson & Cottone (1955). On the other hand, the ATPase activity at pH 7·4 in

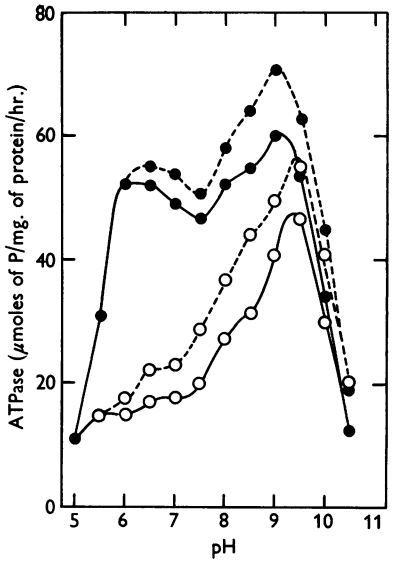

Fig. 4. Hydrolysis of ATP by two preparations of rat-heart sarcosomes. The reaction mixture is the same as that given for Table 3. Sarcosome preparations were isolated from rat heart in 0·21M-sucrose, 0·01M-EDTA, pH 7·4. One preparation was isolated by the standard procedure of Cleland & Slater (1953) and its ATPase activities were measured in the absence (○ - - - ○) and presence (●—●) of 10^{-4}M-DNP. The other preparation was isolated simultaneously and treated in the same way but all homogenizing and mixing operations were carried out with a Potter–Elvehjem homogenizer as described in the text; ATPase activities were measured in the absence (○—○) and presence (● - - - ●) of 10^{-4}M-DNP.

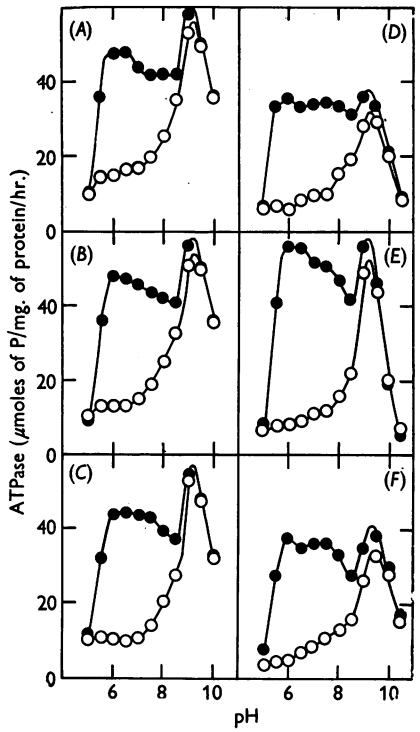

Fig. 5. Hydrolysis of ATP by rat-heart sarcosomes isolated in various media. The reaction medium was the same as in Table 3 and the ATPase activities of the sarcosome preparations were measured in the absence (○) and presence (●) of 10^{-4}M-DNP. Graphs (*A*), (*B*) and (*C*) represent three preparations which were isolated simultaneously in different media from the same rat heart; the heart homogenates were centrifuged for 3 min. at 600 *g* and the sarcosomes were recovered from the supernatant fluids by centrifuging for 7·5 min. at 6000 *g*. Graphs (*D*), (*E*) and (*F*) represent different preparations in which the sarcosomes were recovered from the supernatant fluid by a second centrifuging at 600 *g* for 10–15 min. In all cases the sarcosomes were resuspended in the same medium as used for the isolation procedure. The following media were used: (*A*) 0·25M-sucrose; (*B*) 0·15M-KCl; (*C*) 0·135M-KCl, 0·005M-ATP, pH 7·4; (*D*) 0·23M-sucrose, 0·005M-ATP, pH 7·4 (average of three experiments); (*E*) 0·23M-sucrose, 0·005M-ATP, pH 7·4 [this preparation differs from those given in graph (*D*) in that small rats weighing 50 g. were used in place of adult rats weighing approx. 250 g.]; (*F*) 0·1M-KCl, 0·05M-tris buffer, 0·001M-ATP, 0·005M-$MgCl_2$, 0·001M-EDTA, pH 7·4 (cf. Chappell & Perry, 1954) (average of two experiments).

the presence of Mg^{2+} ions was increased by DNP about 3·3 times when the sarcosomes were isolated in an isotonic medium containing ATP. This result was obtained with the following isolation media: (1) 0·23 M-sucrose, 0·005 M-ATP, pH 7·4, 6·8 or 6·2; (2) 0·23 M-sucrose, 0·005 M-ATP, 0·005 M-$MgCl_2$, pH 7·4; (3) 0·21 M-sucrose, 0·005 M-ATP, 0·01 M-α-oxoglutarate, pH 7·4; (4) 0·135 M-KCl, 0·005 M-ATP, pH 7·4; (5) 0·1 M-KCl, 0·05 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, 0·001 M-ATP, 0·005 M-$MgCl_2$, 0·001 M-EDTA, pH 7·4 (Chappell & Perry, 1954). The sarcosomes isolated in all of these media appeared to have similar ATPase activities. Typical results obtained with sarcosomes isolated in media with and without ATP are shown in Fig. 5.

Further experiments showed that the sarcosomes present in the homogenates of rat heart were not completely homogeneous and that those with the lowest ATPase activity in the presence of Mg^{2+} ions could be centrifuged down at low speeds. A typical experiment on the differential centrifuging of homogenates in 0·25 M-sucrose is shown in Table 3; similar results were obtained with homogenates in sucrose + EDTA, sucrose + ATP and the medium of Chappell & Perry (1954). A considerable fraction of the sarcosomes in heart homogenates was sedimented by centrifuging at 600 *g*; on the other hand, the major portion of the mitochondria in liver homogenates was sedimented only at about 4000 *g*.

The ATPase activity of the clear supernatant fluid obtained after centrifuging the heart homogenate at 20 000 *g* was still stimulated by DNP. In

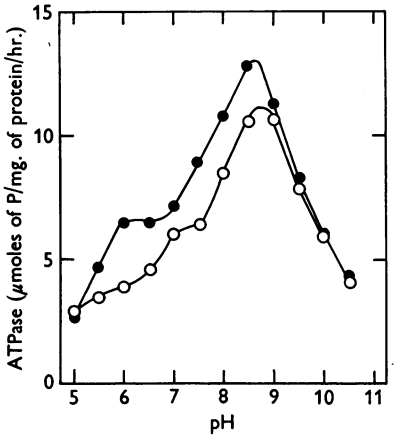

Fig. 6. Hydrolysis of ATP by the supernatant fluid from a rat-heart homogenate after centrifuging at 20 000 *g*. The reaction medium was the same as in Table 3; the ATPase activities of the supernatant fluid were measured in the absence (○) and presence (●) of 10^{-4} M-DNP.

