into ATP in the presence of phosphoenolpyruvate and adenylate kinase (Bücher & Pfleiderer, 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:

$$AMP + 2 \text{ phosphocreatine} \rightarrow ATP + 2 \text{ creatine}.$$

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:

$$AMP + ATP \rightarrow 2 \text{ ADP}$$

and

$$ADP + \text{phosphocreatine} \rightarrow ATP + \text{creatine}$$

(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-

protein antigens and claimed that kephalin phospholipid combined with protein was specifically involved in tumour enhancement (Green & Wilson, 1958). The isolation by chromatography and the properties of a lipid hapten, cytolipin H, from the phospholipid fraction of a human epidermoid carcinoma were described by Rapport, Graf, Skipski & Alonzo (1959), who showed that it was a glycosphingolipid containing fatty acid, sphingosine, glucose and galactose.

In the present work the total lipid composition of Landschutz ascites-carcinoma cells was studied with special reference to the phospholipids and the distribution of fatty acids and aldehydes. Additional information on tumour phospholipids was obtained from analysis of BP8/C3H ascites-sarcoma cells. Previous studies of the phospholipid composition and the fatty acid and aldehyde distribution in a variety of normal tissues (Gray & Macfarlane, 1958, 1961; Gray, 1960*a*, *b*) provided reasonable comparative data. The possible presence of unusual minor lipid components was borne in mind throughout this work, part of which was described by Gray (1961).

METHODS

Analytical methods

The estimations of P, total N, amino N, choline, aldehyde, inositol, fatty acid ester groups and alkali-labile P were carried out as indicated by Gray & Macfarlane (1958). The method for the estimation of ethanolamine and serine (Axelrod, Reichental & Brodie, 1953) as modified by Long & Staples (1961*b*) was used. Chromatography of phospholipids on silicic acid-impregnated paper was done in di-isobutyl ketone–acetic acid–water (40:20:3, by vol.) at 2° and of glycerides in light petroleum (b.p. 60–80°)–di-isobutyl ketone (16:1, v/v) at room temperature (Marinetti, Erbland & Kochen, 1957). Chromatography on Whatman no. 1 acid-washed paper in butan-1-ol–water–aq. NH_3 (sp.gr. 0·88) (100:15:2, by vol.) (Coulon-Morelec, Faure & Maréchal, 1960) was also used to distinguish cardiolipin from phosphatidic acid. Chromatography of water-soluble esters obtained by mild alkaline hydrolysis (Hübscher, Hawthorne & Kemp, 1960) was carried out with the solvent systems described by Dawson (1954). Galactose or glucose or both in phospholipid fractions were identified after hydrolysis of the lipid with 2N-HCl at 100° for 2 hr. The lipid-soluble material was extracted with ether and the aqueous solution was evaporated to dryness with a N_2 or air jet to remove HCl. The residue was redissolved in a known volume of water and a sample chromatographed on Whatman no. 1 paper or on a thin-layer cellulose plate (Stahl, 1956; ideal for quantities of sugar less than 5 μg.) in a pyridine–butan-1-ol–water (4:6:3, by vol.) solvent system. A semiquantitative estimation of reducing sugar in the hydrolysed sample was done by the submicro-method of Park & Johnson (1949) and, when the amount of material permitted, quantitatively by the method of Dubois, Gilles, Hamilton, Roben & Smith (1956). An alternative hydrolysis procedure and estimation as described by Long & Staples (1961*a*) was also used.

Sphingosine in the isolated glycolipids was determined by the method of Long & Staples (1961*a*) and by the following procedure. The glycolipid was hydrolysed in 3N-HCl for 2 hr. at 100° in a sealed tube, cooled and made slightly alkaline with NaOH. The solution was then extracted with chloroform–methanol (19:1, v/v) and the sphingosine present in the chloroform determined by the method of Lea & Rhodes (1954) for the estimation of amino N with ninhydrin. Pure sphingosine standards were used. A qualitative identification of sphingosine was obtained by thin-layer chromatography on cellulose of samples of chloroform-soluble hydrolysis products with butan-1-ol–acetic acid–water (5:3:1, by vol.) solvent. Standard sphingosine markers were also chromatographed and spots were identified with ninhydrin reagent.

