

Quantitative Examination by the Carbon Balance Sheet Method of the Types of Products Formed from Glucose by Miscellaneous Species of Fungi

John Howard Birkinshaw, John Henry Victor Charles, Arthur Clement Hetherington and Harold Raistrick

Phil. Trans. R. Soc. Lond. B 1931 220, 99-125

doi: 10.1098/rstb.1931.0017

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to Phil. Trans. R. Soc. Lond. B go to: http://rstb.royalsocietypublishing.org/subscriptions

99]

Studies in the Biochemistry of Micro-organisms.

Part VI.—Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by miscellaneous species of fungi.

By John Howard Birkinshaw, John Henry Victor Charles, Arthur Clement Hetherington and Harold Raistrick.

In view of the encouraging results obtained from the preparation of carbon balance sheets, particularly for species of Aspergillus and Penicillium, the work was now extended to include a number of varied species of fungi belonging to many other genera. The carbon balance sheets for these miscellaneous species are given in this paper. The conditions of working adopted were exactly the same as those previously used and described in detail in Parts II and III. The results are arranged under the different genera according to the species of fungi investigated, and include the following:—

Class: ASCOMYCETES.

Genus 1. Sordaria, 1 species.

Genus 2. Chætomium, 1 species.

Genus 3. Sclerotinia (conidial form = Botrytis), 1 species.

Class: BASIDIOMYCETES.

Genus 1. Ustilago, 2 species.

FUNGI IMPERFECTI.

Order: HYPHOMYCETALES.

Family: Moniliaceæ.

Genus 1. Eidamia, 2 species.

Genus 2. Sporotrichum, 2 species.

Genus 3. Trichoderma, 2 species.

Genus 4. Cephalothecium, 1 species.

Family: DEMATIACEÆ.

Genus 1. Cladosporium, 5 species.

Genus 2. Helminthosporium, 6 species.

Genus 3. Heterosporium, 2 species.

Genus 4. Alternaria, 3 species.

Genus 5. Fumago, 2 species.

Genus 6. Clasterosporium, 2 species.

Genus 7. Rhacodium, 1 species.

o 2

Family: STILBACEÆ.

Genus 1. Stysanus, 1 species.

Family: Tuberculariaceæ.

Genus 1. Fusarium (see Part V).

Genus 2. Epicoccum, 2 species.

Class: ASCOMYCETES.

The following is the history of the species included in this class:—

Genus 1. Sordaria, 1 species.

Sordaria species, Catalogue No. Ae. 11. Isolated at Ardeer from decaying cotton sludge from methane plant.

Genus 2. Chætomium, 1 species.

Chætomium species, Catalogue No. Ae. 2. Isolated by Mr. F. T. Brooks, of Cambridge, from waste paper.

Genus 3. Sclerotinia (conidial form).

Botrytis cinerea Persoon, Catalogue No. Ae. 4. Isolated at Ardeer from decaying rosebud.

The carbon balance sheets of these three species are given in Table I.

None of these species grows particularly well on the CZAPEK-Dox solution used. The three carbon balance sheets show nothing of any particular biochemical interest except in the case of *Sordaria* species. This fungus gives rise to comparatively large amounts of volatile neutral compounds (alcohol) from glucose and has a fairly high respiration coefficient (1.56). In all other respects the main function of these three species seems to be to convert glucose into carbon dioxide.

Class: BASIDIOMYCETES.

Genus. Ustilago, 2 species.

- (1) Ustilago Mayidis (D.C.) CORDA, Catalogue No. Af. 8. Purchased from Centraalbureau voor Schimmelcultures at Baarn.
- (2) Ustilago avenæ (Pers.) Jensen, Catalogue No. Af. 7. Purchased from Baarn.

The carbon balance sheets for these two species are given in Table II.

These two species, and in particular *Ustilago avenæ*, were exceptionally difficult to cultivate on Czapek-Dox solution. Their carbon balance sheets are uninteresting, as they do not give rise to appreciable amounts of any metabolic products except carbon dioxide.

 ${\bf TABLE~I.} \hbox{---Carbon balance sheets for three species of } {\it Ascomycetes}.$

Specie	s of A s	scomycetes:	Sordaria species.	Chxetomium species.	Sclerotinia
Ca	ıtalogu	e number :	Ae. 11	Ae. 2	Ae. 4
$\mathbf{E}\mathbf{x}\mathbf{p}$	erimer	nt number :	E 1	E 2	E 3
Incubation	n perio	od in days:	90	70	86
Carbon Balance Sheet					
Carbon in solution (start)		gm.	4.901	$5 \cdot 043$	5.043
Carbon in H ₂ SO ₄		,,	0.016	nil	0.002
$\dots \dots \dots \dots$,,	$1 \cdot 641$	$1\cdot 294$	1.680
,, in mycelium		,,	0.364	0.671	1.099
,, in solution (end)	•••	,,	$2 \cdot 747$	$2 \cdot 940$	2 · 107
" accounted for	•••	,,	4.768	$4 \cdot 905$	4.888
" accounted for		per cent.	97.3	$97 \cdot 3$	96.9
Analysis of Solution.					
Carbon in residual glucose		gm.	$1 \cdot 251$	$2\cdot 624$	1.574
in OO in adjustion	•••	_	0.009	0.039	0.001
in malakila asida			0.005	-	0.009
in non voletile saids			0.039	0.130	0.102
in volatile neutral compounds	•••	*** ,,	1.156	0.001	0.015
", in synthetic compounds	•••	,,	0.050	0.077	0.064
Total carbon accounted for			2.510	$2 \cdot 871$	1.765
,, ,, in solution	•••	,,	2.747	2.940	$2 \cdot 107$
Carbon unaccounted for (by difference))	,,	0.237	0.069	0.342
				* :	
Residual Glucose.			0.794	1 490	0.006
Glucose (by polarimeter)	•••	per cent.	0.734	$1 \cdot 430$ $1 \cdot 312$	$0.826 \\ 0.787$
,, (SHAFFER-HARTMANN)	•••	,,	0.625	1.328	$0.787 \\ 0.744$
,, (Wood-Ost)	•••	,,	0.603		
,, (by alkaline iodine)	•••	,,	0.653	1.380	0.813
Acids.				Decrease of	
Titration (N/1 acid)		c.c.	0.2	0.8	0.6
Volatile acids (N/1 acid)					0.32
Barium salts (weight)		gm.	0.038		0.021
Calcium salts (weight)		,,	0.295	0.782	0.457
Volume of oxygen absorbed	•••	c.c.	1974	2049	2739
Respiration coefficient	•••		1.56	$1\cdot 22$	$1 \cdot 15$
Mycelium (weight)	•••	gm.	0.730	$1\cdot 264$	1.903
,, (carbon)		per cent.	49.8	$53 \cdot 1$	57.8