Table 3. *Fractionation of liver and heart homogenates by differential centrifuging*

Homogenates of rat liver and rat heart in 0·25 M-sucrose were prepared with the Potter–Elvehjem homogenizer. The homogenates were centrifuged for 5 min. at 600 *g*, and approx. 75% of the supernatant fluids was decanted into other centrifuge tubes; the remainder of the supernatant was discarded together with the sediment. The retained supernatant from the heart homogenate contained a total of 26·6 mg. of protein, that from the liver homogenate contained a total of 249 mg. of protein in about 12 ml. of sucrose solution. These supernatant fluids were centrifuged again for 10 min. at 600 *g*, the sediments were kept for determination of the ATPase activity, and the supernatants were centrifuged for 10 min. at 1200 *g*. This procedure was repeated at successively higher speeds up to 20 000 *g*. The supernatant fluids were decanted completely each time but no attempt was made to separate the microsomal components from the mitochondria which were sedimented at high speeds. The reaction medium used for the ATPase assay contained KCl, 0·075 M; sucrose, 0·05 M; tris–acetate buffer, pH 7·5, 0·05 M; $MgCl_2$, 0·001 M; EDTA, 6×10^{-4} M; DNP (where shown), 10^{-4} M. Reaction occurred for 15 min. at 20°.

Fraction of the initial supernatant		Protein content (% of total protein)	ATPase activity (μmoles of P/mg. of protein/hr.)		Stimulation of the ATPase activity by DNP (%)
			$MgCl_2$	$MgCl_2$ + DNP	
Rat heart					
Sediment	600 *g*	9	16·0	41·5	159
Sediment	1 200 *g*	7	18·1	42·2	133
Sediment	4 000 *g*	5	28·5	48·3	69
Sediment	8 000 *g*	2	34·9	48·0	38
Sediment	20 000 *g*	3	36·5	51·8	41
Supernatant	20 000 *g*	74	6·4	8·8	37
Rat liver					
Sediment	600 *g*	13	2·6	11·5	340
Sediment	1 200 *g*	6	1·9	14·4	660
Sediment	4 000 *g*	10	1·5	12·3	720
Sediment	8 000 *g*	5	1·8	9·8	440
Sediment	20 000 *g*	4	1·9	5·0	163
Supernatant	20 000 *g*	62	1·1	1·1	0

confirmation of the data given by Lardy & Wellman (1953), the corresponding turbid supernatant from liver homogenates did not show this effect. The pH–activity curve obtained with the supernatant from a heart homogenate was similar to that obtained with intact sarcosomes, although the specific ATPase activity is relatively low because of the presence of soluble proteins (Fig. 6). This would suggest that the supernatant fluid may contain small fragments from the sarcosomes which have been broken during the homogenization of the heart muscle.

The differences in the results obtained with liver mitochondria and heart sarcosomes are probably not due to an inherent instability of the sarcosomes in the isotonic sucrose. The maximum rate of hydrolysis of ATP at pH 7·4 falls slowly when the isolated sarcosomes are allowed to stand in the same medium for 3–4 hr. at 0°, but the degree of stimulation caused by DNP was only slightly decreased (Table 4). The activity of the α-oxoglutaric oxidase of heart sarcosomes in sucrose solution is also stable for 1–3 hr. at 0°, although it deteriorates rapidly at 25° unless EDTA is added to the medium (Slater & Cleland, 1953). Other experiments have shown that the ATPase activity of the sarcosomes is relatively stable when the whole homogenate is allowed to stand for 3 hr. at 0°. It is concluded therefore that any damage which might have been caused to the sarcosomes must have occurred during the preparation of the heart homogenate and not at a later stage in the isolation procedure.

Our attempts to circumvent damage of this type have failed to produce heart sarcosomes with an ATPase activity similar to that of liver mitochondria. However, when young rats weighing 30–40 g. were used in place of the usual adult rats weighing about 250 g., a somewhat greater degree of stimulation by DNP was sometimes found. Fig. 5 shows the ATPase activities of the sarcosomes isolated at 600 *g* in the sucrose–ATP medium from the heart muscle of these young rats. The increase in the degree of stimulation by DNP under these conditions may be due to the ease with which this heart muscle can be homogenized.

Sarcosomes isolated from mouse heart behaved very similarly to those from rat heart. However, guinea-pig-heart sarcosomes had a considerably lower ATPase activity (16 μmoles/mg. of protein/hr. at pH 7·4, in the presence of Mg^{2+} ions and DNP,

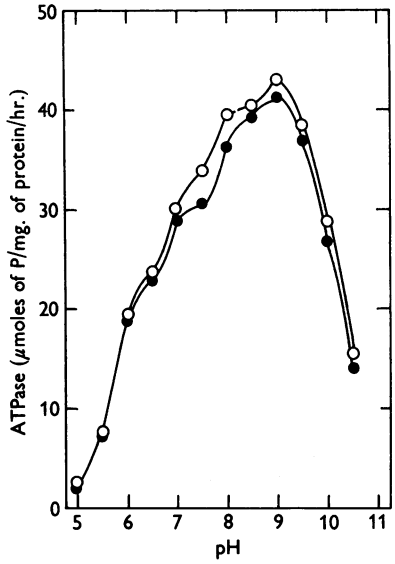

Fig. 7. Hydrolysis of ATP by the Keilin & Hartree (1947) heart-muscle preparation. The reaction medium was the same as in Fig. 2. Average values are given for three preparations isolated from horse heart by the standard procedure of Keilin & Hartree (●) and for three preparations isolated by high-speed centrifuging (○) (cf. Slater, 1949).

Table 4. *ATPase activities of heart sarcosomes after standing at* 0°

Sarcosomes were isolated from rat-heart homogenates in 0·25 M-sucrose or in 0·23 M-sucrose, 0·005 M-ATP (pH 7·4) by centrifuging at 600 *g* for 10 min. as described under Methods. They were resuspended in the same medium as that used in the isolation procedure and allowed to stand at 0°. The ATPase activities at pH 7·5 were determined as in Table 3.

Medium	Time at 0° (hr.)	ATPase activity (μmoles of P/mg. of protein/hr.) $MgCl_2$	ATPase activity (μmoles of P/mg. of protein/hr.) $MgCl_2$ + DNP	Stimulation of the ATPase activity by DNP (%)
Sucrose	0·2	14·9	34·0	128
	3·5	13·8	29·7	115
Sucrose	0·2	15·6	43·9	181
	4·0	15·2	40·6	167
Sucrose + ATP	0·2	10·4	33·7	224
	4·0	9·1	27·3	200
Sucrose + ATP	0·2	9·6	29·5	208
	4·0	9·1	27·2	199

compared with 54 μmoles of P/mg. of protein/hr. for rat heart) and the degree of stimulation by DNP at pH 7·4 was often also higher. There was no difference in the ATPase activities of mitochondria from rat and guinea-pig liver.

