the structure of such complexes would vary with the size of the metal ion. Small metal ions would be completely surrounded by the polyphosphate chain of ATP, intermediate-sized metal ions would rest in a 'shallow cup' formed by the polyphosphate chain, and complexes with large metal ions would have an 'open' configuration. It was pointed out that such differences in the shape of the ATP molecule could profoundly affect the rate of enzyme-catalysed reactions. The non-enzymic reaction described here has an absolute requirement for a bivalent-metal ion, and shows a selective stimulation by K^+ and Rb^+ ions. This implies that in the alkali-metal-stimulated reaction the active complex contains at least two metal ions, and that its structure differs from those described by Melchior (1954). In close analogy, most or all enzyme-catalysed-transphosphorylation reactions from ATP show an absolute requirement for a bivalent metal. A number of such reactions show a selective stimulation by univalent cations, and here the active complex must also involve at least two metal ions. The structures proposed by Melchior (1954) are therefore also insufficient to account for the alkali-metal-ion stimulation of enzyme-catalysed transphosphorylations from ATP.

No complex-formation between the 6-amino group of adenine and the co-ordination points left vacant on the metal is indicated in Fig. 6. The balance of the evidence available at present (e.g. Spicer, 1952; Martell & Schwarzenbach, 1956) is against such an interaction. A similar interaction with the heterocyclic-ring system cannot be ruled out, but metals such as magnesium, calcium and potassium, which are involved in chelate formation with ATP in the cell, show a strong preference for chelating with oxygen as compared with nitrogen (Sidgwick, 1941).

SUMMARY

1. The stimulation by alkali-metal ions of a non-enzymic-phosphate transfer from adenosine tri-

phosphate is described. The alkali-metal-ion stimulation does not abolish the absolute requirement by the reaction for bivalent-metal ions (M^{2+}).

2. Potassium ions produce a greater stimulation than other alkali-metal ions at adenosine triphosphate/ M^{2+} ion ratios in the region of 1.

3. The observed effects are discussed in terms of ion-pair and of chelate formation.

4. The results provide a chemical basis for the alkali-metal-ion dependence or stimulation observed in many enzyme-catalysed-phosphate-transfer reactions involving adenosine triphosphate and similar substances.

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The Component Fatty Acids of *Sporidesmium bakeri* Lipids

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Under certain climatic conditions, such as warm autumn rains after summers of above-average temperatures, pastures in some New Zealand districts have been known to produce a virulent poison causing serious losses in sheep and cattle.

Some of the affected animals show symptoms of photosensitization described as 'facial eczema', but the disease consists essentially in severe liver damage (Cunningham, Hopkirk & Filmer, 1942). Thornton & Percival (1959) have recently re-

ported that a high-sporing strain of *Sporidesmium* species [identified by the Commonwealth Mycological Institute, Kew, as *Sporidesmium bakeri* Syd. (Herb. I.M.I. no. 74473)] contains a hepatotoxin, which produces in both guinea pigs and lambs liver lesions similar to those encountered in 'facial eczema' disease. This discovery gave rise to an extensive study of this fungus, including the examination of its lipids. The fungus belongs to the class of Fungi imperfecti, order Moniliales, family Dematiaceae, the members of which are disting-

uished by the brown-and-black pigmentation of their mycelia and sometimes of their spores. The fatty acid composition of lipids from several members of the related Moniliaceae family such as *Aspergillus nidulans*, *Penicillium javanicum* and others, the mycelia and spores of which are hyaline or bright coloured, is already known (cf. Singh, Walker & Meara, 1955). In this paper we report the fatty acid composition of the lipids from *S. bakeri*, which, as far as we know, is the first member of the Dematiaceae to be thus examined.

Fig. 1. Extraction scheme and weights of lipid fractions.