Table II.—Carbon balance sheets for species of Ustilago.

				\mathbf{Sp}	ecies c	of Usti	lago:	U. Mayidis.	U . aven α .
				Ca	atalogi	ıe nun	nber:	Af. 8	Af. 7
				Exp	erimei	nt nu n	nber:	D 1	D 2
			Inc	ubatio	n perio	od in d	lays:	80	124
	Carbo	n Bala	nce Si	heet.					
Carbon in	solution (start)			•••	•••	•••	gm.	$4 \cdot 901$	$5 \cdot 043$
Carbon in	H.80		•••		•••			Nil	0.001
	CO_2	•••					"	1.440	0.933
	mycelium	•••	•••	•••	•••	•••	,,	0.878	0.526
	solution (end)	•••	•••	•••	•••	•••	,,	2.485	3.416
,, acc	counted for	•••	•••	•••	•••	•••	,,	4.803	4.876
,, acc	counted for	•••	•••	•••	•••	per	cent.	98.0	96.7
	Analg	ysis of	Solute	ion.					
Carbon in	residual glucose						gm.	1.994	$3 \cdot 002$
	CO ₂ in solution	•••	•••		•••		,,	0.005	0.002
	volatile acids				•••		,,	0.022	Nil
	non-volatile acid	S		•••	•••	•••	,,	0.119	0.170
	volatile neutral o			•••	•••	•••	,,	0.006	Nil
	synthetic compo		•••	•••	•••		,,	0.106	0.081
Total carb	on accounted for							$2 \cdot 252$	$3 \cdot 255$
,, ,,	in solution		•••	•••	•••	•••	,,	$2 \cdot 485$	$3 \cdot 416$
Carbon un	accounted for (by	differ	ence)	•••		•••	,,	0.233	0.161
	Res	idual (Hucosi	9.	/				
Hucose (b	y polarimeter)	•••				nar	cent.	1.046	$1 \cdot 602$
,, (Si	HAFFER-HARTMAN		•••	•••				0.997	1.501
	Vood-Ost)	•••	•••		•••		,,	1.016	1.523
	y alkaline iodine)		•••	•••	•••		,,	1.085	1.558
		Acids	3.					Decrease of	Decrease of
Citration (N/1 acid)	•••					c.c.	Decrease of 0.2	Decrease of 0.9
	ids (N/1 acid)			•••		•••	,,	$1.\overline{26}$	0.98
Barium sal	lts (weight)	•••					gm.	0.088	0.007
Calcium sa	lts (weight)	•••	•••	•••	•••		,,	0.580	0.785
	oxygen absorbed		•••	•••	•••		c.c.	2309	1734
tespiration	n coefficient	•••	•••	•••	•••	•••	•••	1 · 17	1.01
Mycelium ((weight)		• • •	•••		•••	gm.	1.561	$1 \cdot 012$
	(carbon)	•••	•••	•••	•••		cent.	$56 \cdot 2$	$52 \cdot 0$
"	(00000000000000000000000000000000000000	•••	•••	•••	•••	Por	cont.	50 Z	02 0

FUNGI IMPERFECTI. Order: HYPHOMYCETALES.

Family: Moniliaceæ. Genus 1. Eidamia, 2 species.

- (1) Eidamia viridescens Horne et Williamson, Catalogue No. Ac. 75. Received from Dr. A. S. Horne, Imperial College of Science and Technology.
- (2) Eidamia catenulata Horne et Williamson, Catalogue No. Ac. 76. Received from Dr. Horne.

The carbon balance sheets for these two species are given in Table III.

The general characteristics of the above species of Eidamia are given by Horne (A. S.), and Williamson (H. S.) in a paper on "The Morphology and Physiology of the Genus Eidamia," 'Annals of Botany,' Vol. 37 (1923), p. 393. To quote from their paper, p. 393, "The genus Eidamia was founded by Lindau to include fungi which bear a general resemblance to Aspergillus, but differ from it in possessing not only conidia, but also spores of a second type." This is particularly interesting since the carbon balance sheets for both species are of the type associated with certain species of Aspergillus. The production of large amounts of volatile neutral compounds, with correspondingly high respiration coefficients, absence of volatile acids, production of moderate amounts of titratable acidity, together with moderate amounts of carbon in the form of non-volatile acids, and a moderate value for "carbon unaccounted for," all suggest the type of carbon balance sheet associated with A. clavatus, or some strains of A. niger. It is also of interest to note that these two different species of Eidamia have similar types of carbon balance sheets.