The Keilin & Hartree (1947) horse-heart-muscle preparation, which consists of sarcosomal fragments, had half the ATPase activity of aged and frozen rat-heart sarcosomes; the pH–activity curves were very similar (cf. Fig. 7 and Fig. 3). Similar results were obtained whether the sarcosomal fragments were sedimented by high-speed centrifuging at neutral pH or by low-speed centrifuging at pH 5·7, as in the procedure of Keilin & Hartree (1947).

The ATPase activity of the Keilin & Hartree preparation was much more stable than that of aged and frozen liver mitochondria (Myers & Slater, 1957), there being little loss after storage for 4 days at 2° in 0·1 M-phosphate, pH 7·4, or in 0·235 M-sucrose, 0·01 M-tris, or for 7 days at −18° in the latter medium. The high ATPase activity of the horse-heart preparation was not observed by Keilin & Hartree (1949), who tested the reaction in the absence of added bivalent cations. We have confirmed the low activity under these conditions and shown that Mg^{2+} ions and a number of other cations activate the reaction (Myers & Slater, 1957). The sarcosomal fragments which constitute the Keilin & Hartree heart-muscle preparation are probably similar to the mitochondrial fragments prepared from liver by Kielley & Kielley (1953).

Respiratory control

We have previously reported an experiment (Slater & Holton, 1953) designed in connexion with an investigation of the adenine nucleotide specificity of oxidative phosphorylation, in which we showed the following relative rates of oxidation with α-oxoglutarate as substrate: no adenine nucleotide, no hexokinase, 1; no adenine nucleotide, with hexokinase, 1·9; ATP, no hexokinase, 2·5; ADP, with hexokinase, 5·2. The stimulation by hexokinase shows that, as with liver mitochondria (Lardy & Wellman, 1952), the rate of oxidation of α-oxoglutarate by heart sarcosomes is limited by the concentration of phosphate acceptor.

The degree of respiratory control obtained with liver mitochondria and heart sarcosomes has now been studied in detail. The respiratory-control index is defined as [rate of respiration in the presence of DNP or phosphate acceptor (ADP or glucose)]/(rate of respiration in the presence of 10^{-4} M-ATP). We used a low concentration of ATP in the control measurement, since in its absence the oxidase systems were often irreversibly inactivated after a short time. Higher concentrations were not used, since they supplied sufficient ADP to maintain an appreciable concentration of phosphate acceptor (cf. Chance & Williams, 1955*a*). Under these conditions the rates of O_2 uptake were essentially constant for about 30 min. at 25°, except when DNP was present, when the rate of O_2 uptake often sharply decreased. Two types of heart sarcosomes were used, one isolated in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4, exactly as in previous papers, and the other isolated in 0·25 M-sucrose, 0·005 M-ATP, pH 7·4.

Before a comparison between the two types of mitochondria could be made, it was necessary to study factors which affected the degree of respiratory control in both types of mitochondria. The following factors were found important:

(1) *Presence of magnesium.* The addition of Mg^{2+} ions increased the respiratory-control index with liver mitochondria and heart sarcosomes, but its effect was much more marked with the former. Qualitatively, the effects of omitting Mg^{2+} ions were also different. With liver mitochondria, the rate of respiration in the absence of acceptor was increased to about the same value as obtained in the presence of acceptor. The latter rate was scarcely affected by omission of Mg^{2+} ions. With heart sarcosomes, however, omission of Mg^{2+} ions did not affect the rate in the absence of phosphate acceptor, but decreased the rate in its presence. The different effects of Mg^{2+} ions in the absence of acceptor suggest that it is necessary for stabilization of liver mitochondria (Baltscheffsky, 1956), but not for heart sarcosomes.

(2) *Presence of fluoride.* In both liver mitochondria and heart sarcosomes, the respiratory-control index was substantially higher in the presence of fluoride (0·012 M).

(3) *Concentration of mitochondria.* Increasing concentration of mitochondria increased the respiratory-control index with both types of preparations (Table 5). With liver mitochondria, this was due both to a decrease in the Q_{O_2} in the absence of phosphate acceptor (or DNP) and to an increase of the Q_{O_2} with increasing concentration of mitochondria, when hexokinase–glucose or ADP was added. This was also observed by Stern & Timonen (1956), who further found that the inactivating effect of dilution could be prevented by the addition of the nuclear fraction, or of a heated nuclear fraction. When DNP was used instead of hexokinase and glucose, the effect of concentration of mitochondria was rather variable, and Table 5 shows an experiment (liver mitochondria II) in which the Q_{O_2} decreased with increasing concentration.

Liver mitochondria had approximately the same respiratory-control index with hexokinase–glucose, DNP, or ADP, but heart sarcosomes gave the highest results with ADP. The concentration of the heart sarcosomes had little effect on the Q_{O_2} in the

absence of acceptor, but the Q_{O_2} in the presence of ADP increased with increasing concentration.

The increase of the Q_{O_2}, in the absence of acceptor, brought about by lowering the concentration of liver mitochondria may be correlated with the greater ATPase activity of these mitochondria in dilute solution (Myers & Slater, 1957).

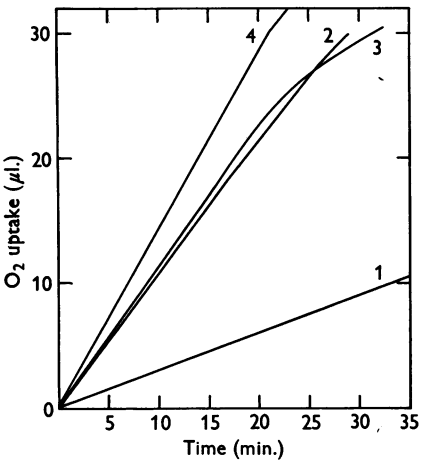

Fig. 8. Respiratory-control index with rat-heart sarcosomes, isolated in 0·23 M-sucrose, 0·005 M-ATP, pH 7·4, and suspended in 0·25 M-sucrose, 0·001 M-ATP, pH 7·4. Reaction mixture: phosphate, pH 7·4 (0·016 M); NaF (0·012 M); $MgCl_2$ (0·006 M); KCl (0·083 M); ATP (10^{-4} M); EDTA (0·001 M); glutamate (DL) (0·007 M); sarcosomes (0·8 mg. of protein/ml.). Reaction vol., 1 ml.; temp., 25°. Curve 1, no further addition; curve 2, 0·02 M-glucose + hexokinase (P:O ratio measured on this flask = 2·13); curve 3, 10^{-4} M-DNP; curve 4, 0·0033 M-ADP.

