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The Conversion of Adenosine 5'-Phosphate into Adenosine Triphosphate as Catalysed by Adenosine Triphosphate-Creatine Phosphotransferase and Adenosine Triphosphate-Adenosine Monophosphate Phosphotransferase in the Presence of Phosphocreatine

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Morrison & Doherty (1961) have reported that the addition of adenosine 5'-phosphate and phosphocreatine to a partially purified rabbit-muscle preparation, treated with charcoal and Dowex 1 (Cl⁻ form) to remove nucleotides, results in the formation of adenosine triphosphate and creatine according to equation (1).

Further, they found that a similar reaction was catalysed by the combined action of creatine kinase (adenosine triphosphate-creatine phosphotransferase, EC 2.7.3.2) and adenylate kinase (adenosine triphosphate-adenosine monophosphate phosphotransferase, EC 2.7.4.3). These findings raise once again the question whether or not the phosphoryl group of phosphocreatine can be transferred directly to AMP.

An enzyme catalysing such a direct transfer was reported by Banga (1943), who claimed that, together with creatine kinase, it could bring about reaction (1). But with the discovery of adenylate

kinase (Colowick & Kalckar, 1943) it became generally accepted that reaction (1) was the sum of reactions (2) and (3),

and

which occurred because of the presence of trace amounts of either ADP or ATP (Chappell & Perry, 1954). Molnar & Lorand (1960) showed that reaction (1) occurs even after exhaustive dialysis of crystalline preparations of creatine kinase and adenylate kinase.

Further investigations have been made of the conversion of AMP into ATP in the presence of phosphocreatine and the results of these are reported in this paper. It has been shown that reaction (1) occurs with highly purified preparations of AMP and purified preparations of the enzymes that have been treated repeatedly with charcoal

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and Dowex 1 (Cl^- form), and it would appear that reaction is due to trace amounts of ATP which are firmly bound to creatine kinase and which are incompletely removed by methods usually considered to be effective for the removal of nucleotides. It has also been confirmed that creatine kinase does not bring about the phosphorylation of AMP to ADP.

EXPERIMENTAL

Materials

Chemicals. The preparation and purification of a number of the chemicals used in this work have been described by Morrison & Doherty (1961). Bovine albumin, obtained from Armour Laboratories, London, was treated with charcoal by the following procedure: the protein solution (25 mg./ml.) was stirred with acid-washed Norit A (1 g./10 ml.) for 30 min. at 2° , filtered on a Buchner funnel and then stirred for 17 hr. with a similar amount of Norit A. Both treatments were then repeated and charcoal was removed by high-speed centrifuging.

Enzymes. The methods for the preparation of myosin adenosine triphosphatase, apyrase, crystalline creatine kinase and rabbit-muscle fraction 2 have been described by Morrison & Doherty (1961). Adenylate kinase was obtained by three different procedures. For the preliminary experiments, a modification of the method of Colowick & Kalckar (1943) was used; the 50–80% saturated $(\text{NH}_4)_2\text{SO}_4$ fraction was dissolved in and dialysed against water for 17 hr. at 2° . This solution was stirred with Norit A (1 g./25 ml.) for 30 min. at 2° , filtered on a Buchner funnel and the charcoal treatment repeated with stirring for 16 hr. The filtered solution was then stirred for 10 min. with Dowex 1 (Cl^- form) (100 mg./ml.) and the resin removed by filtration. Further purification was achieved as follows. The enzyme solution was diluted with water to give a concentration of 10 mg. of protein/ml., and to this solution was added, with stirring, 0.5 vol. of calcium phosphate gel (25 mg./ml.; prepared according to Keilin & Hartree, 1938). After standing for 5 min. at 2° , the gel was removed by centrifuging at 1500 g. This treatment resulted in the removal of a little over half the total amount of protein with only a small loss of enzymic activity. The enzyme was stored at 1° . Adenylate kinase was also prepared by the method of Callaghan (1957), but the final chromatography on a calcium phosphate-gel column was omitted. Although this preparation showed a single protein peak when subjected to electrophoresis at pH 8.6 and pH 5.2, a number of minor components were detected at pH 7.2. The preparation was free of creatine-kinase activity. Before use, the enzyme was treated with acid-washed charcoal as described for creatine kinase by Morrison & Doherty (1961). Crystalline adenylate kinase was obtained by the method of Noda & Kubo (1957) and stored in the $(\text{NH}_4)_2\text{SO}_4$ solution, from which it was crystallized. The enzyme was dissolved to a concentration of 1 mg./ml. in a solution containing 0.01 M-cysteine (pH 7.2), 0.01 M-N-ethylmorpholine buffer (pH 7.2) and albumin (1 mg./ml.). At this concentration, the enzyme was stable for 1 week at 1° . Subsequent dilutions were made stepwise in an albumin solution (1 mg./ml.) just before use. Because of the small amount of enzyme obtained by the above method and the large loss of protein that occurred on stirring solutions with charcoal, this pre-

