not disturbed. This procedure was applied to the C20:3 acid of Ginkgo. It was isomerized, recovered, esterified, ozonized and hydrogenated to yield C_6 and C_7 aldehydes in equivalent amounts, but only C_5 aldehyde-ester. The structure of this acid as 5,11,14-eicosatrienoic was therewith established. The ultraviolet spectrum of the original ester showed no absorption while that after isomerization showed the normal conjugated diene peak at 233 mμ.

The infrared spectrum of the eicosatrienoate showed less than 5% *trans* double bond. This sample was recovered and hydrogenated analytically[10]. It absorbed 91% of the theoretical value for three double bonds. The hydrogenated ester was recrystallized and identified as methyl arachidate by melting point and mixed melting point.

The fatty acids of *Ginkgo biloba*

Component	% of total in		Double bond position	Relative amounts of isomers in	
	nut	leaf		nut	leaf
C14:0	2.6	1.0			
C16:0	11.3	22.3			
C16:1	6.2	3.1	7		minor
			9	major	major
			11	minor	
C16:2	tr.	tr.	7, 10	minor	major
			9, 12	major	minor
C16:3		4.8	7, 10, 13		major
			9, 12, 15		minor
C18:0	0.7				
C18:1	30.0	6.8	9	major	major
			11	minor	minor
C18:2	42.3	20.8	[a]5, 11	minor	
			9, 12	major	major
C18:3	1.6	32.0	[a]5, 11, 14	major	
			9, 12, 15	major	major
			11, 14, 17	minor	
C20:2	0.9	tr.	[a]5, 11	major	major
			11, 14	minor	minor
			14, 17		minor
C20:3	4.1	6.3	[a]5, 11, 14	major	major
C20:4	tr.	1.7	[a]5, 11, 14, 17	major	major
			9, 12, 15, 18	minor	minor

[a] Tetramethylene interrupted isomer.

Ozonization-hydrogenation, ozonization-oxidation, and ozonization-hydrogenation after isomerization elucidated the structure of the eicosatrienoic acid. These procedures were used also with the other fatty acid fractions and were given priority in that sequence when the amount of material did not suffice to carry out all the procedures mentioned above. Experience in GLC with numerous authentic isomers showed that shifts of retention time for isomers encountered in Ginkgo were consistent with the chemical evidence. The analytical results are listed in the Table.

The composition of the total fatty acids from nuts of the Ginkgo was similar to that of the leaves except for the drastic decrease of linolenic acid. This conforms with the observation that the presence of this acid is often associated with that of chlorophyll.

The occurrence of unsaturation in the C_5 position which is isolated from the other double bonds points toward biosynthetic mechanisms which so far have not been recognized. It may be noted that in spite of the presence of 5,11,14-C20:3, an acid, 5,8,11,14-C20:4 (arachidonic) is not formed by this plant.

Correlation of the unusual isomers found in Ginkgo with its early and unique place in the evolution of plant life would be most interesting. Work in that direction is underway in this laboratory[11].

Zusammenfassung. In Blättern und Nüssen von *Ginkgo biloba* wurde eine Gruppe ungesättigter Fettsäuren gefunden, die in ihrer Struktur von der üblichen Anordnung der Doppelbindungen in Fettsäuren abweichen. Jede der ungewöhnlichen Säuren hat die «isolierte» Doppelbindung in Δ^5-Position, was auf eine neuartige Biosynthese dieser Dien-, Trien- und Tetraensäuren hinweist.

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[10] H. ROTH in *Methoden der Organischen Chemie* (Ed. E. MÜLLER, Thieme-Verlag, Stuttgart 1953) vol. II, p. 292.

[11] *Acknowledgment.* The authors wish to thank L. H. WEINSTEIN and T. H. HAINES for Ginkgo leaf extracts, T. AKIYA for nuts, and G. MIZUNO for IR-investigation of the samples.

On the Origin of the Mg++-Activated ATPase of Muscle Mitochondrial Preparations

In contrast to those of the liver, muscle mitochondria have been found to exhibit a Mg++-stimulated ATPase activity, even if freshly prepared and having a good phosphorylating capacity[1]. It was therefore concluded that such ATPase must either represent a normal property, specific for muscle mitochondria, or derive from some contaminating material present in the mitochondrial preparations of muscle tissue. Such contamination appears to be fairly possible, because of the existence in the muscle fibre of distinct Mg++-stimulated ATPase activities associated with the myofibrils and the sarcotubular system.

In order to disclose a possible occurrence of contaminating material in muscle mitochondrial preparations, and to gain information on its origin, the effect of calcium on the Mg++-stimulated ATPase activity has been investigated in pigeon breast muscle homogenate fractions obtained by differential centrifugation at various gravitational forces. In the presence of Mg++, calcium does in fact activate myofibrillar ATPase while strongly inhibiting that of the sarcotubular system[2]. Further, calcium has long been known to cause structural damage in typical mitochondrial preparations, such as those of liver, resulting in a substantial increase of the Mg++-stimulated ATPase.

Material and Methods. Mitochondria were prepared from pigeon breast muscle. The tissue was minced and homogenized in 6 vol of *M* sucrose with a lucite Potter

[1] G. F. AZZONE, E. CARAFOLI, and U. MUSCATELLO, Exp. Cell Res. *21*, 456 (1960).