(4) *Substrate.* In agreement with the results of Lardy & Wellman (1952), a greater respiratory-control index was obtained with glutamate than with α-oxoglutarate, succinate, or β-hydroxybutyrate as substrate.

(5) pH. A higher respiratory-control index was obtained at pH 7·2 than at 6·2 or 6·7.

An experiment demonstrating respiratory control with rat-heart sarcosomes is shown in Fig. 8. In other experiments with guinea-pig-heart sarcosomes, which have a lower ATPase activity, the rate of O_2 uptake showed a sudden decline when all the ADP was phosphorylated to ATP. This also occurred with liver mitochondria (cf. Chance & Williams, 1955*b*).

Diphosphopyridine nucleotide content of mitochondria

The DPN content of liver mitochondria was determined by precipitation of the protein with $HClO_4$, centrifuging and measurement of the DPN content of the neutralized supernatant with alcohol dehydrogenase, as described by Racker (1950). Since Chance & Williams (1955*b*) have found that isolated liver mitochondria contain most of the DPN in the reduced form, and DPNH is unstable in acid solution, the liver mitochondria were incubated for a short time with ADP before addition of the $HClO_4$ in order to oxidize the DPNH (Chance & Williams, 1955*b*). Table 6 shows the effect of time of

Table 5. *Effect of concentration of mitochondria on the respiratory-control index*

The reaction mixture contained phosphate, pH 7·4 (0·016 M); NaF (0·012 M); $MgCl_2$ (0·006 M); KCl (0·083 M); ATP (10^{-4} M); EDTA (0·001 M); glutamate (DL) (0·007 M); DNP (where added, 10^{-4} M); ADP (where added, 0·0027 M); glucose (added with hexokinase, 0·02 M). Temperature, 25°.

Preparation	Protein (mg./ml.)	Q_{O_2} (μl. of O_2/mg. of protein/hr.) ATP (*a*)	ATP + hexokinase (*b*)	ATP + DNP (*c*)	ATP + ADP (*d*)	Respiratory-control index (*b*/*a*), (*c*/*a*) or (*d*/*a*)
Liver mitochondria I	0·67	14·9	32·8	—	—	2·2
	1·34	13·4	50·8	—	—	3·8
	2·68	10·4	52·2	—	—	5·0
Liver mitochondria II	1·0	13·0	—	52·0	—	4·0
	2·0	8·0	—	41·0	—	5·1
	4·0	7·0	—	41·0	—	5·9
Liver mitochondria III	1·15	12·7	—	34·4	—	2·7
	2·30	8·3	—	38·1	—	4·6
Liver mitochondria IV	1·32	12·7	—	—	37·7	3·0
	3·96	10·9	—	—	75·9	7·0
Heart sarcosomes I*	0·30	41	—	—	110	2·7
	0·60	43	—	—	135	3·2
	0·90	37	—	—	132	3·6
Heart sarcosomes II†	0·26	21	—	—	36	1·8
	0·52	23	—	—	52	2·25
	0·78	24	—	—	58	2·4

* Isolated in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4, by the method of Cleland & Slater (1953).
† Isolated in 0·23 M-sucrose, 0·005 M-ATP, pH 7·4.

incubation on the DPN found. The results show that between 43 and 65 % of the total DPN was not recovered by deproteinizing before the addition of the ADP. The actual percentage of the pyridine nucleotide present in the reduced form in the isolated mitochondria might be greater than these values, since it is not certain that all of the DPNH would be destroyed by the isolation procedure. Pre-incubation with ADP had little or no effect on the amount of DPN extracted from the heart sarcosomes. The value found for sarcosomes isolated in sucrose–EDTA is the same as that obtained by Holton (1955). Sarcosomes isolated in the medium of Chappell & Perry (1954) contained about the same amount of DPN.

Difference spectrum of sarcosomes from 300 to 350 mμ

Although Holton (1955) showed the presence of large amounts of DPN in heart sarcosomes, the difference spectrum (anaerobic *minus* aerobic state) did not include the expected maximum in the region 320–350 mμ when the reducing substrate was β-hydroxybutyrate. This question has now been investigated by the procedure described under Methods.

Heart sarcosomes were suspended in a 0·32-osmolar mixture containing DL-β-hydroxybutyrate, ATP, EDTA and sucrose. Upon aeration of the sarcosome suspension, negative changes of extinction occurred at wavelengths between 300 and 350 mμ with both rat- and rabbit-heart sarcosomes. These changes were reversed by stopping the aeration (Fig. 9). The kinetics of the extinction changes were closely similar to the simultaneous changes recorded at 440–450 mμ, which are mainly due to reactions of cytochrome a_3. Thus they may safely be taken to represent oxidation and reduction of respiratory catalysts.

The difference spectra (anaerobic *minus* aerobic steady states) for two different sarcosome suspensions are shown in Fig. 10. They show a marked peak at about 320 mμ. This is about the same position as that found in liver mitochondria by Chance & Williams (1955*c*, Fig. 1).

Calcium and iron content of mitochondria

Table 7 compares the calcium and iron contents of the two types of mitochondria. For comparison, the calcium content of sarcosomes isolated in 0·25 M-sucrose is given. Sarcosomes isolated by the

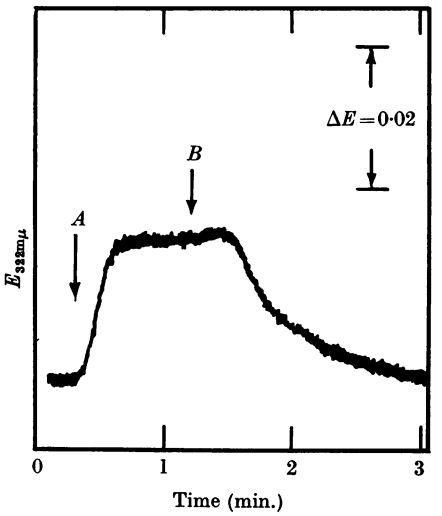

Fig. 9. Kinetics of extinction changes at 322 mμ in an aerated sarcosome suspension. Actual tracing from pen recorder. An upward deflexion represents a decrease of the extinction. Rat-heart sarcosomes, 6·6 mg. of protein/ml.; β-hydroxybutyrate (DL), 0·0085 M; ATP, 0·0079 M; sucrose, 0·27 M; EDTA, 0·001 M. Band width, 4·4 mμ, expts. 56/38. Aeration commenced at arrow *A* and stopped at arrow *B*.