paration was not generally treated with charcoal. When the crystalline enzyme was dissolved in albumin solution (1 mg./ml.) and treated with charcoal, the loss of activity was considerably reduced. Preparations treated in this way, together with creatine kinase, were still able to catalyse reaction (1).

Methods

Methods for the estimation of creatine and protein and for the detection of low concentrations of ADP or ATP or both have been described by Morrison & Doherty (1961). The latter procedure involved a linked enzyme system that consisted of creatine kinase and myosin adenosine triphosphatase and in which the nucleotides acted catalytically.

Electrophoresis. This was carried out by Mr R. F. Adams of this Department, using a Perkin-Elmer model 38A apparatus.

Chromatography. Nucleotides were chromatographed on Whatman 3MM paper by ascending chromatography in isobutyric acid-aq. NH_3 (sp.gr. 0.88)-water (66:1:33, by vol.) and detected by visual inspection in ultraviolet light.

RESULTS

Preliminary experiments

Jandorf, Klemperer & Hastings (1941) have reported that it is necessary to stir muscle extracts with charcoal for 12 hr. to ensure complete removal of NAD. In the present work, partially purified rabbit-muscle preparations (fraction 2, Morrison & Doherty, 1961), as well as solutions of crystalline creatine kinase and purified adenylate kinase, have been stirred with charcoal (1 g./10 ml.) for two 12 hr. periods, but no change in enzyme activity was detected. During this investigation it was found that higher concentrations of adenylate kinase prepared by an adaptation of the method of Colowick & Kalckar (1943) and treated with charcoal were capable of catalysing the reaction without the addition of creatine kinase. Subsequently, it was found that the activity was completely lost on dilution of the enzyme, but could be restored by the addition of creatine kinase. The presence of creatine kinase in this preparation was confirmed by direct analysis.

The rate of creatine release from phosphocreatine in the presence of AMP, creatine kinase and adenylate kinase was markedly increased by the addition of Mn^{2+} , Mg^{2+} , Ca^{2+} or Co^{2+} ions (Table 1), whereas Al^{3+} , Fe^{3+} , Be^{2+} , Sr^{2+} , Ba^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} and Li^+ ions did not cause any activation. For subsequent experiments, Mg^{2+} was chosen as the activating metal ion and the relationship between Mg^{2+} ion concentration and overall enzymic activity is given in Table 2. The optimum pH for the reaction was pH 7.2.

The nucleotide resulting from the phosphorylation of AMP in the presence of phosphocreatine and the two kinases has been identified as ATP by

chromatography of the reaction mixture and comparison of its behaviour with that of an authentic sample of ATP. A similar result was obtained when the purified enzymes were replaced by a partially purified rabbit-muscle preparation (fraction 2, Morrison & Doherty, 1961). A study of the stoichiometry of the reaction, under conditions where phosphocreatine was in excess, showed that 2 moles of creatine are released/mole of AMP phosphorylated. Thus the equilibrium of the reaction lies completely to the right.

Kinetics of the release of creatine from phosphocreatine in the presence of adenosine monophosphate, creatine kinase and adenylate kinase

The time-curve for the release of creatine from phosphocreatine (Fig. 1) is similar to that obtained by Chappell & Perry (1954), who took no special precautions to exclude ADP and ATP. The autocatalytic nature of the curve suggests that ADP is formed during the reaction and that the velocity increases until such time as creatine kinase is saturated with this substrate; the reaction rate

then becomes linear and is a function of the concentration of this enzyme. Extrapolation of the linear part of the curve to the base line gives a measure of what subsequently will be called the lag period.

It was important to gain some indication of the concentration of nucleotide that could prime the overall reaction. This could not, of course, be determined directly so that the amount of ATP which would give a small reduction in the lag period was determined. It was found that, at a concentration of 50 μM or above, ATP reduced the lag period slightly without affecting the rate of creatine release over the linear part of the curve (Fig. 2). At 10 μM , ATP was without effect.

