Tableau I. Foie: Activité spécifique et incorporation en pour-cent de l'activité de l'acétate injecté. Chaque valeur représente la moyenne de quatre souris.

		Temps écoulé après injection (en minutes)					
		5	10	15	30	45	60
Acides gras totaux	Activité spécifique (en c.p.m.)	358	75	435	116	198	93
	Incorporation (en %)	24,7	6,0	24,3	8,9	12,1	5,1
Acides gras extraits des phospholipides	Activité spécifique (en c.p.m.)	202	59	352	114	174	96
	Incorporation (en %)	—	1,8	7,8	3,3	4,7	2,4
Acides gras extraits des graisses neutres	Activité spécifique (en c.p.m.)	491	86	572	142	252	118

Tableau II. Muscles: Activité spécifique et incorporation en pour-cent de l'activité de l'acétate injecté. Chaque valeur représente la moyenne de quatre souris.

		Temps écoulé après injection (en minutes)					
		5	10	15	30	45	60
Acides gras totaux	Activité spécifique (en c.p.m.)	3,4	1,1	5,1	3,1	2,8	3,0
	Incorporation (en %)	0,10	0,03	0,12	0,08	0,04	0,07

Tableau III. Foie: Activité spécifique et incorporation en pour-cent de l'activité de l'acétate injecté. Chaque valeur représente la moyenne de quatre souris.

		Temps écoulé après injection (en minutes)						
		5	10	15	30	45	60	90
Acides gras totaux	Activité spécifique (en c.p.m.)	57	224	416	233	144	284	59
	Incorporation (en %)	5,5	17,5	24,6	13,3	7,7	17,1	4,2

Les injections combinées de C^{14} et P^{32} montrent la différence existant entre l'incorporation de ces deux éléments. L'incorporation du P^{32} dans les phosphatides présente une lente augmentation; elle n'atteint une valeur maximale qu'entre 6 et 12 h après l'injection.

Ces travaux ont été subsidiés par l'Institut de Physique nucléaire belge.

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Summary

The determination of the rate of incorporation of C^{14} in the fatty acids of the glycerides and the phosphatides confirms their rapid metabolism in the liver of mice (maxima at about 10–60 min).

Oxidases of *Polystictus versicolor*

Many basidiomycetes produce abundant polyphenol oxidase in culture[1]. Since it has been suggested that this enzyme may act as a terminal oxidase in respiration[2] it was considered of interest to see whether or not this was so for *Polystictus versicolor* (Linn.) Fr. which is known to produce abundant laccase[3].

Flasks containing a liquid medium of 2% malt extract and 2% peptone were inoculated with mycelium that had been blended in a Waring blendor. In shake culture the fungus grew as small pellets. Since the respiration rate of the pellets was not increased by allowing them to respire in pure oxygen it was considered that oxygen was not limiting the respiration rate within the pellet. This method of growth ensured homogeneous material[1] and the washed pellets were pipetted directly into Warburg flasks for respiration measurements. The respiration was measured by determining the oxygen uptake of the intact mycelium in the presence and absence of respiratory inhibitors. The usual precautions to ensure maximum effectiveness and degree of selectivity of the inhibitors were employed[2].

As can be seen from the table, cyanide which inhibits heavy metal containing enzymes caused approximately 80% inhibition of the respiration. 95% carbon monoxide with 5% oxygen mixtures caused 43% to 65% inhibition, most of which was reversible by light and this is characteristic of cytochrome oxidase mediated respiration[3]. Salicylaldoxime, dieca and phenylthiourea which inhibit copper containing enzymes caused only 4% to 13% inhibition. This inhibition might indicate the participation of a copper-containing enzyme in respiration but could also be due to a non-selective effect of the inhibitors on the cytochrome oxidase.

[1] G. LINDEBERG, Physiol. Plant. *1*, 196 (1948).
[2] W. O. JAMES, Biol. Revs. Cambridge Phil. Soc. *28*, 245 (1953).
[3] G. FÅHRAEUS, Physiol. Plant. *5*, 284 (1952).

[1] J. W. FOSTER, *Chemical Activities of Fungi* (Acad. Press Inc., New York, 1949).
[2] W. O. JAMES, Ann. Rev. Plant. Physiol. *4*, 59 (1953).
[3] W. O. JAMES, Biol. Revs. Cambridge Phil. Soc. *28*, 245 (1953).

Table I.–Effect of Various Inhibitors on the Respiration of *Polystictus versicolor*

Expt. No.	pH at which inhibitor applied	Inhibitor	% inhibition
L 8	7	95% CO with 5% O_2 in the light	10
	7	95% CO with 5% O_2 in the dark	65
L 10	7	95% CO with 5% O_2 in the light	0
	7	95% CO with 5% O_2 in the dark	43
L 9a	7	1 mM KCN	70
L 9b	7	1 mM KCN	80
L 9c	7	1 mM KCN	80
L 11	5	0·2 mM diethyldithiocarbamate Na salt (dieca)	12
	5	saturated solution phenylthiourea	4
	5	saturated solution phenylthiourea	13
L 12a	5	1 mM salicylaldoxime	13
L 12b	5	1 mM salicylaldoxime	4

Spectroscopic evidence of the presence of cytochromes was obtained. Bands corresponding to the alpha absorption bands of the reduced cytochromes *a*, *b* and *c* were observed with a Hilger microspectroscope when light from a 500 W metal filament lamp was passed through a thick suspension of the mycelium to which sodium hydrosulphite had been added[1].