Genus 2. Sporotrichum, 2 species.

- (1) Sporotrichum carneolum, Catalogue No. Ag. 23. Isolated from meat by Mr. F. T. Brooks, of Cambridge.
- (2) Sporotrichum bombycinum (CORDA) RAB., Catalogue No. Ag. 25. Received from Baarn via Mr. F. T. Brooks, of Cambridge.

The carbon balance sheets for these two species, which are given in Table IV, are both of the same type. Neither species gives rise to an appreciable amount of any metabolic product other than CO₂, and both of them produce an actual decrease in the original acidity of the medium. They both grow quite well on the CZAPEK-Dox medium used, but require a considerable time to metabolize even a moderate amount of glucose.

Table III.—Carbon balance sheets for species of Eidamia.

				Spe	ecies of	Eida	nia:	E. viridescens.	$E.\ catenulata$
				Ca	talogu	e num	ber:	Ac. 75	Ac. 76
				Exp	erimen	t num	ber:	102	103
	,		Incu	batio	n perio	d in d	ays:	45	35
	Carbo	n Balanc	e She	et.					
Carbon i	n solution (start)			•••	•••	•••	gm.	$4 \cdot 834$	$4 \cdot 834$
Carbon i	n H ₂ SO ₄							0.013	0.006
	$n \stackrel{\text{11}}{\text{CO}}_{2} \dots \qquad \dots$	•••		•••		•••	,,	1.802	1.063
	n mycelium	•••			•••	•••	,,	0.583	0.443
	n solution (end)		•	•••	•••	•••	,,	$2 \cdot 376$	3.168
,, 1	in solution (end)	•••	•	•••	•••	•••	,,	2 010	9.100
,, ε	accounted for		•	•••	•••	•••	,,	4.774	4.682
,, a	accounted for	•••	•	•••	•••	per	cent.	98.8	96.9
	Analy	sis of So	lutio	n.					
Carbon i	n residual glucose	•••			•••		gm.	0.777	1.812
	n CO ₂ in solution	•••			•••	•••		0.009	0.011
,, i	n volatile acids	•••		•••	•••	•••	,,	Nil	Nil
,,	n non-volatile acids			•••		•••	,,	0.254	0.141
	n volatile neutral c					•••	"	0.952	0.839
	n synthetic compou			•••	•••	•••	,,	0.125	0.061
Potal car	rbon accounted for						-	2 · 117	9.061
	in		•	•••	•••	••• ,	"	1	2.864
,,	,, in solution	;	•	•••	•••	•••	"	$2 \cdot 376$	$3 \cdot 168$
Carbon	unaccounted for (b	y differe	nce)	•••	•••	•••	,,	0.259	0.304
	Res	idual Glu	cose.						
Glucose	(by polarimeter)				•••	per	cent.	0.367	0.911
,,	SHAFFER-HARTMAN			•••		-	1	0.388	0.906
	(Wood-Ost)			•••	•••		"	0.388	0.978
,,	(by alkaline iodine)			•••	•••		,,	0.425	0.963
		Acid	· · · · · · · · · · · · · · · · · · ·		T. C				
Titration	n (N/1 acid)	21000					c.c.	4.3	$2 \cdot 7$
	acids (N/1 acid)	•••		•••	•••	•••	1	Nil	0.44
	salts (weight)	•••	-	•••	•••	•••	om .	Nil	0.008
	salts (weight)		•	•••	•••	•••	gm.		
		•••	•			•••	,,	1.027	0.621
	of oxygen absorbed			•••	•••	•••	c.c.	2129	1190
Kespirat	ion coefficient		•	•••	•••	•••	•••	1.59	$1 \cdot 69$
Myceliur	n (weight)		-			,	otm:	1.146	0.001
•	(combon)	•••	•	•••	•••	non.	gm.	1.146	0.901
,,	(carbon)	•••	•	• • •	•••	per	cent.	50.9	$49 \cdot 4$

BIOLOGICAL

THE BIOCHEMISTRY OF MICRO-ORGANISMS.

105

Table IV.—Carbon balance sheets for species of Sporotrichum.