Table 6. *Diphosphopyridine nucleotide content of rat-liver mitochondria and heart sarcosomes*

A volume (0·2 ml.) of a solution containing 0·024 M-ADP, 0·012 M-EDTA, pH 7·4, was added to 2 ml. of mitochondrial or sarcosomal suspension, and the mixture allowed to stand for different times at 22°, with frequent shaking; $HClO_4$ (0·2 ml. of 70 %, v/v) was then added, and the DPN content of the deproteinized extract determined according to the procedure of Holton (1955). The unincubated samples were treated with $HClO_4$ before the addition of ADP and EDTA. A control solution was prepared in the same way, with the substitution of 2 ml. of isolation medium for the mitochondria. This solution was used in the reference cell in the determination of the DPN.

	DPN (μmoles/g. of protein)			
Time of incubation with ADP (min.) ...	0	5	10	15
Preparation				
Liver mitochondria I	2·64	—	4·66	—
Liver mitochondria II	1·84	4·56	4·60	4·44
Liver mitochondria III	1·50	—	4·32	—
Heart sarcosomes I*	6·7	—	—	—
Heart sarcosomes II†	6·1	—	6·4	—

* Isolated in 0·1 M-KCl, 0·05 M-tris, 0·001 M-ATP, 0·005 M-$MgCl_2$, 0·001 M-EDTA, pH 7·4 (Chappell & Perry, 1954).
† Isolated in 0·21 M-sucrose, 0·01 M-EDTA, pH 7·4 (Cleland & Slater, 1953).

method of Cleland & Slater (1953) contain much less calcium, which is bound by the EDTA in the medium (Slater & Cleland, 1953). EDTA does not remove iron from mitochondrial preparations.

Both types of mitochondria contain large amounts of iron, most of which is probably not in the form of haem (cf. Green, 1956). The amount of iron in liver mitochondria corresponds to about 1 mole/50 000 g. of protein.

DISCUSSION

Chance (1956) has recently compared the properties of liver mitochondria, isolated by himself, with those of heart-muscle mitochondria (sarcosomes) isolated by ourselves in the following words: 'In summary, four of Slater's experimental data on his own preparations distinguish them from the liver preparations; their "less marked" respiratory control, their low pyridine nucleotide content, their low P:O value, and the kinetics of cytochrome *b*. All these differences are consistent with the idea that the muscle sarcosomes studied so far by Slater have been somehow damaged in the course of their preparation. Direct evidence of damage to their respiratory-control mechanism is afforded by evidence cited in Lardy's report on the great range of respiratory control obtainable in intact muscle.'

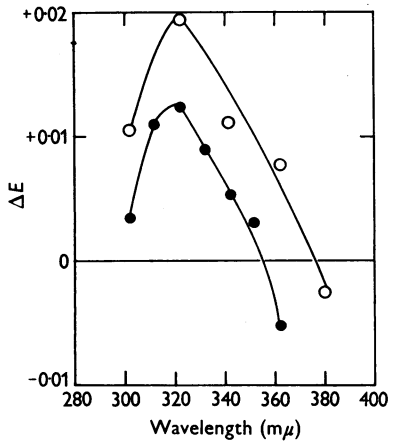

Fig. 10. Difference spectrum (anaerobic *minus* aerobic steady states) of sarcosome suspensions reduced by DL-β-hydroxybutyrate. ●, Rabbit-heart sarcosomes, 9·2 mg. of protein/ml.; DL-β-hydroxybutyrate, 0·0025 M; ATP, 0·005 M. Band widths, 4·3–4·7 mμ, expts. 56/35. ○, Rat-heart sarcosomes, 8·9 mg. of protein/ml.; DL-β-hydroxybutyrate, 0·0065 M; ATP, 0·006 M; sucrose, 0·28 M; EDTA, 0·001 M. Band widths, 4·3–4·5 mμ, expts. 56/36.

Table 7. *Calcium and iron content of rat-liver mitochondria and heart sarcosomes*

	Liver mitochondria	Heart sarcosomes
Calcium (μmoles/g. of protein)	61	99*
Iron (μmoles/g. of protein)	22	18

* From Slater & Cleland (1953).

In considering these differences it should be remembered that Chance has not reported any experiments on heart sarcosomes. The sarcosomes referred to by Chance & Williams (1955*c*) were isolated from blowfly thoracic muscle. We have found that these preparations phosphorylate much more poorly than heart sarcosomes (Lewis & Slater, 1954). The only other study of oxidative phosphorylation in heart that we are aware of was made by Maley & Plaut (1953, 1954), who obtained slightly higher P:O ratios than ours with α-oxoglutarate, and considerably higher ratios with succinate. Unfortunately, none of the other properties discussed in this paper have been studied with the sarcosomes isolated by Maley & Plaut, and we have not succeeded in isolating sarcosomes with such high P:O values. In what follows therefore the properties of our own preparations of heart sarcosomes are compared with those of liver mitochondria. Concerning liver mitochondria, we are essentially in agreement with other workers.

(1) *Respiratory control with isolated mitochondria.* Fig. 8 demonstrates a stimulation of 4·8-fold in the rate of oxidation of glutamate by heart sarcosomes, brought about by the addition of ADP. This is somewhat lower than the degree of stimulation which we obtained under similar conditions with liver mitochondria (about sevenfold); Lardy & Wellman (1952) found a stimulation of about tenfold, and Chance & Williams (1955*a*), under rather different conditions, report an even greater stimulation. The greater ATPase activity of heart sarcosomes in the presence of Mg^{2+} ions and absence of DNP, compared with liver mitochondria, might lead one to expect an even bigger difference in the respiratory-control indices of the two types of mitochondria. That it is not larger can be partly explained by the higher respiratory activity of the heart sarcosomes. A similar respiratory-control index was obtained with our usual preparations of sarcosomes, and with those preparations in which an attempt was made to keep the ATPase activity in the absence of DNP as low as possible.