Materials

Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells (benzpyrene-induced) were grown in mice at the Microbiological Research Establishment, Porton, Wilts. The cells were collected in 0·9% NaCl and freeze-dried. I am indebted to Dr D. A. L. Davies, M.R.E., Porton, for kindly supplying large quantities of the freeze-dried cells.

Extraction

Landschutz ascites-carcinoma cells. The freeze-dried cells were reconstituted with water (1:9, w/v) and the wet mass was extracted with chloroform–methanol (1:1, v/v) (2 vol.) followed by chloroform–methanol (2:1, v/v) (1 vol., two extractions). The chloroform phase of the total extract (*a*) was washed three times with 0·1M-KCl (0·2 vol.) and an interfacial layer of presumably lipoprotein (*b*) which separated at this stage was collected. The two fractions were then treated as follows.

(*a*) The main lipid extract was evaporated to dryness under vacuum, redissolved in light petroleum (b.p. 60–80°) (10 ml./g.) and dialysed as described by van Beers, de Iongh & Boldingh (1958) to separate most of the neutral lipid from the phospholipid. The phospholipid was fractionated on silicic acid as described by Gray (1960*b*) and the neutral lipid by the system of Hirsch & Ahrens (1958). The isolation of the fatty acids and aldehydes from the kephalin- and choline-containing phospholipids was carried out as described by Gray (1960*b*). The fatty acids were identified by gas chromatography as methyl esters and the aldehydes as dimethyl acetals (Gray, 1960*b*, *c*, 1961).

(*b*) The lipoprotein material recovered from the interface of the chloroform layer and the 0·1M-KCl washing solution was soluble in mixtures of chloroform, methanol and water and could be precipitated from solution by the addition of water or chloroform. The lipid was not chemically bound to the protein and could easily be extracted by evaporating a chloroform–methanol–water solution (5:5:1, by vol.) of the material to dryness, repeating with chloroform–methanol (1:1, v/v) and extracting the dry residue with chloroform. The total lipid extract of this interfacial material was dialysed as described by van Beers *et al.* (1958) and the phospholipids only were fractionated on silicic acid. The fatty acids of the total kephalin fraction, the total lecithin fraction (both containing small amounts

of the corresponding plasmalogens) and the total neutral-lipid fraction were isolated and analysed.

BP8/C3H ascites-sarcoma cells. The procedure as for the Landschutz ascites-carcinoma cells was carried out but the interfacial-lipoprotein and the neutral-lipid fractions were not investigated after separation from the main phospholipid fraction.

EXPERIMENTAL AND RESULTS

The total lipid from the Landschutz ascites-carcinoma cells consisted of approximately equal amounts of phospholipid and neutral lipid (Table 1) but the interfacial lipoprotein contained almost twice as much neutral lipid as phospholipid.

The phospholipid mixtures from the Landschutz ascites-carcinoma cells (main extract and interfacial material) and from the BP8/C3H ascites-sarcoma cells were each fractionated on silicic acid columns and the usual type of separation curve (e.g. Gray, 1960*b*) was obtained for each of the three mixtures. The eluent fractions were collected and pooled on the basis of these curves supplemented by analysis by paper chromatography. The phospholipid compositions were computed as described by Gray & Macfarlane (1961) and are given in Table 2.

The cardiolipin and phosphatidic acid in the phospholipids (main extract) of the Landschutz ascites-carcinoma cells were separated during the chromatography of the total phospholipids, a separation not normally achieved on silicic acid columns. Paper chromatography (Coulon-Morelec *et al.* 1960) showed that phosphatidic acid was also present in the cardiolipin fractions of the interfacial material of the Landschutz ascites-carcinoma cells and in the cardiolipin fractions of the BP8/C3H ascites-sarcoma cells. The phospholipid distribution in the main extract of the Landschutz ascites-carcinoma cells was different from that in the interfacial material, the relative proportions of cardiolipin to phosphatidic acid, sphingomyelin and lysolecithin being much higher in the latter. The major component of the neutral lipid from Landschutz ascites-carcinoma cells [fractions (*a*) or (*b*)] was triglyceride with considerable amounts of cholesterol and monoglyceride also present (Table 3). Only traces of diglyceride were detected.