			i	Species	s of Sp	orotric	hum:	S. carneolum.	S. bombycinus
				C	atalog	ue nur	nber :	Ag. 23	Ag. 25
				Exp	perime	nt nur	nber :	C 2	C 3
			Inc	cubatio	on peri	od in	days:	83	82
	Carbo	n Bala	ance S	heet.	4				
Carbon in solu	tion (start)	•••	•••		•••	•••	gm.	4.901	4.901
Carbon in H ₂ S	Ο ₄	•••						0.001	0.001
,, in CO ₂		•••	•••	•••	•••	•••	,,	1.715	$1.\overline{192}$
	elium		•••	•••	•••	•••	,,	0.780	1.041
,, in myc	tion (end)	•••	•••	•••	•••	•••	,,	$2 \cdot 303$	2.439
,, In solu	non (ena)	•••	•••	•••	•••	•••	. ,,	2.909	7.439
,, accoun	ted for	•••	•••	•••	•••	•••	,,	4.799	4.673
" accoun	ted for	•••	•••	•••	•••	per	cent.	97.9	95 • 4
	Anal	ysis of	Soluti	on.					
Carbon in resid	lual glucose		•••				gm.	1.780	1.802
,, in CO ₂	in solution	•••	•••				_	0.016	0.038
,, in vola	tile acids	•••	•••	•••	•••	•••	,,	0.024	0.016
	volatile acid		•••		•••	•••	"	0.223	0.182
	tile neutral o					•••	,,	0.001	0.003
	hetic compo		•••	•••	•••	•••	,,	0.137	0.152
Total carbon a	ccounted for		•••					2.181	2 · 193
:	1 solution	•••		•••	•••	•••	,,		I .
,, ,, lr	r solution	•••	•••	•••	•••	•••	,,	2.303	2 · 439
Carbon unacco	unted for (by	y differ	ence)	•••	•••	•••	,,	0.122	0.246
	Res	idual (Hucose	з.					
Glucose (by po	larimeter)	•••	•••		•••	per	cent.	0.896	0.910
,, (Shafi	ER-HARTMA	NN)	•••	•••		-		0.890	0.901
	-Оѕт)	•••	•••				,,	0.920	0.916
,, (by alk	aline iodine		•••		•••		,,	0.938	0.963
		Acid	8.			Section of the section of		Decrease of	Decrease of
Citration (N/1	acid)						0.0	0.1	0.7
olatile acids (N/L acid)		•••	•••	•••	•••	c.c.	2.58	$1 \cdot 19$
Barium salts (w	reight)	•••	•••	•••	•••	•••	,,	0.110	
alcium salts (v		•••	•••	•••	•••	•••	gm.		0.066
		•••	•••	•••	•••	•••	,,	0.899	0.994
olume of oxyg							c.c.	2788	1797
Respiration coe		•••	•••	•••	•••	•••		1.16	1.28
ycelium (weig	ht)	-	-				07700	1 500	9 001
/1·		•••	•••	•••	•••	•••	gm.	1.526	2.001
" (carb	on)	•••	• • •	•••	•••	per	cent.	51 · 1	$52 \cdot 0$

Genus 3. Trichoderma, 2 species.

- (1) *Trichoderma* species, Catalogue No. Ag. 47. Isolated at Ardeer from pale green spots on wood.
- (2) Trichoderma lignorum (Tode.) Harz, Catalogue No. Ag. 22. Isolated at Ardeer from beech bark.

The carbon balance sheets for these two species are given in Table V.

Neither of the species of *Trichoderma* investigated grew well on CZAPEK-Dox solution, as judged by the weight of mycelium produced. They both give the same type of balance sheet, which indicates, in both cases, the production of considerable amounts of volatile neutral compounds. There are no other items of particular interest in either of the balance sheets.

Genus 4. Cephalothecium, 1 species.

(1) Cephalothecium roseum Corda, Catalogue No. Ag. 21. Isolated at Ardeer from a decaying twig.

The carbon balance sheet for this species, which is given in Table VI, shows no characteristics of any particular interest. This species grew well on CZAPEK-Dox solution, but was only able to metabolise very slowly the glucose supplied, since, even after 67 days, only about 65 per cent. of the glucose had been utilised. It apparently gives rise to no metabolic product other than carbon dioxide, in appreciable amounts.

107

Table V.—Carbon balance sheets for species of Trichoderma.

				Specie	s of Tr	ichode	rma:	T. species.	T. lignorum	
				C	atalogu	ıe nun	ber:	Ag. 47	Ag. 22	
				Exp	perimen	nt nun	ber :	C 4	C 6	
			Inc	eubatio	on perio	od in d	lays:	50	79	
	Carbo	n Bala	nce S	heet.						
Carbon in solutio	n (start)	•••	•••	•••	•••		gm.	$5 \cdot 043$	5.043	
Carbon in H ₂ SO ₄	•••	•••		•••	•••		,,	0.009	0.011	
,, in CO ₂	•••			•••	•••		,,	$1 \cdot 474$	1.320	
,, in myceli		•••					,,	$0 \cdot 220$	0.375	
,, in solution			•••	•••		•••	,,	3.086	3.141	
" accounte	, ,	•••		•••	•••		,,	4.789	4.847	
" accounted			•••	•••		per	cent.	95.0	96.1	
	Amal	unia of	Salart	ion			-			
~	,	ysis of	Borur	юн.		•		1 OF	2 151	
Carbon in residua		•••	•••	• • •	•••	• • •	gm.	1.978	$2 \cdot 454$	
" in CO ₂ in	solution	•••	• • •	• • •	•••	•••	,,	0.008	0.006	
,, in volatil		•••	• • •	•••	•••	•••	,,	0.028	0.011	
,, in non-vo	latile acid	s	• • •	•••		•••	,,	0.098	0.118	
,, in volatil	e neutral c	ompou	$_{ m nds}$	•••	•••		. ,,	0.620	0.383	
,, in synthe	tic compo	ands	•••	•••	•••	•••	,,	0.147	0.104	
Total carbon acc	ounted for							2.879	3.076	
in a	olution		•••	•••	•••	•••	,,	3.086	$3 \cdot 141$	
,, ,, in s	oration	•••	•••	•••	•••	•••	,,	. 3.000	9.141	
Carbon unaccoun	ted for (by	differ	ence)	•••	•••	•••	,,	0.207	0.065	
	Res	idual G	lucose	г.						
Glucose (by polar	imeter)		•••	•••	•••	per e	ent.	1.090	$1 \cdot 209$	
	-Hartman	IN)	•••	•••	•••	-	,	0.989	$1\cdot 227$	
,, (Wood-C		•••	•••	•••	•••		,	1.028	$1 \cdot \overline{212}$	
	ine iodine)		•••	•••	•••		,	1.094	$1 \cdot 247$	
		Acids	,						Decrease of	
D:1	:4\	420000	•					9.5	0.5	
Citration (N/1 aci		•••	•••	•••	•••	•••	c.c.	2.5	0.0	
Volatile acids (N/		•••	•••	. •••	•••	•••	,,	1.77	0.049	
Barium salts (wei	gnt)	•••	•••	•••	•••	•••	gm.	0.131	0.042	
Calcium salts (we	ight)	•••	•••	•••	•••	•••	,,	0.498	0.684	
Volume of oxygen	absorbed			•••			c.c.	1984	2061	
Respiration coeffic		•••		•••		• • • • • • • • • • • • • • • • • • • •		$1 \cdot 40$	$1 \cdot 20$	
M 1' / '1'	\							0.407	0.759	
Mycelium (weight		•••	•••	•••	•••		gm.	0.407		
,, (carbon)		• • •			per c	ent.	$54 \cdot 1$	$49 \cdot 4$	