These results are consistent with the formation or presence of catalytic amounts of ATP or ADP or both in the reaction system. If it be assumed that the reaction is due to either of these compounds, then it follows that they are (a) formed as a result of the phosphorylation of AMP by phosphocreatine, (b) present in the enzyme preparations and are not removed by dialysis or treatment with charcoal and Dowex 1 (Cl^- form) or (c) present in one of the other components of the system. In the first instance, experiments were carried out to determine whether either of the kinases was able to catalyse a direct transfer of the phosphoryl group of phosphocreatine to AMP. Although it has been reported that creatine kinase does not catalyse this reaction (Ennor & Rosenberg, 1954; Oliver, 1954; Chappell

Table 1. *Effect of metal ions on the velocity of reaction (1)*

The reaction mixture contained: *N*-ethylmorpholine buffer (pH 7.2), 50 μmoles ; phosphocreatine, 5 μmoles ; albumin, 1 mg.; creatine kinase, 2 $\mu\text{g.}$; adenylate kinase (prepared by an adaptation of the procedure of Colowick & Kalckar, 1943), 1 $\mu\text{g.}$; 2.5 μmoles of metal ion; total volume, 1.0 ml. Incubation was for 5 min. at 37°.

Metal ion	Creatine released ($\mu\text{mole/ml.}$)
None	0.04
Ca^{2+}	0.55
Co^{2+}	0.51
Mg^{2+}	0.61
Mn^{2+}	0.79*

* Mn^{2+} ion interferes with the method for the estimation of creatine.

Table 2. *Effect of concentration of magnesium on the velocity of reaction (1)*

The reaction mixture was the same as that described in Table 1 except that the concentration of magnesium was varied. Incubation was for 4 min. at 37°.

Concn. of magnesium (mm)	Creatine released ($\mu\text{mole/ml.}$)
0	0.040
0.001	0.066
0.01	0.095
0.1	0.444
1.0	0.702
2.5	0.630
10.0	0.301

Fig. 1. Rate of release of creatine from phosphocreatine in the presence of AMP, creatine kinase and adenylate kinase. The reaction mixture contained: *N*-ethylmorpholine buffer (pH 7.2), 50 μmoles ; phosphocreatine, 2.5 μmoles ; MgSO_4 , 1 μmole ; AMP, 1 μmole ; albumin, 1 mg.; creatine kinase, 2 $\mu\text{g.}$; adenylate kinase (Callaghan preparation), 1 $\mu\text{g.}$ Total volume was 1.0 ml. Incubation was at 37°.

& Perry, 1954), the methods used to detect ADP formation depended on the stoichiometric assay of ADP, and a low concentration of this nucleotide could have escaped detection.

No release of creatine was obtained when phosphocreatine and AMP were incubated for 3 hr. with 0.5 mg. of either creatine kinase or adenylate kinase under the conditions described in Fig. 1, even in the presence of apyrase. Each of the incubated reaction mixtures was also analysed for the presence of ATP or ADP by the sensitive procedure of Morrison & Doherty (1961). Although the sensitivity of this method was reduced by the presence of AMP, it was estimated that less than $1 \mu\text{mole}$ of these compounds/ml. was formed. Thus it may be concluded that the overall reaction is not initiated by the formation of ADP from AMP and phosphocreatine.

On the basis that reaction occurs because of the presence of ATP or ADP in one or more of the reaction components, attempts were made to stop the reaction by converting these compounds into AMP as a result of prior incubation with apyrase.

Effect of apyrase on the reaction. The possibility of contamination of phosphocreatine was not considered, as this compound was chemically synthesized from creatine. When the reaction components, less phosphocreatine, were preincubated with apyrase ($6 \mu\text{g.}$) for 10 min. under the conditions described in Fig. 1, and the reaction was started by addition of phosphocreatine, there was

no release of creatine. A similar result was obtained in the presence of apyrase when preincubation was omitted. The inhibition by apyrase was not overcome by increasing the concentration of creatine kinase. However, a fivefold increase in adenylate kinase resulted in the release of creatine, although at a considerably slower rate than that obtained in the absence of apyrase, and preincubation of the reaction components with apyrase did not affect the results. It follows that if ADP or ATP or both are present as contaminants of the reaction components, they cannot be hydrolysed by apyrase. The inhibition of the overall reaction by this enzyme is also consistent with the idea that one or both nucleotides are formed during the reaction.