(2) *Diphosphopyridine nucleotide content of isolated mitochondria.* Chance's (1956) statement concerning the low DPN content of heart sarcosomes is not supported by any of the published data on the sarcosomes. The high activity of the α-oxoglutaric oxidase system in the sarcosomes strongly suggested that they contained DPN, and this is confirmed by the direct analyses reported by Holton (1955) and in this paper. In fact, the sarcosomes contain somewhat more DPN than liver mitochondria. Although

Chance (1956) specifically refers to the 'low pyridine nucleotide content' of heart sarcosomes, it is possible that he may have had in mind enzymically reducible DPN (see Chance & Williams, 1956), and his comment was probably inspired by Holton's (1955) failure to demonstrate in the difference spectrum of sarcosomes a maximum which could be ascribed to reactions of endogenous DPN. It now appears that this failure was due to the restricted range of wavelengths studied. In work reported above, measurements extending between 300 and 445 mμ have detected a marked peak at 320 mμ in the difference spectrum of sarcosomes reduced by β-hydroxybutyrate. If the whole of the extinction at 320 mμ is assumed to result from reduction of bound DPN, its magnitude corresponds to between 55 and 80 % of that calculated from the measured DPN content of the sarcosomes. This calculation assumes that the extinction coefficient at 320 mμ of sarcosome-bound DPNH in a turbid suspension is equal to that of DPNH at 340 mμ in clear solution. In view of this and other uncertainties of the calculation (for instance, sarcosome-bound DPNH may be expected not to be fully oxidized in the aerobic steady state), these spectrophotometric results represent satisfactory agreement with the direct analyses. It is clear, then, that the DPN previously found by Holton (1955) in the sarcosomes is reducible by the β-hydroxybutyric dehydrogenase in these granules.

(3) *The low* P:O *ratio in heart sarcosomes.* The P:O ratios obtained with our sarcosomes are lower than those found with liver mitochondria, particularly with succinate as substrate.

In our attempts to determine the P:O ratio of heart sarcosomes (Slater & Holton, 1954), we were conscious of the fact that it would be difficult to isolate the sarcosomes from the muscle, where they are trapped between the myofibrils, without damaging some. There was a strong possibility therefore that our preparation would contain some damaged sarcosomes, even if most were intact. We therefore studied, in some detail, the effect of damaging sarcosomes in various ways: e.g. by mechanical damage, by swelling in hypotonic media, or by incubation (Slater & Cleland, 1953). The significant result was that while all these treatments of heart sarcosomes drastically reduced the activity of the α-oxoglutaric oxidase system, they had only a slight effect on the P:O ratio. Thus we felt confident that the damaged sarcosomes, which were probably present, would contribute little to the oxygen uptake with α-oxoglutarate as substrate, and since this oxygen uptake would be accompanied by only a slightly lower phosphorylation ratio, the final effect of the damaged sarcosomes on the measured phosphorylation ratio would be insignificant. Thus the measured ratio would represent the true ratio of the undamaged sarcosomes.

It must be emphasized that this argument was applied only to the heart sarcosomes oxidizing α-oxoglutarate. Our results indicated that the P:O ratio for the step α-oxoglutarate$\rightarrow$succinate was very close to 3·0. This is not very different from liver mitochondria, and is not inconsistent with the generally accepted view that there are four phosphorylation steps in the α-oxoglutaric oxidase system (see Slater, 1956). This argument was not applied to the blowfly thoracic-muscle sarcosomes, since the P:O ratio was found very susceptible to various treatments (Lewis & Slater, 1954).

The argument was also not applied to the heart sarcosomes oxidizing succinate, since damage to the particles increased the respiratory activity (by decreasing the steady-state concentration of the inhibitory oxaloacetate) with a marked loss of phosphorylative activity. Thus the presence of damaged sarcosomes will appreciably lower the experimental P:O ratio when succinate is the substrate. It is significant that, in contrast with the very reproducible results obtained with α-oxoglutarate (Slater & Holton, 1954), our P:O ratios with succinate and rat-heart sarcosomes vary over a rather wide range, between 0·7 and 1·4 in sixteen experiments, with most values near 1. It is impossible for us to say whether our generally low values represent a difference between intact sarcosomes and liver mitochondria, or are due to damaged sarcosomes in our preparation. However, if the latter is the case it does mean that our sarcosomal preparation contains a large proportion of damaged sarcosomes (a mixture of 33 % damaged particles with zero phosphorylation and double the succinic oxidase activity of intact particles, and 66 % intact sarcosomes with a P:O ratio of 2 is necessary to give an experimental ratio of 1). Despite exhaustive attempts, we have not succeeded in isolating rat-heart sarcosomes which consistently have a P:O ratio much above 1 with succinate. We are continuing experiments in an attempt to explain the difference between our results and those of Maley & Plaut (1953, 1954) in this respect. Guinea-pig-heart sarcosomes often have higher ratios (K. Minnaert, unpublished work).

In our previous paper (Slater & Holton, 1954), we expressed the view that Copenhaver & Lardy's (1952) procedure, involving a short equilibration time and relatively small oxygen uptakes, appreciably underestimated the oxygen uptakes, and that consequently their values for the P:O ratios were too high. Although this was certainly the case when we used what we understood to be Copenhaver & Lardy's procedure, it now seems clear that no serious error accompanied their use of this technique (Lardy & Copenhaver, 1954). Our own

measurements shown in Table 1 do not, however, show any appreciable difference between the P:O ratios of heart and liver mitochondria oxidizing α-oxoglutarate, although our ratios with liver mitochondria oxidizing succinate and β-hydroxybutyrate are a little higher than those of Copenhaver & Lardy (1952).

(4) *Kinetics of cytochrome* b. There is a remarkable difference between the kinetics of cytochrome *b* in the two preparations. Holton (1955) has shown that if an anaerobic suspension of heart sarcosomes in a medium containing succinate and ADP is aerated, the cytochrome *b* is only partially oxidized (it remained nearly 100% reduced in one experiment), but becomes more oxidized during the subsequent aerobic steady state. In contrast, cytochromes *c* and a_3 are immediately and completely oxidized, and remain oxidized during the aerobic steady state. The corresponding experiment has not been done with liver mitochondria, but Chance & Williams's (1955*a*) data suggest that under these conditions cytochrome *c* would be 13% reduced, and cytochrome *b* 16% reduced. The degree of reduction of cytochrome *b* in the aerobic steady state in the presence of ADP is then a clear-cut difference between the mitochondria isolated from the two tissues. It is important to note that the high degree of reduction of cytochrome *b* in the aerobic steady state is characteristic of the phosphorylating heart sarcosomes. The addition of DNP caused the cytochrome *b* to be largely oxidized in the steady state.

The explanation of the subsequent change in the steady state of cytochrome *b* has not been established. It is possible, however, that it coincides with the phosphorylation of the ADP to ATP. If this is the case, then it follows that as ADP is phosphorylated to ATP, the cytochrome *b* becomes more oxidized. This is the exact opposite of the behaviour of cytochrome *b* in liver mitochondria (Chance & Williams, 1955*a*).