Table VI.—Carbon balance sheet for a species of Cephalothecium.

			Spe	cies of 6	Cephalo	otheciv	um:	$C.\ roseum.$
				Cat	talogue	num	ber:	Ag. 21
				Expe	eriment	num	ber:	C 1
			Inc	ubation	period	l in da	ays:	67
	Car	bon Ba	lance	Sheet.	Marie Commission of the Commis			*
Carbon	in solution (sta	rt)	•••	•••	•••	•••	gm.	5.018
Carbon	in H ₂ SO ₄	•••	•••	•••	•••	•••	,,	0.001
,,	in CO ₂						,,	1.780
,,	in mycelium	•••	•••		•••		,,	0.853
,,	in solution (end		•••	•••	•••	•••	,,	$2 \cdot 287$
,,	accounted for				•••		,,	$4 \cdot 921$
,,	accounted for	•••	•••	•••	•••	per	cent.	98 • 1
	Ar	alysis	of Solv	ution.				лен Монто (Монто — Монто (Монто ((Монто (Монто (М
Carbor	n in residual glu	-					gm.	$1 \cdot 774$
,,	in CO ₂ in solut		•••			•••		0.049
,,	in volatile acid	s	•••	•••	•••	•••	,,	0.053
,,	in non-volatile		•••	•••	•••	•••	,,	0.175
,,	in volatile neu				•••	•••	,,	0.077
,,	in synthetic co			•••		•••	,,	0.147
Total	carbon accounte	d for	•••				.	$2 \cdot 275$
,,	,, in soluti		•	•••	•••	•••	,,	$2 \cdot 287$
Carbo	n unaccounted f	or (by	differe	nce)	, •••	•••	,,	0.012
		 Residua	ıl Gluc	ose.				
Glucos	se (by polarimet					nar	cent.	0.918
,,	(Shaffer-Ha	RTMAN	N)	•••	•••	her		$0.918 \\ 0.887$
,,	(Wood-Ost)		•••		•••		,,	0.868
,,	(by alkaline i		•••	•	•••		,,	0.875
		A	cids.			***************************************		Decrease of
Titrat	cion (N/1 acid)						c.c.	0.7
	ile acids $(N/1 \text{ ac})$	id)	•••	•••	•••	•••		$2 \cdot 20$
	m salts (weight)		•••	•••	•••	•••	om ,	0.269
	ım salts (weight			•••	•••	•••	gm.	$0.269 \\ 0.763$
Volur	ne of oxygen ab	sorbed					c.c.	2867
	ration coefficien		•••	•••	•••	•••		$1 \cdot 19$
								1 001
Myce	lium (weight)			• • •	• • •		gm.	$1 \cdot 624$

FUNGI IMPERFECTI.

Order: HYPHOMYCETALES.

Family: Dematiaceæ.

Genus 1. Cladosporium, 5 species.

- (1) Cladosporium species S, Catalogue No. Ag. 1. Isolated from cold storage meat by Mr. F. T. Brooks, of Cambridge.
- (2) Cladosporium species 1020, Catalogue No. Ag. 3. Isolated from cold storage meat by Mr. F. T. Brooks.
- (3) Cladosporium species 60, Catalogue No. Ag. 5. Isolated from dead leaves by Mr. F. T. Brooks.
- (4) Cladosporium species 55, Catalogue No. Ag. 9. Isolated from a maize shoot by Mr. F. T. Brooks.
- (5) Cladosporium species from lichen, Catalogue No. Ag. 109. Isolated at Ardeer from a lichen.

The carbon balance sheets for these five species are given in Table VII.

All the species of *Cladosporium* grew quite reasonably well on Czapek-Dox solution, but with one exception did not metabolize very quickly the glucose provided. The single exception is *Cladosporium* species 55, which completely destroyed all the glucose in 76 days. From a biochemical point of view, the balance sheets for these five species, which are all of the same type, are very uninteresting, since no compounds of any sort other than carbon dioxide are produced in an appreciable amount. None of these species produced appreciable amounts of acid, either of a volatile or non-volatile nature, and in three cases out of five there is an actual decrease in the titratable acidity of the medium. Of minor interest is the uniformly high percentage of carbon in the mycelium.

Genus 2. Helminthosporium, 6 species.

- (1) Helminthosporium geniculatum Tracy et Earle, Catalogue No. Ag. 93.
- (2) Helminthosporium teres Sacc., Catalogue No. Ag. 94.
- (3) Helminthosporium inaequalis Shear., Catalogue No. Ag. 95.
- (4) Helminthosporium species, Catalogue No. Ag. 96.
- (5) Helminthosporium interseminatum BERK. et RAV., Catalogue No. Ag. 97.
- (6) Helminthosporium gramineum RABENH., Catalogue No. Ag. 98.