Efforts were therefore made to determine whether the albumin, which was used to stabilize adenylate kinase, or the AMP was contaminated by ADP or ATP.

Attempt to detect the presence of adenosine diphosphate or adenosine triphosphate in albumin and adenosine monophosphate. When charcoal-treated albumin was added to the reaction mixture at a concentration five times as great as that used normally, the lag period was unaffected. Moreover, no nucleotide could be detected in 5 mg. of albumin when assayed in the creatine kinase-myosin adenosine triphosphatase system. The sensitivity of this system is limited to the detection of one part of ADP or ATP in 2500 parts of AMP and was thus inadequate for the detection in AMP of concentrations of nucleotide that would prime the overall reaction. It might have been expected that if AMP were contaminated by ADP or ATP, then the presence of these compounds could be confirmed by adding AMP in concentrations greater than that required for saturation of the enzyme with which it reacts. The lag period of the reaction would be reduced without any effect on the steady-state rate. However, the addition of AMP in concentrations greater than 1 mM increased the lag period and decreased the steady-state rate, presumably because of the inhibition of one or both enzymes. Thus no conclusions could be drawn about whether AMP contained ATP or ADP. On the other hand, AMP that had been purified by chromatography twice on a column of Dowex 1 (Cl^- form) (see the Methods section) and isolated in the crystalline form gave results identical with those obtained above. Thus it seemed reasonable to conclude that AMP was not the source of contaminating nucleotide(s).

The possibility remained that one or other of the kinases contained ATP or ADP. Analysis (Morrison & Doherty, 1961) of crystalline adenylate kinase (1 mg.) and charcoal-treated creatine kinase (5 mg.) revealed no nucleotide in solutions of the native enzymes, in boiled solutions or in the supernatants after precipitation of the protein with

Fig. 2. Effect of added ATP on the length of the lag period. The reaction mixture contained: *N*-ethylmorpholine buffer (pH 7.3), 200 μmoles ; albumin, 2 mg.; phosphocreatine, 5 μmoles ; magnesium acetate, 5 μmoles ; AMP, 2 μmoles ; creatine kinase, 20 $\mu\text{g.}$; crystalline adenylate kinase (not treated with charcoal), 1.2 $\mu\text{g.}$; added ATP; total volume, 2.0 ml. Samples (0.2 ml.) were taken for the estimation of creatine. Incubation was at 37° . ●, No additions or ATP (10 μM); ▲, ATP (50 μM); ■, ATP (300 μM).

perchloric acid. Attention was therefore directed to a study of the kinetics of the reaction, under conditions where the concentration of one enzyme was held constant and that of the other varied, in the hope that such studies would yield evidence for or against contamination.

No conclusions could be reached about whether adenylate kinase contributed ADP or ATP to the system from those experiments in which the concentration of adenylate kinase was varied while that of creatine kinase was held constant (Fig. 3). However, increasing concentrations of adenylate kinase decreased the lag period and the velocity over the linear portion of the curve was a linear function of the enzyme concentration. On the other hand, the results obtained when the concentration of creatine kinase was varied in the presence of a fixed concentration of adenylate kinase are indicative of the addition of nucleotide with the former enzyme (Fig. 4). Thus when the concentration of creatine kinase was increased from 10 to 50 $\mu\text{g./2 ml.}$, there was no effect on the steady-state velocity, but there was a marked reduction in the lag period. Further, it may be concluded that creatine kinase catalyses the second step of what is considered to be a two-step reaction.

If it be assumed that the extra addition of 40 $\mu\text{g.}$ of creatine kinase adds sufficient ATP or ADP to the system to increase the concentration by at least 50 μmM (cf. Fig. 2), then 5 mg. of the enzyme would contain about 10 μmoles of nucleotide. As this amount of free nucleotide can be readily detected by the assay system of Morrison & Doherty (1961), and as no nucleotide could be detected in 5 mg. of

the enzyme, then one of two conclusions must be drawn. Either the bound nucleotide is much more effective in reducing the lag period or it is bound to the protein in such a way as to preclude detection by the method used.