Chance & Williams (1955*a*) have found an important difference between the kinetics of cytochrome *b* in phosphorylating mitochondrial preparations, where it is rapidly reduced, and in non-phosphorylating sarcosomal fragments (Keilin & Hartree heart-muscle preparation) where it is slowly reduced (Slater, 1950; Chance, 1952). Since the exact role of cytochrome *b* in the respiratory chain is still obscure, the significance of this difference in its kinetics remains uncertain. However, the high degree of cytochrome *b* in the aerobic steady state found by Holton (1955) in heart sarcosomes suggests that the rate of reduction of this cytochrome is not impaired in these granules.

(5) '*Direct evidence of damage*'. Since Lardy's (1956) paper refers to skeletal muscle, we cannot agree with Chance that it provides direct evidence of damage to our heart preparations. Skeletal muscle is intermittent in its activity, compared with the almost constant activity of the intact heart. Melrose, Dreyer, Bentall & Baker (1955) have reported experiments which provide good evidence of metabolic control in the intact heart (see Slater, 1957). However, it is not certain whether the control of the metabolic rate by muscular activity in heart and skeletal muscle is due to respiratory control or to the effect of muscular activity on the rate of glycolysis (Cori, 1956). If the latter is blocked, no substrate is supplied to the respiratory enzymes. In any case, the experiment described in Fig. 8 shows that isolated sarcosomes are capable of a considerable degree of respiratory control.

ATPase activity of liver and heart mitochondria

The enzyme systems catalysing the hydrolysis of ATP in the two types of mitochondria are very similar. Under the same conditions, the pH–activity curves were of the same general shape, although the positions of all the optima were about 0·2–0·3 pH unit lower in the sarcosomes than in the liver.

With regard to the question whether or not the isolated sarcosomes are damaged, the most interesting difference between the two types of mitochondria is the stimulation of the ATPase activity by added Mg^{2+} ions. As a consequence, the degree of stimulation by DNP, in the presence of magnesium at pH 7·4, was only about twofold with sarcosomes prepared in the way used in previous studies, compared with at least tenfold with liver mitochondria. This difference could be interpreted to support the contention that the sarcosomes are damaged. It is indeed probable that the sarcosomes isolated by the method of Cleland & Slater (1953) are slightly damaged since the ATPase activity in the absence of DNP can be lowered to some extent by varying the isolation procedure. However, we have not been able to isolate sarcosomes which show more than a fourfold increase in the latent ATPase activity at pH 7·4 on addition of 10^{-4}M-DNP to a reaction medium containing Mg^{2+} ions. Similar results were obtained by Chappell & Perry (1954) with pigeon-breast-muscle sarcosomes. The procedure used in the isolation of the sarcosomes from the hearts of young rats and guinea pigs was relatively mild and would not be expected to damage the sarcosomes more than the liver mitochondria would be damaged during their isolation.

It is possible that the moderately high ATPase activity observed in the absence of DNP is an intrinsic property of these sarcosomes. Perry (1956) has suggested that the sarcosomes isolated from pigeon-breast muscle contain a contractile system similar to actomyosin in that it requires ATP to function normally. It is possible that this contractile system is responsible for a portion

of the ATPase activity observed in the absence of DNP.

Perry's (1956) studies may also offer an explanation for the effect of including ATP in the isolation medium, since it is likely that the contracted sarcosomes would be more resistant to damage during the isolation procedure. The denser sarcosomes which are precipitated from the homogenate by centrifuging at low speeds probably represent those sarcosomes in which the most water has been squeezed out by the process of contraction.

Differences between liver and heart mitochondria

Both types of mitochondria exhibit a considerable degree of respiratory control with glutamate and a high phosphorylative activity with α-oxoglutarate. When fully activated by DNP, or by freezing and thawing, the pH–activity curves for the hydrolysis of ATP are very similar. There are, however, a number of differences between the two preparations, the most important of which are:

(1) The higher respiratory activity of the heart preparations, which is probably a consequence of their greater content of cytochromes (this is obvious to the eye, since liver mitochondria appear much paler than the heart preparations) and of DPN, and which may also be related to their greater ATPase activity after complete activation by ageing, freezing and thawing.

(2) The kinetics of cytochrome *b* considered above.

(3) The lower P:O ratio obtained with sarcosomes oxidizing succinate.

(4) The higher ATPase activity of sarcosomes in the presence of Mg^{2+} ions and absence of DNP.

Tapley & Cooper (1956) have shown that heart sarcosomes are much more resistant than liver mitochondria to swelling induced by thyroxine. There is also evidence from the effect on the P:O ratio with ferrocytochrome *c* as substrate that heart sarcosomes are more resistant than liver mitochondria to morphological changes induced by hypotonicity (Slater, 1956).

Less important differences are the low myokinase (Cleland & Slater, 1953) and rhodanese (Moyle, 1953) activity of heart sarcosomes.

It seems likely that the high respiratory activity of isolated heart sarcosomes is a physiological characteristic, and is not brought about during their isolation. Although it is not possible at present to say that this is also the case with the other differences between liver and heart mitochondria, the work reported above can be as well explained in terms of physiological as of artifactual contrasts. It would thus seem preferable for calculations relating to muscle physiology to be based on biochemical properties of muscle sarcosomes, as these become better known, rather than on the properties of liver mitochondria.

SUMMARY

1. Succinate and α-oxoglutarate were oxidized faster by rat-heart sarcosomes (mitochondria) than by rat-liver mitochondria. β-Hydroxybutyrate was oxidized at about the same rate by the two preparations.

2. Heart sarcosomes oxidizing succinate and β-hydroxybutyrate yielded lower P:O ratios than liver mitochondria oxidizing the same substrates. The maximum rate of phosphorylation per unit weight (Q_P) was greater in heart sarcosomes than in liver mitochondria.

3. The properties of the enzyme systems in the two types of mitochondria after activation by the addition of dinitrophenol or by freezing and thawing, bringing about the hydrolysis of added adenosine triphosphate (ATP), were very similar. In the absence of dinitrophenol or other activating treatment, magnesium stimulated these enzyme systems in heart sarcosomes, but had little effect on the hydrolysis of ATP by liver mitochondria.

4. Factors affecting the degree of respiratory control in the two types of mitochondria are similar. The addition of adenosine diphosphate to heart sarcosomes oxidizing glutamate stimulated the rate of respiration about fivefold. The greater degree of respiratory control in liver mitochondria may be satisfactorily explained by their lower ATPase activity.

5. Both spectrophotometric measurements of endogenous pigments in the intact mitochondria and direct chemical analyses showed that liver and heart mitochondria contained similar amounts of diphosphopyridine nucleotide. They also contain similar amounts of iron. Heart sarcosomes contain a considerably higher concentration of the cytochrome system and a greater specific ATPase activity after full activation.