All these cultures were purchased from the Centraalbureau voor Schimmelcultures at Baarn.

The carbon balance sheets for these six species, which are given in Table VIII, are all of a similar type, and enable one to classify biochemically, species of *Helminthosporium*

Table VII.—Carbon balance sheets for species of Cladosporium.

		.1.			
Species of Cladosporium:	Species S.	Species 1020	Species 60	Species 55	Species fr. lichen
Catalogue number:	Ag. 1	Ag. 3	Ag. 5	Ag. 9	Ag. 109
Experiment number:	B 4	В 5	В7	В 9	B 23
Incubation period in days:	81	72	52	76	50
Carbon Balance Sheet.					
Carbon in solution (start) gm.	4.901	5.043	5.043	5.043	$4 \cdot 952$
Carbon in H ₂ SO ₄ ,, ,, in CO ₂ ,, ,, in mycelium ,, ,, in solution (end) ,,	Nil 1·503 1·282 2·033	Nil 1·230 0·909 2·680	Nil 1 · 451 1 · 260 2 · 115	0.004 2.524 2.025 0.181	Nil 0·892 0·521 3·499
" accounted for ' "	4.818	4.819	4.826	4.734	$4 \cdot 912$
" accounted for … per cent.	98.3	96.0	96.2	94 · 4	99 · 2
Analysis of Solution.					
Carbon in residual glucose gm., in CO_2 in solution ,, in volatile acids ,, in non-volatile acids ,, in volatile neutral compounds ,, in synthetic compounds ,,	1 · 684 0 · 019 0 · 009 0 · 080 Nil 0 · 051	$\begin{array}{c} 2 \cdot 432 \\ 0 \cdot 015 \\ 0 \cdot 007 \\ 0 \cdot 066 \\ 0 \cdot 001 \\ 0 \cdot 040 \end{array}$	$\begin{array}{c} 1.620 \\ 0.016 \\ 0.060 \\ 0.103 \\ 0.007 \\ 0.049 \end{array}$	0·021 0·028 0·002 0·103 Nil 0·006	$\begin{array}{c} 3.186 \\ 0.009 \\ 0.009 \\ 0.190 \\ 0.006 \\ 0.101 \end{array}$
Total carbon accounted for ,, ,, ,, in solution ,,	1·843 . 2·033	$2.561 \\ 2.680$	$1.855 \\ 2.115$	0·160 0·181	$3.501 \\ 3.499$
Carbon unaccounted for (by difference) ,,	0.190	0.119	0.260	0.021	Surplus o
Residual Glucose.				-	
Glucose (by polarimeter) per cent. ,, (Shaffer-Hartmann) ,, (Wood-Ost) ,, ,, (by alkaline iodine) ,,	0.934 0.842 0.880 0.863	$1 \cdot 318$ $1 \cdot 216$ $1 \cdot 222$ $1 \cdot 277$	0.874 0.810 0.824 0.859	0.018 0.011 — 0.019	1.354 1.593 1.374 1.598
Acids.		Decrease	Decrease	Decrease	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$0.1 \\ 1.05 \\ 0.069 \\ 0.517$	of 0.1 0.86 0.036 0.396	of $0 \cdot 3$ $1 \cdot 91$ $0 \cdot 294$ $0 \cdot 585$	of $1 \cdot 4$ $0 \cdot 18$ $0 \cdot 017$ $0 \cdot 804$	$0.2 \\ 0.35 \\ 0.075 \\ 0.728$
Volume of oxygen absorbed c.c. Respiration coefficient	$\begin{array}{c} 2281 \\ 1 \cdot 25 \end{array}$	1905 $1 \cdot 22$	$ \begin{array}{c c} 2148 \\ 1 \cdot 28 \end{array} $	$\frac{3988}{1 \cdot 20}$	1524 1·10
Mycelium (weight) gm. ,, (carbon) per cent.	$\begin{array}{c} 2 \cdot 341 \\ 54 \cdot 7 \end{array}$	$\begin{array}{c} 1 \cdot 663 \\ 54 \cdot 8 \end{array}$	$\begin{array}{c} 2 \cdot 230 \\ 56 \cdot 5 \end{array}$	$\begin{array}{c} 3 \cdot 600 \\ 56 \cdot 3 \end{array}$	$\begin{array}{c} 0 \cdot 974 \\ 53 \cdot 5 \end{array}$

Table VIII.—Carbon balance sheets for species of *Helminthosporium*.