Cell free extracts of the mycelium were prepared by grinding the well washed mycelium with clean sand and ice cold buffer in a chilled mortar (9 parts of 0·05 *M* phosphate buffer pH 7·0 to 1 part of moist mycelium). Cell debris and sand were removed by centrifuging at 500 g for 5 min. Extra-cellular laccase preparations were prepared by the method of LINDEBERG and HOLM[2].

Table II.–Activities of Enzyme Extracts from a 7-day Old Culture of *Polystictus versicolor*

Enzyme system	Substrate	pH	$Q \cdot O_2$ dry weight (μl O_2 per mg per h)
Cytochrome oxidase	Hydroquinone	7·0	24
Extra-cellular laccase	Pyrocatechol	5·2	30
Laccase from intact mycelium	Pyrocatechol	5·2	1·0

For measurements of enzyme activities the Warburg flasks contained 1 ml enzyme preparation, 5 mg substrate in the sidearm, 0·15 ml 15% KOH in the centre well. All reagents were dissolved in 0·05 *M* phosphate buffer to give a total volume of fluid of 3 ml. For the cytochrome oxidase tests 0·3 ml of 4×10^{-4} *M* cytochrome *c* (*Evans*) was added. The laccase activity of the medium (extracellular laccase) was related to the amount of mycelium from which it had come.

Since the laccase preparation obtained from the intact mycelium had a $Q \cdot O_2$ of only 1·0 it is unlikely that laccase activity could account for the observed uptake of oxygen by the intact mycelium which had a $Q \cdot O_2$ (dry weight) varying from 15 to 28. Laccase preparations having a $Q \cdot O_2$ of 30 were obtained from the culture medium (cf. FÅHRAEUS[1]) but these could play no part in the respiration of the intact mycelium. On the other hand, cytochrome oxidase was present in sufficient quantity to account for the total observed oxygen uptake of the intact mycelium.

These experiments indicate that the electron transference to molecular oxygen is mediated in this fungus mainly, if not completely, by a cytochrome *c*-cytochrome oxidase system. Although small quantities of laccase are present in the mycelium and much larger quantities in the culture medium, it is unlikely that these act as terminal oxidases in the respiration of this fungus.

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Department of Botany, University of Liverpool, December 6, 1954.

Zusammenfassung

Obgleich *Polystictus versicolor* reichlich Laccase bildet, zeigen die Ausfälle in respirationshemmenden Versuchen, dass die Cytochromoxydase die entscheidende Oxydase dieses Pilzes ist.

[1] G. FÅHRAEUS, Physiol. Plant. *5*, 284 (1952).

[1] D. KEILIN and E. F. HARTREE, Nature *164*, 254 (1949).

[2] G. LINDEBERG and G. HOLM, Physiol. Plant. *5*, 100 (1952).

Über den Umsatz von Anthranilsäure mit Brenztraubensäure

v. EULER[1] hat sich in mehreren Arbeiten mit den Umsetzungsprodukten von Verbindungen, die möglicherweise aus Buttergelb (p-Dimethylamino-azobenzol) in der Leber entstehen und den in der Leber nachgewiesenen Stoffwechselprodukten, wie etwa der Brenztraubensäure, eingehend befasst. Es wurden die Verbindungen, die aus Brenztraubensäure und p-Phenylendiamin[2], p-Aminophenol[2], den Aminobenzoesäuren[2], dem Sulfanilamid[3] usw. *in vitro* entstehen, beschrieben und ihr konstitutioneller Aufbau bewiesen.

Für den Umsatz von Anthranilsäure (AS) mit Brenztraubensäure (BTS) haben v. EULER und HASSELQUIST[3] einen detaillierten Reaktionsmechanismus angeführt. Der entstehenden Verbindung der Bruttozusammensetzung $C_{13}H_{11}O_6N$, die antibiotisch und im Kressenwurzeltest wirksam ist, wurde die Konstitution I einer 1,2-Dihydrochinaldin-2,4,8-tricarbonsäure zugeschrieben. Sie ist phototrop (gelb-grün) und gab bei der Chromsäureoxydation die Chinaldin-4,8-dicarbonsäure VI bzw. bei der Destillation mit Eisenpulver Chinaldin. Diese Chinaldin-4,8-dicarbonsäure sollte sich beim Liegen grün färben.

Beim Umsatz der Säure I mit Diazomethan entstand nach den Angaben der schwedischen Autoren die Verbindung $C_{17}H_{19}O_6N_3$, der die Konstitution II zukommen sollte. Durch Erhitzen dieser Verbindung über ihren

[1] H. v. EULER, Naturw. Rdsch. *4*, 133 (1953). In dieser Arbeit wurde scheinbar irrtümlich das Reaktionsprodukt aus AS und 2 Molen BTS zum Unterschied von der Originalarbeit als Chinaldin-4,8-dicarbonsäure angeschrieben, während es dort als 1,2-Dihydrochinaldin-2,4,8-tricarbonsäure formuliert worden war.

[2] H. v. EULER, H. HASSELQUIST und E. ERIKSSON, Ark. Kemi *5*, 251 (1953).

[3] H. v. EULER und H. HASSELQUIST, Ark. Kemi *5*, 193 (1953).