EXPERIMENTAL

Growth and collection of Sporidesmium bakeri

A high-sporing strain of *Sporidesmium bakeri* was surface-cultured on an infusion of 200 g. of potato and 200 g. of carrot/1000 ml. of water, in Roux bottles each containing 200 ml. of medium. After an incubation at 24° for 7 days, there was maximum spore development and the fungal mats were separated from the liquid culture medium by filtration through a Büchner filter. Two batches of 138 and 140 bottles of culture were obtained. In addition a lipid sample was examined, which represented a by-product obtained after the removal of the hepatotoxin from a large culture of *Sporidesmium* (700 Roux bottles).

Extraction and separation of the lipids

The common procedures used for the extraction and separation of the lipids from biological material were slightly modified to include partitioning of the lipid between aqueous 80% methanol and light petroleum, which was used by White (1958) for the concentration of hepatotoxic substances. The reason for applying this additional step was to establish whether the hepatotoxin has affinity to any specific fatty acids. The scheme employed in two batches of the fungus is outlined in Fig. 1. The only difference between the handling of the two batches was that the first was freeze-dried whereas the second was dehydrated by refluxing three times with acetone (1500 ml.) for 30 min. The third lipid sample corresponded to fraction B in Fig. 1.

Fraction B obtained from batch 2 contained an appreciable proportion of phospholipid (1.04% of P). This phospholipid was separated from the remaining lipids by dialysis against light petroleum (b.p. 40–60°) in a rubber membrane (van Beers, de Iongh & Boldingh, 1957). The

yield of both components amounted to only 70.4% of the total material dialysed. This was due to the appearance during the dialysis of ethyl ether-insoluble material, which was discarded.

Analytical methods

The determination of fatty acids in the form of their methyl esters was carried out by gas-liquid chromatography. The various lipid fractions were saponified and after the removal of unsaponifiable matter the liberated fatty acids were converted into methyl esters as described by Hilditch (1956). The esters were separated, with a Pye Argon Chromatograph (Cambridge), the amount of each component being determined by measuring peak areas with a planimeter. The chromatographic columns (1.2 m. long, 4 mm. internal diameter) were packed with 45–85 mesh Celite 545 mixed with 20% (w/w) of Apiezon M (James & Martin, 1956) and operated at 200°, which made possible the separation of methyl oleate from methyl stearate. Another set of columns was packed with Celite, as above, impregnated with 20% of polydiethylene glycol succinate, which separated methyl linoleate from linolenate (Lipsky & Landowne, 1958). The use of these two liquid phases provided therefore means of determining the amounts of saturated and of mono-, di- and tri-ene esters in the C₁₈ series.

Total phosphorus was determined colorimetrically according to the method of Allen (1940), and choline as the reineckate salt (Entenman, Taurog & Chaikoff, 1944).

RESULTS AND DISCUSSION

The weights of lipid fractions are shown in Table 1. The yield of lipids (4–6% of the dry weight of fungal mats) appears low when compared with that

Table 1. *Component fatty acids of three batches of Sporidesmium bakeri expressed as molar percentages*

Saturated acids	Batch 1. Fractions A + B	Batch 2				Batch 3. Fraction B
		Fraction A	Fraction B		Total	
			'Glycerides'	'Phospholipids'		
<i>n</i> -C ₈	0.1	0.5	—	—	0.1	0.3
<i>n</i> -C ₉	1.0	—	—	—	—	—
<i>n</i> -C ₁₀	0.1	0.8	—	—	0.2	0.1
Branched-chain C ₁₁	0.1	0.9	—	—	0.2	—
<i>n</i> -C ₁₁	0.4	1.5	—	—	0.3	0.3
Branched-chain C ₁₂	0.1	—	—	0.3	0.1	0.1
<i>n</i> -C ₁₂	0.4	—	0.2	—	0.1	1.2
Branched-chain C ₁₃	0.5	0.4	—	0.4	0.2	—
Branched-chain C ₁₄	1.2	—	—	—	—	—
<i>n</i> -C ₁₄	0.2	—	0.9	0.3	0.6	1.6
Branched-chain C ₁₅	—	0.5	0.2	—	0.2	0.1
<i>n</i> -C ₁₅	0.2	0.2	0.7	0.4	0.5	0.9
Branched-chain C ₁₆	—	—	—	—	—	0.2
<i>n</i> -C ₁₆	29.5	11.9	22.5	15.0	18.3	23.3
Branched-chain C ₁₇	—	—	0.5	—	0.3	0.1
<i>n</i> -C ₁₇	—	6.2	0.6	—	1.7	0.4
<i>n</i> -C ₁₈	8.1	3.0	8.0	3.7	5.9	8.3
Unsaturated acids						
Palmitoleic	0.7	1.6	1.3	1.1	1.3	1.7
Oleic	15.0	6.7	9.9	5.7	8.2	11.3
Linoleic	41.3	62.1	52.5	71.0	59.0	46.5
Linolenic	1.1	3.7	2.7	2.1	2.8	3.6