Species of ${\it Helminthosporium}$:	$H.\ geni-$	H. teres.	H. in-aequalis.	H. species.	H. inter- seminatum.	H. gra- mineum.
Catalogue number:	Ag. 93	Ag. 94	Ag. 95	Ag. 96	Ag. 97	Ag. 98
Experiment number :	B 15	B 16	B 17	B 18	B 19	B 20
Incubation period in days:	53	65	61	63	66	37
Carbon Balance Sheet.						
Carbon in solution (start) gm.	$4 \cdot 952$	$4\cdot 952$	$4 \cdot 952$	$4 \cdot 952$	4.952	$4 \cdot 952$
Carbon in H ₂ SO ₄ ,,	0.015	0.003	0.005	0.002	0.001	0.003
$,, \text{in CO}_2 \dots ,,$	$2 \cdot 017$	1.547	$2 \cdot 062$		1.763	1.350
" in mycelium … "	0.906	0.700	1.460	0.526	0.630	0.659
", in solution (end) … "	1.827	$2 \cdot 473$	$1 \cdot 242$	2.706	2 · 489	2.783
" accounted for … "	4.765	$4 \cdot 723$	4.769		4.883	4.795
,, accounted for per cent.	$96 \cdot 2$	95 • 4	96.3		98.8	96.8
Analysis of Solution.						
Carbon in residual glucose gm.	$0 \cdot 242$	$2 \cdot 206$	0.964	2.350	$2 \cdot 214$	2.384
' OO	$0.242 \\ 0.007$	0.002	0.904	0.005	0.012	0.032
in realetile eside	$0.007 \\ 0.029$	$0.002 \\ 0.013$	$0.008 \\ 0.013$	0.003	0.012	0.032
in non realatile soids	$0.029 \\ 0.094$	$0.013 \\ 0.077$	$0.013 \\ 0.109$	0.013	$0.033 \\ 0.148$	0.048
in realistile noutral com	0.094	0.011	0.109	0.000	0.140	0.010
"	0.818	0.141	0.048	0.107	0.042	0.258
,, in synthetic compounds,	0.015	0.020	0.015	0.026	0.058	0.043
Total carbon accounted for ,,	$\frac{1 \cdot 205}{1 \cdot 205}$	$\phantom{00000000000000000000000000000000000$	1.157	$2 \cdot 581$	$2 \cdot 529$	2.843
,, ,, in solution ,,	1.827	$2 \cdot 473$	1.242	2.706	2.489	2.783
Carbon unaccounted for (by ,, difference)	0.622	0.014	0.085	0.125	Surplus of 0.040	Surplus o
Residual Glucose.						
Glucose (by polarimeter) per cent. ,, (Shaffer-Hartmann)	0.100	1.072	0.495	1.120	0.822	1.135
per cent.	$0 \cdot 121$	$1 \cdot 103$	0.483	1.175	1.107	1.192
,, (Wood-Ost) ,,		0.954		$1 \cdot 134$	0.968	1.126
" (by alkaline iodine) "	0.162	$1 \cdot 106$	0:493	1.189	1.060	1.167
Acids.		Decrease	Decrease	Decrease		Decrease
Citration (N/1 acid) c.c.	0.3	1.1	0.8	$1\cdot 2$	0.3	0.7
Valatila agida (NV/1 agid)	$1 \cdot 44$		0.87	0.68	1.74	1.99
Barium salts (weight) gm.	0.154	0.063	0.057	0.03000	0.237	0.229
Calcium salts (weight) ,,	0.449	0.413	0.571	0.510	0.548	0.783
Volume of oxygen absorbed c.c.	2417	2554	3351	2746	3106	1974
Respiration coefficient	1.56	1.13	1.16	. —	1.07	1.31
Mycelium (weight) gm.	1.577	1.362	$2 \cdot 826$	1.008	1.148	$1 \cdot 285$
,, (carbon) per cent.	$57 \cdot 4$	$51 \cdot 4$	$51 \cdot 7$	$52 \cdot 2$	$53 \cdot 7$	$51 \cdot 3$

with those fungi which produce moderate amounts of volatile neutral compounds (alcohol). All the species grow reasonably well on CZAPEK-Dox solution. None of the species gives rise to appreciable amounts of either carbon as non-volatile acids or as titratable acidity, and in fact, four of the species actually produce a decrease in the initial acidity of the medium. Five out of six species give negligible, or even negative amounts of "carbon unaccounted for," but the other species, Helminthosporium geniculatum, provides the first instance so far recorded, in this paper, of any of the miscellaneous fungi worthy of further intensive investigation. This species has a figure for "carbon unaccounted for" of 0.622 gm., corresponding to a yield of 13 per cent. of the glucose fermented. This, together with 0.818 gm. of "carbon as volatile neutral compounds," corresponding to a yield of 17 per cent., gives a total yield of metabolic products other than carbon dioxide of 30 per cent. An investigation has been carried out on the nature of the compounds included in the "carbon unaccounted for" produced by this species, and is reported in Part XVII.

Genus 3. Heterosporium, 2 species.

- (1) Heterosporium gracile Sacc., Catalogue No. Ag. 99. Purchased from Baarn.
- (2) Heterosporium variabile Cooke, Catalogue No. Ag. 100. Purchased from Baarn.

The carbon balance sheets for these two species are given in Table IX. Both species grow well on the CZAPEK-Dox medium, and both give balance sheets of a similar type, *i.e.*, the type producing no volatile neutral compounds. Ag. 100 might, if occasion offered, prove a suitable species for further investigation, since it gives a figure for "carbon unaccounted for" of 0·381 gm. (11 per cent.), and a figure for carbon in non-volatile acids of 0·228 gm. (7 per cent.).

Genus 4. Alternaria, 3 species.

- (1) Alternaria species, Catalogue No. Ag. 35. Isolated at Ardeer from tobacco leaf spots.
- (2) Alternaria species, Catalogue No. Ag. 16. Isolated at Ardeer from black spots on butter.
- (3) Alternaria species, Catalogue No. Ag. 49. Isolated at Ardeer from a rotting orange.

The carbon balance sheets for these three species are given in Table X, and are all of the same type. The main metabolic product is that included in "volatile neutral compounds," and while only moderate in amount (limits 0.262 gm. to 0.171 gm.) it is perfectly definite. These three species also have respiration coefficients corresponding to this feature (limits 1.31 to 1.26), but do not give rise to any other metabolic products except a small amount of some compound included in "carbon unaccounted for" (limits 0.285 gm. to 0.209 gm.).