Further evidence for the presence of nucleotide in crystalline preparations of creatine kinase could be deduced from the increase in the $E_{280}:E_{260}$ ratio of solutions of the enzyme as a result of treatment with charcoal (Table 3). A similar increase was observed after prolonged dialysis of the enzyme against buffer containing EDTA, but no such increase occurred when albumin solutions were treated with charcoal. The $E_{280}:E_{260}$ ratios obtained for solutions of once-crystallized enzyme were, even after charcoal treatment, lower than the value of 1.85 reported by Noda, Kuby & Lardy (1954) for a recrystallized preparation.

Fig. 3. Rate of release of creatine from phosphocreatine in the presence of constant amounts of AMP and creatine kinase and various concentrations of adenylate kinase. Conditions were the same as those described in Fig. 2, except that 10 $\mu\text{g.}$ of creatine kinase and crystalline adenylate kinase (●, 0.4 $\mu\text{g.}$; ▲, 0.48 $\mu\text{g.}$; ○, 0.64 $\mu\text{g.}$; △, 0.8 $\mu\text{g.}$), which had not been treated with charcoal, were added.

Fig. 4. Rate of release of creatine from phosphocreatine in the presence of constant amounts of AMP and adenylate kinase and various concentrations of creatine kinase. The reaction mixture was the same as that described in Fig. 2 except that the following amounts of creatine kinase were added: ○, 5 $\mu\text{g.}$; △, 10 $\mu\text{g.}$; ●, 50 $\mu\text{g.}$

Table 3. Spectrophotometric detection of nucleotide material in solutions of once-crystallized creatine kinase

Treatment	$E_{280}:E_{260}$ ratio
None	1.65
Enzyme (10 mg./ml.) stirred with Norit A (1 g./10 ml.) for 100 min.	1.77
Filtrate from above treated further with Norit A (1 g./10 ml.) for 16 hr.	1.77
Enzyme (10 mg./ml.) dialysed against 1 mM-EDTA in 1 mM-N-ethylmorpholine buffer (pH 8.0) for 3 days and then against 1 mM buffer for 3 days	1.74

In contrast with the results obtained with creatine kinase that had been treated with charcoal, it was found that analysis of 5 mg. of once-crystallized enzyme did reveal the presence of nucleotide. The amount was small, corresponding to less than $0.1 \mu\text{mole}$ of nucleotide/g. of protein.

Identification of adenosine triphosphate. The isolation of a compound, with chromatographic properties similar to those of ATP, from eluates of charcoal that had been stirred with solutions of the enzyme supports the above conclusions. The compound was isolated by the following procedure. Once-crystallized creatine kinase (200 mg.) was dissolved in 5 ml. of water and stirred for 100 min. with 500 mg. of Norit A at 2° . The charcoal was collected by filtration on a Buchner funnel and washed with water, and the adsorbed material eluted from the charcoal by stirring three times for 1 hr. with portions (5 ml.) of 10 % (v/v) 3-methylbutan-1-ol. The aqueous layer from the combined eluates was evaporated to dryness under reduced pressure and the residue dissolved in a small quantity of water. The solution, which absorbed ultraviolet light, was applied to Whatman 3 MM paper and chromatographed (see the Methods section). The behaviour of the material was identical with that of an authentic sample of ATP. The amount of material was too small for further spectrophotometric studies, but it may be concluded tentatively that it is ATP.

DISCUSSION

The conversion of AMP into ATP in the presence of phosphocreatine by partially purified rabbit-muscle preparations (Morrison & Doherty, 1961) and by the combined action of crystalline preparations of adenylate kinase and creatine kinase can be ascribed to reactions (2) and (3), which occur presumably because ATP is present as a contaminant of creatine kinase and is not completely removed by treatment with charcoal and Dowex 1 (Cl^- form). Though it cannot be categorically stated that this is the case, such a conclusion appears reasonable from the evidence that the crystalline enzyme contains ATP and activity is not lost as a result of treatment with charcoal and resin. Reactions (2) and (3) constitute an autocatalytic system and thus only small amounts of ATP would be required to prime the reaction. The results of kinetic studies are consistent with the reaction of added AMP and small amounts of ATP to yield ADP as an intermediate (Fig. 1). In addition, these studies are in accordance with the presence of low concentrations of ATP in creatine kinase treated with charcoal (Fig. 4).

The possibility of adenylate kinase also containing ATP has not been eliminated, but the presence

or absence of nucleotide in this enzyme is immaterial as far as the overall reaction is concerned. Provided that one of the enzymes can contribute ATP to the system, reaction will occur.