The component fatty acids of five different phospholipids present in the Landschutz ascites-carcinoma cells were determined (Table 4). The major saturated acid in all fractions was stearic acid. Amounts of oleic acid and linoleic acid were almost identical in the lecithin, kephalin and phosphatidic acid fractions with only slight variations in the kephalin plasmalogen and choline plasmalogen; they were the major components (33 and 36% respectively) of the cardiolipin fatty acids, which also contained palmitic acid (12%) and stearic acid (12%). Both kephalin plasmalogen and choline plasmalogen were rich in C_{20} and C_{22} polyenoic acids. The kephalin, lecithin and choline

Table 1. *Recovery of lipid from extracts of freeze-dried ascites-tumour cells*

Experimental details are given in the text.

	Lipid recovered (g./100 g. of freeze-dried cells)	
	Phospho-lipid	Neutral lipid
Landschutz ascites-carcinoma cells		
Main lipid extract (*a*)	6·6	7·0
Interfacial lipoprotein (*b*) (35% lipid) (1·6 g./100 g. of freeze-dried cells)	0·2	0·36
BP8/C3H ascites-sarcoma cells	5·7	—

Table 2. *Distribution of phospholipids in Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells*

Experimental details are given in the text. Values are expressed as percentages of total lipid P.

Phospholipid	Landschutz ascites cells: Main extract	Landschutz ascites cells: Interfacial lipoprotein	BP8/C3H ascites cells
Cardiolipin	2	9	4
Phosphatidic acid	2		
Phosphatidylethanolamine	17	14	18
Phosphatidylserine	6	6	10
Phosphatidylinositol	6	9	5
Ethanolamine plasmalogen	5	3	3
Serine plasmalogen	1		4
Phosphatidylcholine	40	32	40
Choline plasmalogen	6	4	3
Sphingomyelin	12	18	12
Lysolecithin	2	5	1
Phosphorus	264 mg./100 g. dry wt. of cells	8 mg./100 g. dry wt. of cells	228 mg./100 g. dry wt. of cells

Table 3. *Analysis of neutral lipids in Landschutz ascites-carcinoma cells*

Experimental details are given in the text.

Lipid	Percentage by wt. of total neutral lipid
Monoglyceride	17
Diglyceride	4
Triglyceride	56
Cholesterol	22
Cholesterol esters	1
Hydrocarbons	0·2

plasmalogen from BP8/C3H ascites-sarcoma cells had similar fatty acid distributions to the corresponding phospholipids from the Landschutz ascites-carcinoma cells, but the kephalin plasmalogen contained a high proportion (57%) of saturated acids including an unusually high amount of dodecanoic acid (8%). Both kephalin plasmalogens contained more saturated acids (mainly stearic acid) than the corresponding choline plasmalogens.

Palmitaldehyde was the major component of the aldehydes in the plasmalogens from both types of tumour cell (Table 5) with stearaldehyde and olealdehyde accounting for most of the remainder. Odd-numbered and branched-chain aldehydes occurred in traces only. Linolealdehyde was present in all fractions and the plasmalogens from the BP8/C3H ascites-sarcoma cells contained minor amounts of eicosanal. The glycerides from the main extract and the total neutral lipid (mainly triglyceride) from the interfacial lipoprotein of the Landschutz ascites-carcinoma cells contained approximately equal amounts of palmitic acid and stearic acid (Table 6). The diglyceride and the neutral-lipid fractions were more unsaturated than the monoglyceride or the triglyceride fractions; approximately 10% of the fatty acids from the diglyceride fraction and 21% of those from the neutral lipid (from the interfacial lipoprotein) were C_{20} and C_{22} polyenoic acids.

Preliminary studies on lipids containing glucose or galactose or both

Rapport *et al.* (1959) isolated a lipid hapten containing glucose and galactose from phospholipid extracts of a human epidermoid carcinoma grown in conditioned rats. The lipid, which they named cytolipin H, contained fatty acid, sphingosine, galactose and glucose in the molar proportions 1:1:1:1, but no phosphorus. It was present only as a very minor component. The possible presence of a similar lipid in the ascites cells prompted an examination of the various phospholipid fractions isolated by silicic acid chromatography. Acid-hydrolysed samples of the phospholipid fractions were analysed for reducing sugar (Park & Johnson, 1949) and the sugars were identified by paper chromatography or by thin-layer chromatography on cellulose. Two distinct carbohydrate-containing lipids (Table 7) were found in both Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells. One of these was eluted by chloroform containing 10–15% (v/v) of methanol from a silicic acid column just after the main cardiolipin fraction. Glucose was the only sugar present. The other compound was eluted by chloroform containing 35–50% (v/v) of methanol with the phosphatidylinositol fraction. Galactose and probably galactosamine were present, but glucose was detected in trace amounts only.