of other fungi of the Moniliales order (Singh *et al.* 1955), but it is known that the lipid content of fungi is greatly influenced by the nature of the substrate (cf. Garrido & Walker, 1956).

The fatty acid composition of the three batches is shown in Table 1. The composition of batch 1 is given as a total (fraction A + fraction B) whereas the composition of the various fractions obtained from batch 2 (i.e. methanol-soluble lipid, glyceride and phospholipid) are given separately. The total composition of batch 2 has also been calculated on the basis of the weight of fatty acids obtained from each fraction. The composition of batch 3 (light petroleum-soluble lipid only) is given for comparison.

The fat moulds so far examined include *Aspergillus nidulans* (Singh & Walker, 1956), *Phycomyces blakesleeanus* (Bernhard & Albrecht, 1948), *Neurospora crassa* (Todd, Stone, Hechter & Nussbaum, 1957), *Penicillium* species (Hilditch & Meara, 1944), *P. javanicum* (Ward & Jamieson, 1934), *P. flavocinereum* (Singh, Shah & Walker, 1956*a*), *P. lilacinum* (Singh, Shah & Walker, 1956*b*) and *P. spinulosum* (Shimi, Singh & Walker, 1959). In general, the main fatty acid constituents (% by wt.) are approximately as follows: palmitic (15–40), stearic (5–15), oleic (5–44), linoleic (13–43) and linolenic (trace–32). In *Phycomyces blakesleeanus*, however, there also occurs an isomer of linolenic acid, namely octadec-6:9:12-trienoic acid, tetracos-17-enoic acid and a hexacosenoic acid.

The present results show that the fatty acids from *Sporidesmium bakeri* Syd. lipids contain more linoleic acid (42–60%) than previously found in other moulds. Earlier work has shown that in general increased amounts of linoleic acid in moulds are associated with decreased amounts of palmitic acid. The fatty acids from *S. bakeri* Syd., however, display a high content of linoleic acid along with a considerable amount of palmitic acid (20–30%). The high linoleic acid content is accompanied by a reduction in the amount of oleic acid. The three batches of *Sporidesmium* show a similar pattern of fatty acid composition, but the 'phospholipid' fraction is notable for its high linoleic acid content (71%) compared with the 'glyceride' fraction, thereby resembling the relationships found between phospholipids and glycerides in liver fats.

The more sensitive gas-liquid-chromatographic technique, as compared with ester fractionation, has revealed the presence of small amounts of straight-chain fatty acids containing an odd number of carbon atoms in the molecule and branched-chain fatty acids (apparently of both *iso* and *anteiso* configuration), showing that fungi elaborate these constituents, as do animals and bacteria.

As mentioned above, the lipids in the fraction

containing the hepatotoxin were examined separately to determine whether the fatty acids were selectively associated with the poison. The results show (batch 2, fraction A, Table 1) that the fatty acids in the hepatotoxin fraction comprise a similar range of fatty acids to that found in the other fractions. However, the amount of linoleic acid is higher and of palmitic acid and stearic acid is lower. This could be attributed to solubility factors.