[2] U. MUSCATELLO, E. ANDERSSON-CEDERGREN, G. F. AZZONE, and A. VON DER DECKEN, J. Biophys. Biochem. Cyt. *10*, Suppl. 4, 201 (1961).

homogenizer. Molar sucrose was selected as the homogenizing medium after a number of preliminary experiments, since this molarity has been found to result in a better separation of the various subcellular structures than the lower ones, as judged by the distribution of cytochrome oxidase activity and by the RNA/protein ratio[3]. The fractions were isolated by differential centrifugations in a Spinco Model L Ultracentrifuge as indicated in the text. The pellets were re-suspended, without washing, in 0.25 *M* sucrose by gentle homogenization with a teflon pestle. All the operations were carried out at 0–2°C.

ATPase activity was tested in a medium of the following composition, in a final volume of 2 ml: 50 m*M* KCl; 25 m*M* Tris-HCl buffer, pH 7.4; 2.5 m*M* ATP; 2.0 m*M* $MgCl_2$, and 0.4 m*M* $CaCl_2$, when indicated. Incubation time, 15 min at 25°C. The reaction was stopped by addition of 1 ml of cold N perchloric acid.

Inorganic phosphate was estimated according to the method of MARTIN and DOTY, as modified by LINDBERG and ERNSTER[4]. Protein content was measured by either the biuret reaction[5], or the LOWRY procedure[6].

Results and Discussion. When fractionating a pigeon breast muscle homogenate in *M* sucrose, the pattern of distribution of cytochrome oxidase shows that most of the mitochondria are sedimented below 50,000 *g*[3]. Therefore, in the table the results are reported which refer to the fractions isolated by applying gravitational forces up to 50,000 *g*.

The effect of Ca^{++} on the Mg^{++}-stimulated ATPase activity of various mitochondrial fractions from pigeon breast muscle

Fractions	ATPase activity (μ moles P_i/mg protein/h)		
	without Ca^{++}	with 0.4 m*M* Ca^{++}	% *A*
400 *g*– 2,000 *g* × 10 min	15.85	16.63	+ 4.8
2,000 *g*–10,000 *g* × 10 min	26.41	23.72	−10.2
10,000 *g*–26,000 *g* × 20 min	62.10	54.20	−12.8
26,000 *g*–50,000 *g* × 30 min	45.68	34.46	−24.2

Experimental conditions: The enzymatic activity was tested in a medium of the following composition, in a final volume of 2 ml: 50 m*M* KCl; 25 m*M* tris-HCl buffer, pH 7.4; 2.5 m*M* ATP; 2.0 m*M* $MgCl_2$, and resuspended enzyme corresponding to 0.1–0.9 mg protein. Incubation time, 15 min at 25°C. The values represent the average of six experiments.

As shown in the Table, the addition of Ca^{++} resulted in a marked inhibition of the Mg^{++}-stimulated ATPase activity of all the muscle mitochondrial preparations, with the exception of the one obtained at low centrifugal force which occasionally showed a slight activation. However, in no case was this activation found to be of an order of magnitude comparable with that usually observed in liver mitochondria. The inhibition appeared to increase progressively in the fractions obtained at higher centrifugal forces, and to reach maximal values in the range of sedimentation typical of microsomes (35 to 50% of inhibition at 105,000 *g* for 1 h). The fact that the specific activity of the fraction isolated between 26,000 *g* and 50,000 *g* was lower than that of the fraction obtained between 10,000 *g* and 26,000 *g* could possibly be explained by the presence of subcellular structures, different from mitochondria and microsomes (lisosomes?), demonstrated by electron microscope investigation of the pellet.

Since the Mg^{++}-stimulated ATPase activity associated with the sarcotubular system is the only one known to be strongly inhibited by addition of Ca^{++}[2], it may be concluded that fragments of sarcotubular origin are likely to contaminate mitochondrial preparations from muscle tissue obtained by the conventional isolation procedures. In this connection, it should be added that electron microscopical examinations of the fractions have shown that, even at the lowest gravitational forces employed, vesicular structures closely resembling those of sarcotubular origin are commonly present, intermingled with mitochondria. Thus sarcotubular fragments could be responsible, at least in part, for the remarkably high ATPase activity in the presence of Mg^{++} of such preparations. It should be further emphasized that the contamination need not be large in order to give appreciable interference, since the specific activity is far higher in sarcotubular than in mitochondrial preparations[2].

The above considerations do not exclude, however, the existence in muscle mitochondria proper of a Mg^{++}-stimulated ATPase such as that of liver mitochondria. The fact that the mitochondrial fractions isolated at low gravitational force appeared to be only slightly inhibited, or even sometime activated, by addition of Ca^{++}, strengthens the above possibility.

Riassunto. Nelle preparazioni mitocondriali di muscolo è presente una notevole attività ATPasica stimolata dal Mg^{++}, anche se i mitocondri sono intatti e ben fosforilanti. Questa attività ATPasica, stimolata da Mg^{++}, appare essere inibita dall'aggiunta di Ca^{++}. Poichè questo comportamento è tipico della attività ATPasica associata ai microsomi, gli autori concludono che l'attività ATPasica stimolata da Mg^{++} delle preparazioni mitocondriali di muscolo è molto probabilmente espressione di un inquinamento delle preparazioni stesse da parte di frammenti originanti dal reticolo sarcoplasmico.

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Etude de la distribution du calcium dans l'os haversien avec le radioanalyseur à microsonde électronique

Le radioanalyseur à microsonde électronique peut être utilisé avec succès pour l'étude des tissus biologiques minéralisés. La distribution du calcium a été déterminée au moyen de cet instrument dans la dentine et l'émail par BOYDE, SWITSUR et FEARNHEAD[1], dans l'os adulte par TOUSIMIS[2], et dans le cartilage épiphysaire par BROOKS, TOUSIMIS et BIRKS[3].

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