The ability of protein-bound nucleotide to participate in the reaction is not unique, for Yagi & Noda (1960) have shown that ADP bound to myofibrils is converted into ATP on the addition of phosphocreatine and creatine kinase. However, it must be concluded that the ATP bound to creatine kinase cannot function as a substrate for all enzymes involved in the metabolism of ATP, for it is not hydrolysed by either apyrase or myosin (cf. Molnar & Lorand, 1960).

If it be accepted that reaction occurs because of the introduction into the system of catalytic amounts of ATP with the creatine kinase, then it follows that even prolonged treatment of this enzyme with charcoal cannot completely remove the nucleotide. This suggests a very strong binding of ATP to the enzyme and emphasizes the need for caution in reaching the conclusion that a reaction proceeds by a pathway that does not involve the participation of nucleotides. This is especially so when a reaction can be accounted for by well-established reactions involving these compounds.

Molnar & Lorand (1960) have obtained results similar to those reported above with crystalline preparations of the same two enzymes that have been dialysed exhaustively. They believed that this treatment would remove contaminating nucleotides and quoted the lack of effect of prior treatment of the components of the system with apyrase as evidence in support of this belief. However, the difficulty of removing nucleotides from protein by dialysis has been discussed by Martonosi, Gouvea & Gergely (1960). In contrast with the results of Molnar & Lorand (1960), we found that apyrase would inhibit the overall reaction, which is in accordance with the idea that ADP is formed as an intermediate of the reaction. No details are available of the conditions used by Molnar & Lorand (1960) and hence comparison of the two sets of results cannot be made. However, an enzyme inhibition of a two-step reaction is not of the simple type, as it depends, not only on the concentration of the inhibiting enzyme, but also on the concentrations of the enzymes catalysing the two reactions. Thus it is possible to achieve 0–100 % inhibition of the reaction by altering the experimental conditions. The observation by Molnar & Lorand (1960) that creatine kinase catalyses an exchange reaction between $[^{14}\text{C}]$ creatine and phosphocreatine is in disagreement with the results of Noda, Nihei & Morales (1960), but is consistent with the presence of ATP in the crystalline enzyme.

Crystalline preparations of pyruvate kinase have been reported to catalyse the conversion of AMP

into ATP in the presence of phosphoenolpyruvate and adenylate kinase (Bücher & Pfeleiderer, 1955; Molnar & Lorand, 1960). From the present results it is possible that crystalline pyruvate kinase, like creatine kinase, is contaminated by ATP.

SUMMARY

1. Adenosine 5'-phosphate is converted into ATP by the combined action of adenylate kinase (adenosine triphosphate-adenosine monophosphate phosphotransferase) and creatine kinase (adenosine triphosphate-creatine phosphotransferase) in the presence of phosphocreatine, according to the equation:

The conversion is not prevented as a result of treatment of the kinases with charcoal and Dowex 1 (Cl⁻ form) to remove nucleotides.

2. It has been confirmed that neither creatine kinase nor adenylate kinase catalyses the direct phosphorylation of AMP by phosphocreatine.

3. It appears that reaction occurs because crystalline creatine kinase contains a sufficient amount of ATP, even after treatment with charcoal and Dowex 1, to prime an autocatalytic system consisting of these reactions:

and

(catalysed by adenylate kinase and creatine kinase respectively).

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The Lipid Composition of Tumour Cells

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The chemistry of tumour tissues, especially with regard to enzyme systems, protein synthesis, and protein and nucleotide metabolism, has been extensively investigated (Greenstein, 1954), and detailed comparisons of these processes with those in normal tissues have given much information on the general metabolic behaviour of tumour tissue. The lipids in tumour tissues have received very little attention, as a result in part of the lack of suitable analytical techniques for the separation and identification of components, especially minor ones. The development of chromatographic methods, including the establishment of gas chromatography as a routine analytical tool, have now made

possible the quantitative study of complex lipid mixtures, and a growing awareness of the importance of lipid, especially phospholipid, in cell membranes and in the structure of such subcellular particles as mitochondria has stimulated studies of the phospholipid metabolism and composition of tumour tissues (Kogl, Smak, Veerkamp & van Deenen, 1960; Veerkamp, Mulder & van Deenen, 1961). A quantitative study of the phospholipids of tumour tissues with special reference to their intracellular distribution was made by Wallach, Soderberg & Bricker (1960) on Ehrlich ascites-carcinoma cells. Green (1957) found that phospholipids were important determinant groups in lipo-