6. The differences between the enzymic behaviour of heart sarcosomes and liver mitochondria are discussed. The higher respiratory activity of isolated sarcosomes is probably a physiological characteristic, and is not brought about during their isolation. Although it is not possible at present to be certain that this is also the case with the other differences between liver and heart mitochondria, the work reported in this paper is more readily explicable in terms of physiological rather than artifactual contrasts, and offers no support for the idea that their patterns of enzymic behaviour are identical *in vivo*.

We wish to acknowledge with thanks the skilled assistance of Mrs M. Jong-Tuynman in the experiments on respiratory control and on ATPase. We also wish to thank Mr K. Minnaert and Dr J. L. Purvis for some of the measurements of the P:O ratios with liver mitochondria.

REFERENCES

Baltscheffsky, H. (1956). *Biochim. biophys. Acta*, **20**, 434.
Beechey, R. B. (1957). *Biochem. J.* **66**, 10P.
Chance, B. (1952). *Nature, Lond.*, **169**, 215.
Chance, B. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 300.
Chance, B. & Williams, G. R. (1955*a*). *J. biol. Chem.* **217**, 409.
Chance, B. & Williams, G. R. (1955*b*). *J. biol. Chem.* **217**, 383.
Chance, B. & Williams, G. R. (1955*c*). *J. biol. Chem.* **217**, 395.
Chance, B. & Williams, G. R. (1956). *Advanc. Enzymol.* **17**, 65.
Chappell, J. B. & Perry, S. V. (1954). *Nature, Lond.*, **173**, 1094.
Cleland, K. W. & Slater, E. C. (1953). *Biochem. J.* **53**, 547.
Copenhaver, J. A. & Lardy, H. A. (1952). *J. biol. Chem.* **195**, 225.
Cori, C. F. (1956). In *Enzymes: Units of Biological Structure and Function*, p. 573. New York: Academic Press.
Green, D. E. (1956). In *Enzymes: Units of Biological Structure and Function*, p. 465. New York: Academic Press.
Holton, F. A. (1955). *Biochem. J.* **61**, 46.
Holton, F. A. (1956). *Biochem. J.* **64**, 57P.
Keilin, D. & Hartree, E. F. (1947). *Biochem. J.* **41**, 500.
Keilin, D. & Hartree, E. F. (1949). *Biochem. J.* **44**, 205.
Kielley, W. W. & Kielley, R. K. (1953). *J. biol. Chem.* **200**, 213.
Lardy, H. A. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 287.
Lardy, H. A. & Copenhaver, J. A. (1954). *Nature, Lond.*, **174**, 231.
Lardy, H. A. & Wellman, H. (1952). *J. biol. Chem.* **195**, 215.
Lardy, H. A. & Wellman, H. (1953). *J. biol. Chem.* **201**, 357.
Lehninger, A. L. & Smith, S. W. (1949). *J. biol. Chem.* **181**, 415.
Lewis, S. E. & Slater, E. C. (1954). *Biochem. J.* **58**, 207.
Mahler, H. R. & Elowe, D. G. (1954). *J. biol. Chem.* **210**, 165.
Maley, G. F. & Plaut, G. W. E. (1953). *J. biol. Chem.* **205**, 297.
Maley, G. F. & Plaut, G. W. E. (1954). *Biochim. biophys. Acta*, **14**, 443.
Melrose, D. G., Dreyer, B., Bentall, H. H. & Baker, J. B. E. (1955). *Lancet*, **269**, 21.
Montgomery, C. M. & Webb, J. L. (1956). *J. biol. Chem.* **221**, 347.
Moyle, J. M. (1953). *Nature, Lond.*, **172**, 508.
Myers, D. K. & Slater, E. C. (1957). *Biochem. J.* **67**, 558.
Perry, S. V. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 300.
Plaut, G. W. E. & Plaut, K. A. (1952). *J. biol. Chem.* **199**, 141.
Racker, E. F. (1950). *J. biol. Chem.* **184**, 313.
Sacktor, B. (1954). *J. gen. Physiol.* **37**, 343.
Slater, E. C. (1949). *Biochem. J.* **45**, 1.
Slater, E. C. (1950). *Biochem. J.* **46**, 484.
Slater, E. C. (1956). *Proc. 3rd Int. Congr. Biochem., Brussels*, 1955, p. 264.
Slater, E. C. (1957). *Ned. Tijdschr. Geneesk.* **101**, 732.
Slater, E. C. & Cleland, K. W. (1953). *Biochem. J.* **55**, 566.
Slater, E. C. & Holton, F. A. (1953). *Biochem. J.* **55**, 530.
Slater, E. C. & Holton, F. A. (1954). *Biochem. J.* **56**, 28.
Stern, H. & Timonen, S. (1956). *Exp. Cell Res.* **9**, 101.
Tapley, D. F. & Cooper, C. (1956). *Nature, Lond.*, **178**, 1119.
Walker, P. G. (1954). *Biochem. J.* **58**, 699.
Witter, R. F., Watson, M. L. & Cottone, M. A. (1955). *J. biophys. biochem. Cytol.* **1**, 127.

Purine and Pyrimidine Transaminases in *Escherichia coli**

By A. H. SCHEIN† and E. M. BROWN

Department of Biochemistry, University of Vermont College of Medicine, Burlington, Vermont, U.S.A.

(*Received* 9 *April* 1957)

Biological conversion of amino purines and pyrimidines into their hydroxy compounds has hitherto been ascribed exclusively to the action of hydrolytic deaminases. The report that suspensions or extracts of *Escherichia coli* (strains B and Crookes) catalyse transamination between α-oxoglutarate and adenine, guanine or cytosine (Gunsalus & Tonzetich, 1952) would point to the existence of important alternate metabolic pathways for these constituents of nucleic acids. The transamination reaction, as usually understood, involves an intermolecular oxidation–reduction. This reaction is not possible with an intact purine or pyrimidine ring; therefore transamination of these compounds must involve a mechanism not previously described or must be preceded by a change in the ring such as reduction. It seemed therefore of importance to repeat and to extend the experiments of Gunsalus & Tonzetich (1952). We have not been able to confirm their observations by employing similar bacteria and similar experimental conditions. Nor was transamination evident, when, in a limited number of experiments, mammalian-liver homogenates or slices replaced bacterial preparations.

* This investigation was supported by the research grant A 139 and A 139 C from the National Institutes of Health, Public Health Service, and by a Lederle Medical Student Research Fellowship (E.M.B.).

† Exchange Senior Lecturer in Biochemistry, The Medical College of St Bartholomew's Hospital, London, E.C. 1.