SUMMARY

1. The component fatty acids of the lipids elaborated by *Sporidesmium bakeri* Syd., a fungus held responsible for the 'facial eczema' disease affecting sheep and cattle, have been determined by gas-liquid chromatography.

2. The main component acids have been shown to be linoleic acid (41–59%), palmitic acid (18–30%), oleic acid (8–15%) and stearic acid (6–8%) (molar percentages). In addition, minor amounts of linolenic acid, palmitoleic acid and of several straight-chain fatty acids containing an odd number of carbon atoms in the molecule and branched-chain fatty acids ranging from C₉ to C₁₇ have been detected.

3. The fatty acid composition of *S. bakeri* lipids differs from that of other moulds inasmuch as an unusually high content of linoleic acid is accompanied by a substantial amount of palmitic acid.

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Constitutive Enzymes of the Developing Chick Embryo: Adenosine Deaminase

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The study of enzyme activities in developing tissues yields information only about the overall potential metabolic pattern and gives no clue to the nature of the chemical or biological stimulus which promotes the change in the differential rate of accumulation of a certain enzyme. If a specific stimulus to promote enzyme synthesis could be found, it would be possible to measure the capacity for enzyme synthesis of an embryonic tissue at any stage of its development. It is this capacity of embryonic cells to be controlled by their environment, at different stages of development, that may influence the cells' ultimate function. An attractive way of viewing the complex processes of differentiation is as 'a series of mutually dependent endogenously induced enzyme formations' (Rusch, 1954). The phenomenon of adaptive increase in enzyme activity is well known in bacteria (e.g. Monod, 1947; Spiegelman, 1948), as are increases in the activity of mammalian enzymes due to certain non-specific stimuli, such as the variation of dietary protein (e.g. Mandelstam & Yudkin, 1952), changes of altitude (Tappan, Reynafarje D., Potter & Hurtado, 1957), hormones or disease (Knox, Auerbach & Lin, 1956). However, relatively few cases of substrate-induced enzyme adaptations, other than that of tryptophan peroxidase (e.g. Knox, 1951), have been found in mammals.

If the development of the ability to produce certain enzymes during normal growth is due to a type of enzymic adaptation (otherwise known as enzyme induction) it might be expected that this capacity (with respect to any particular enzyme) could be prematurely stimulated by exposure of embryonic cells to an excess of substrate.

In the chick embryo only three cases of substrate-induced enzyme adaptation have so far been reported (Gordon & Roder, 1953; Gordon, 1956; Roeder, 1957). Arginase was found to be increased by 30–143% in the whole chick embryo during 5–14 days of incubation by injecting arginine into the egg (Roeder, 1957). Tryptophan peroxidase was increased tenfold in the developing chick embryo by injections of tryptophan during the third to ninth days of incubation (Gordon, 1956). Gordon & Roder (1953) found that adenosine-deaminase activity increased eightfold in whole chick embryos at 8 days of incubation after the eggs had been injected with adenosine 24 hr. earlier. These authors also found a sevenfold increase of enzyme activity in the liver at 14 days of incubation after seven consecutive daily injections of small amounts of adenosine.

No adaptive increase in lactic-dehydrogenase activities in the liver of the chick embryo was observed after injection of sodium pyruvate or lactate into the egg at various stages of development; similarly no adaptive increase in liver glutaminase was observed after injections of glutamine (J. B. Solomon, unpublished results). It was concluded that lactic dehydrogenase and glutaminase were constitutive enzymes, in that they are normally produced by the cell and are not susceptible to stimulation by the presence of their substrates. In view of the paucity of examples of enzyme induction in any type of embryo (Markert, 1958), we have attempted to confirm the results of Gordon & Roder (1953) on adenosine deaminase.

A preliminary report of these results has already been published (Palmer & Solomon, 1959).