Samples of the cardiolipin fraction (fraction G, Table 7) were subjected to acid hydrolysis (e.g. 2N-hydrochloric acid at 100° for 2 hr.) and the solutions were extracted with ether to remove lipids. Analyses and paper and thin-layer chromatography of samples from both the aqueous and ether solutions indicated that sphingosine was present as well as glucose. These results and the chromatographic behaviour of the intact material suggested the presence of a glucose cerebroside and the following isolation scheme was carried out. A sample of the cardiolipin fraction G (P, 10 mg.) was subjected to a mild alkaline hydrolysis (Hübscher *et al.* 1960) to remove most of the phospholipid. The glucose lipid, unhydrolysed phospholipid (P, 1 mg.) and the fatty acids from the cardiolipin (as methyl esters) in chloroform were evaporated to dryness and the residue was washed with light petroleum (b.p. 60–80°) into a latex-rubber finger. The fatty acids were removed by dialysis against light petroleum. The residue was washed from the finger with chloroform, evaporated to dryness and redissolved in chloroform–methanol (9:1, v/v) and chromatographed on a silicic acid column (15 g.; 1·1 cm. diam. × 32·0 cm.) with chloroform–methanol (9:1, v/v) as solvent. Development of the column was carried out with the same solvent (cf. Long & Staples, 1961*a*) and fractions from the column were monitored by chromatographing samples on thin-layer silicic acid plates with chloroform–methanol–water (40:10:1, by vol.) as solvent. Fractions which contained the glucose lipid were combined, evaporated to dryness, dissolved in chloroform–methanol (1:1, v/v) and chromatographed on an alumina column (30 g.; 1·1 cm. × 32·0 cm.) in the same solvent. After a small amount of lipid material was eluted the solvent was changed to chloroform–methanol–water (25:25:2, by vol.) and the glucose lipid was eluted as a sharp band. The solvent was evaporated off and the residue was dissolved in a small amount of chloroform–methanol (1:1, v/v), a large volume of acetone was added and the solution placed in a

Table 4. *Fatty acid composition of different phospholipids from Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells*

Experimental details are given in the text. Values are expressed as percentages of total methyl esters.

		Landschutz ascites-carcinoma cells								BP8/C3H ascites-sarcoma cells			
		Main extract						Interfacial material					
	Designation	Choline plasmalogen	Lecithin	Kephalin plasmalogen	Kephalin	Cardiolipin	Phosphatidic acid	Lecithin	Kephalin	Choline plasmalogen	Lecithin	Kephalin‡ plasmalogen	Kephalin
Myristic acid	14:0	0·2	0·2	0·1	0·1	0·8	0·5	0·3	0·5	0·1	0·1	0·2	—
Palmitic acid	16:0	7·6	17·1	3·2	4·7	11·8	4·7	18·4	8·2	8·0	20·8	6·9	2·9
Stearic acid	18:0	9·3	22·0	21·1	31·8	11·8	27·4	22·6	45·7	6·1	17·2	32·5	26·5
Total normal saturated acids	—	17·1	39·3	24·4	36·6	24·4	32·6	41·3	54·4	14·2	38·1	39·6	29·4
†Total odd-numbered saturated acids	—	0·2	0·2	0·1	0·2	0·1	Trace	0·7	1·7	0·2	0·5	2·9	0·4
†Total branched-chain saturated acids	—	0·1	Trace	Trace	Trace	0·2	Trace	0·3	0·6	0·4	0·7	7·0	1·1
Palmitoleic acid	16:1	0·9	1·1	0·6	0·3	0·7	1·0	1·2	1·2	0·4	0·4	0·3	Trace
Oleic acid	18:1	11·5	16·4	13·5	17·9	33·0	17·7	17·2	19·1	10·9	22·4	13·0	15·8
Total monoenoic acids	—	12·4	17·6	14·1	18·2	33·7	18·7	18·4	20·3	11·3	22·8	13·3	15·8
Linoleic acid*	18:2	15·4	18·5	12·3	19·6	36·0	20·0	19·4	13·9	16·3	19·8	11·0	9·8
Eicosadienoic acid	20:2	0·5	0·6	—	0·6	2·5	2·8	1·2	—	1·1	1·6	0·7	2·0
Eicosatrienoic acid	20:3	4·2	2·7	3·5	Trace	Trace	3·0	1·7	0·9	2·8	1·8	2·9	1·3
Arachidonic acid	20:4	32·3	13·3	28·8	12·0	1·9	17·2	6·2	39·2	9·0	9·0	6·8	13·2
Eicosapentaenoic acid	20:5	0·7	Trace	Trace	1·0	Trace	—	—	—	Trace	Trace	1·4	Trace
Total C_{20} polyenoic acids	—	37·7	16·6	32·3	12·6	4·4	23·0	14·6	7·1	43·1	12·4	11·8	16·5
Docosapentaenoic acid	22:5	3·3	1·7	4·6	2·7	Trace	1·4	0·9	0·3	3·1	1·0	1·9	5·7
Docosahexaenoic acid	22:6	9·6	4·6	10·2	5·8	Trace	3·9	3·7	0·8	10·5	3·4	6·7	18·4
Total C_{22} polyenoic acids	—	12·9	6·3	14·8	8·5	Trace	5·3	4·6	1·1	13·6	4·4	8·6	24·1
Average no. of double bonds/fatty acid molecule	—	2·7	1·5	2·5	1·7	1·2	1·7	1·4	0·8	2·9	1·2	1·3	2·3

* Includes trace amounts of linolenic acid. † Almost exclusively C_{15} and C_{17} acids. ‡ Also contained decanoic acid (7·5%).

Table 5. *Aldehyde composition of plasmalogens from Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells*

Experimental details are given in the text. Values are expressed as percentages of total dimethyl acetals.

Aldehyde	Designation	Landschutz ascites-carcinoma cells		BP8/C3H ascites-sarcoma cells	
		Choline plasmalogen	Kephalin plasmalogen	Choline plasmalogen	Kephalin plasmalogen
Lauraldehyde	12:0	—	0·8	—	Trace
Tridecanal	13:0	—	0·3	—	—
11-Methyldodecanal / 10-Methyldodecanal	br13:0	—	0·2	—	—
Myristaldehyde	14:0	0·3	0·2	0·1	0·4
12-Methyltridecanal / 11-Methyltridecanal	br14:0	Trace	0·7	Trace	0·1
Pentadecanal	15:0	0·7	0·7	1·1	Trace
13-Methyltetradecanal	iso-br15:0	—	—	—	—
12-Methyltetradecanal	anteiso-br15:0	0·7	1·0	0·1	0·2
Palmitaldehyde	16:0	43·0	55·0	43·6	38·9
Palmitolealdehyde	16:1	—	0·9	0·4	0·1
14-Methylpentadecanal / 13-Methylpentadecanal	br16:0	0·3	0·2	0·2	0·2
Heptadecanal	17:0	1·2	1·1	1·8	1·7
Heptadecenal	17:1	0·1	0·4	2·0	1·1
16-Methylheptadecanal	iso-br17:0	0·6	0·2	0·8	0·8
15-Methylheptadecanal	anteiso-br17:0	0·5	0·5	0·5	0·8
Stearaldehyde	18:0	25·6	22·0	24·5	33·9
Olealdehyde	18:1	24·0	10·1	21·8	18·9
iso-Olealdehyde	iso18:1	—	3·8	—	—
Linolealdehyde	18:2	2·6	1·1	2·2	1·5
Eicosanal	20:0	—	—	0·8	1·5

Table 6. *Fatty acid composition of the glycerides from Landschutz ascites-carcinoma cells*

Experimental details are given in the text. Values are expressed as percentages of total methyl esters.

	Designation	Main lipid extract			Interfacial material. Total neutral lipid
		Monoglyceride	Diglyceride	Triglyceride	
Lauric acid	12:0	0·1	0·1	0·2	0·2
Myristic acid	14:0	1·6	1·3	1·5	1·7
Palmitic acid	16:0	23·8	19·4	22·2	15·1
Stearic acid	18:0	25·6	16·4	22·0	13·7
Total normal saturated acids	—	51·1	37·2	45·9	30·7
Total odd-numbered saturated acids	—	0·3	0·3	1·6	1·3
Total branched-chain saturated acids	—	0·6	0·6	1·7	0·3
Palmitoleic acid	16:1	0·3	1·4	2·0	1·7
Oleic acid	18:1	26·7	25·8	26·0	19·3
Eicosamonoenoic acid	20:1	1·1	1·2	0·7	0·6
Total monoenoic acids	—	28·1	28·4	28·7	21·6
Linoleic acid*	18:2	14·8	22·4	16·6	24·3
Eicosadienoic acid	20:2	0·8	1·5	0·9	1·1
Eicosatrienoic acid	20:3	0·8	1·6	1·0	1·3
Arachidonic acid	20:4	1·6	3·6	1·7	6·5
Total C_{20} polyenoic acids	—	3·2	6·7	3·6	8·9
Docosapentaenoic acid	22:5	0·4	0·7	0·5	2·3
Docosahexaenoic acid	22:6	0·7	2·4	0·8	9·7
Total C_{22} polyenoic acids	—	1·1	3·1	1·3	12·0

* Includes trace amounts of linolenic acid.

cold bath at 0°. A flocculent precipitate settled out, the supernatant was removed, the residue washed with dry ether and the procedure repeated twice more. The final residue was a white waxy solid (20·7 mg.) which by analysis gave sphingosine:glucose:fatty acid molar proportions of 1:1:1; calculated as a simple glucose cerebroside, the material, based on dry wt., was approximately 95% pure. Gas chromatography of the fatty acid methyl esters indicated the presence of lignoceric acid (major component), nervonic acid, behenic acid and small amounts of C_{20} and C_{18} compounds.

Table 7. *Distribution of glucose and galactose in the phospholipids of BP8/C3H sarcoma cells separated by chromatography on silicic acid*

Experimental details are given in the text.

Eluting solvent (chloroform–methanol, v/v)	Fractions collected	Phospholipids	Total P (mg.)	Sugar	Total (approx.) reducing sugar (mg.)
19:1	A–D	Cardiolipin;	13	Not detected	—
9:1	E–F	phosphatidic acid;	16	Glucose (trace)	<1
17:3	G	phosphatidylserine (5%)	16	Glucose	6
4:1	H	Phosphatidylethanolamine; phosphatidylserine; plasmalogens	370	Not detected	—
13:7	I–J	Phosphatidylinositol	18	Galactose Galactosamine (?)	20
3:2	K–L	Phosphatidylinositol; lysokephalin (trace); phosphatidylcholine	150	Galactose Galactosamine (?)	31
1:1	M–O	Phosphatidylcholine; choline plasmalogen	459	Galactose (trace)	—
2:3	P	Phosphatidylcholine; choline plasmalogen; sphingomyelin	59	Not detected	—

Thin-layer chromatography (Mangold & Malins, 1960) of the free acids on silicic acid, with light petroleum–diethyl ether–acetic acid (70:30:1, by vol.) as solvent, indicated the presence of some hydroxy acids. The other glycolipid contained galactose and probably galactosamine. The quantities of glucose cerebroside in the Landschutz ascites-carcinoma cells (0·025% by wt. of total phospholipid) and the BP8/C3H ascites-sarcoma cells (0·1% by wt. of total phospholipid) were very small. No trace of cytolipin H was detected.

DISCUSSION

The phospholipid compositions of the tumour cells did not show any marked differences from the compositions of normal tissues. Lysolecithin, though not a usual constituent of tissue phospholipids, has been found in normal brain (Thompson, Niemiro & Webster, 1960) and plasma (Newman, Ching-Tong Liu & Zilversmit, 1961), so no particular significance can be attached to its presence in the tumour cell. Also breakdown of the lecithin or choline plasmalogen to lysolecithin during the freeze-drying of the cells cannot be ruled out. Phosphatidic acid has been found in small amounts in normal liver phospholipids (Hübscher & Clark, 1960) but not in other normal tissues, and thus the small amounts consistently found in tumour cells may possibly represent a metabolic abnormality. However, the fatty acid composition of the phosphatidic acid from the Landschutz ascites-carcinoma cells is very similar to that of the kephalin, suggesting that the former compound could have been formed by enzymic breakdown of the kephalin with loss of nitrogen base, possibly as an artifact of the isolation procedure.

Wallach *et al.* (1960) found a constant proportion of amino acids other than serine in the phospholipids from Ehrlich ascites-tumour cells and they suggested that the acids formed a peptide which was linked by a peptide bond to the ethanolamine or serine of the kephalin phospholipids. Amino acids other than serine were not detected in the phospholipids of the Landschutz ascites-carcinoma cells or of the BP8/C3H ascites-sarcoma cells.

The considerable quantity of monoglyceride (17% of total neutral lipid) present in the Landschutz ascites-carcinoma cells does appear to be in excess of that normally found in mammalian tissues (Hirsch & Ahrens, 1958; Pietruszko, 1962); the fatty acid distributions (Table 5) in the mono- and tri-glyceride fractions were very similar. The presence of substantial amounts of monoglyceride, phosphatidic acid and lysolecithin may reflect a tendency to general breakdown in tumour lipids, perhaps by enzymes released by changes in membrane permeability. The triglycerides of the Landschutz ascites-carcinoma cells contained a lower ratio of palmitic acid to stearic acid, and less oleic acid (less than 30%) than those from normal tissues (40–60%) (Gray & Macfarlane, 1961).

A comparison of the fatty acid distributions in phospholipids of the Landschutz ascites-carcinoma cells with those in normal tissues shows some distinct differences. The definite preference for the saturated C_{16} acid (palmitic acid) over the C_{18} acid (stearic acid) shown by lecithins and choline plasmalogens from normal tissues, which is independent of species and also therefore of a wide varia-

tion in dietary fat (Gray & Macfarlane, 1961), is not shown by those from the Landschutz ascites-carcinoma cells in which the saturated C_{18} acid is the major component. The quantities of oleic acid and linoleic acid in the different tumour phospholipids are far less variable than in normal tissues. Even the tumour cardiolipin tends to conform more to the general pattern by containing palmitic acid (12%) and stearic acid (12%), and almost equal quantities of oleic acid and linoleic acid, whereas cardiolipin in normal mammalian tissues has a very specific fatty acid pattern (Gray & Macfarlane, 1961). The general, less selective, distribution of fatty acids in the different tumour phospholipids suggests a loss in specificity of the enzyme systems responsible for the incorporation of the fatty acids into phospholipids. The similarity in phospholipid composition, and of the fatty acid and aldehyde distribution in them, in both the Landschutz ascites-carcinoma cells and the BP8/C3H ascites-sarcoma cells is in agreement with the established view (Greenstein, 1954) that tumours tend chemically to resemble each other more than they do normal tissues or than normal tissues resemble each other.

The significance of the two glycolipids detected in the tumours is not known and their concentrations are so low that they could have been overlooked in previous studies on normal tissues. However, their relative amounts appear to vary in different tumours and, as they could be of immunological interest, they are being investigated further. The fact that the cerebroside isolated from the tumours contains glucose is of interest, because, as far as the author is aware, cerebrosides specifically with glucose residues have been isolated only from the spleens of persons with Gaucher's disease (Halliday, Deuel, Tragerman & Ward, 1940; Marinetti, Ford & Stotz, 1960).

SUMMARY

1. The lipid was extracted from freeze-dried Landschutz ascites-carcinoma cells and BP8/C3H ascites-sarcoma cells. The phospholipids from both tumours and the neutral lipid from the Landschutz ascites-carcinoma cells were fractionated quantitatively on silicic acid columns.

2. The distribution of lipid phosphorus was computed from analysis of fractions and was not significantly different from that in the majority of normal tissues.

3. The constituent fatty acids and aldehydes from the separated phospholipids and glycerides were identified and estimated by gas chromatography.

4. The results showed a far less selective distribution of fatty acids in the phospholipids and glycerides than was usually found in normal tissues. A preponderance of C_{18} saturated acid as compared with C_{16} saturated acid was found in most phospholipids.

5. Small amounts of two chromatographically-distinct carbohydrate-containing lipids were found in the phospholipid fractions from the tumour cells.

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