Table IX.—Carbon balance sheets for species of Heterosporium.

		Sp	ecies o	f Heter	rospor	ium:	$H.\ gracile.$	$H.\ variabile$
			Ca	talogu	e nun	ber:	Ag. 99	Ag. 100
			Exp	erimen	t nun	ber:	B 21	B 22
		Inci	ıbatioı	a perio	d in d	ays:	68	44
0.1.		07						
Carbon in solution (start)	n Bala	nce sn	eei.			am.	4.952	$4\cdot 952$
carbon in solution (start)	•••	•••	•••	•••	•••	gm.	1.302	1 502
Carbon in H_2SO_4	•••	•••	•••	•••	•••	,,	0.001	0.001
,, in CO ₂	•••	•••	•••	•••	•••	,,	$2 \cdot 256$	1.545
" in mycelium …	•••		•••		•••	,,	$1 \cdot 155$	1.031
,, in solution (end)	•••	•••	•••	•••	•••	,,	1.394	2 · 268
" accounted for	•••		•••	•••	٠	,,	4.806	4.845
,, accounted for	•••				\mathbf{per}	cent.	97.3	97.8
Anal	ysis of	Soluti	on.			·		
		Double	<i>510</i> •				0.075	1.585
Carbon in residual glucose	•••	•••	•••	•••	•••	gm.	0.975	
,, in CO ₂ in solution	•••	•••	•••	•••	•••	,,	0.016	0.004
,, in volatile acids		•••	•••	•••	•••	,,	0.041	0.021
" in non-volatile acid		•••	•••	•••	•••	,,	0.134	0.228
,, in volatile neutral of		ınds	•••	•••	•••	,,	Nil	0.002
" in synthetic compo	unds	•••	•••	•••	•••	, ,,	0.071	0.047
Total carbon accounted for	·	•••	•••	•••		,,	$1 \cdot 237$	1.887
,, ,, in solution	•••	•••	•••	•••	•••	,,	1.394	2.268
Carbon unaccounted for (b	y differ	rence)		•••	. •••	,,	0.157	0.381
Res	idual (Hucose			THE PERSON NAMED IN COLUMN TWO IS NOT THE PERSON NAMED IN COLUMN TWO IS NAMED IN COLUMN TW			
Glucose (by polarimeter)		•••			ner	cent.	0.340	0.889
(CTT A TENED III A DETECT)		•••			-		0.488	0.792
(Woon Oam)			•••	•••		,,	0.438	0.726
,, (by alkaline iodine)		•••	•••	•••		,,	0.445	0.831
AND THE RESIDENCE OF THE PROPERTY OF THE PROPE	Acid	s.			N		Decrease of	
Citration (N/1 acid)						c.c.	0.6	0.9
Volatile acids (N/1 acid)	•••	•••	•••	•••			3.95	1.26
Barium salts (weight)	•••	•••	•••	•••	•••	,,	0.135	0.097
Calcium salts (weight)	•••	•••	•••	•••	•••	gm.	0.431	0.037
		•••	•••	•••		-,,	0.491	0.002
Volume of oxygen absorbed	l		•••	•••	•••	c.c.	3893	2465
Respiration coefficient	•••	•••	•••	•••	•••		1.09	1.17
Mycelium (weight)						gm.	2.330	2.036
,, (carbon)				•••		cent.	$49 \cdot 6$	50.6
,, (,	•••		•••	•••	LOI	-0110	20 0	1 50 5

Table X.—Carbon balance sheets for species of Alternaria.

			Specie	s of A	l <i>ltern</i> a	ria:	A. species.	A. species.	A. species.
			Cat	alogue	e num	ber :	Ag. 35	Ag. 16	Ag. 49
			Expe	rimen	t num	ber:	В 6	B 11	B 12
		Incu	bation	perio	d in d	ays:	67	44	64
a ng at the sea and a	Carbon E	Balance	Sheet.						
arbon	in solution (start)	•••	•••	•••	•••	gm.	$5 \cdot 043$	$5 \cdot 043$	$5 \cdot 043$
arbon	in H ₂ SO ₄		•••			,,	0.006	0.006	0.005
,,	in CO ₂		•••			,,	$1 \cdot 188$	1.816	$2 \cdot 346$
,,	in mycelium					,,	$0 \cdot 433$	1.064	1.193
,,	in solution (end)	•••	•••	•••	•••	,,	$3 \cdot 210$	1.998	$1 \cdot 266$
,,	accounted for	•••		•••	•••	,,	4.837	4.884	4.810
,,	accounted for			•••	per	cent.	95 • 9	96.9	95 • 4
	Analysis	of So	lution.						
arbon	in residual glucose			•••		gm.	$2 \cdot 494$	1.300	0.527
,,	in CO ₂ in solution						0.011	0.015	0.030
"	in volatile acids				•••	"	0.024	0.098	0.021
	in non-volatile acid				•••	,,	0.091	0.072	0.145
,,	in volatile neutral			•••	•••	,,	0.262	0.261	0.171
,,	in synthetic compo			•••	•••	,,	0.059	0.043	0.087
Cotal c	arbon accounted fo	r		•••	•••	,,	$2 \cdot 941$	1.789	0.981
,,	" in solution	•••	•••	•••	•••	,,	3 · 210	1.998	$1 \cdot 266$
Carbon	unaccounted for (b	y diffe	erence)	•••	•••	,,	0.269	0.209	0.285
	Residi	ıal Glu	cose.						
Glucos	e (by polarimeter)	•••		• • •	per	cent.	$1\cdot 426$	0.684	0.226
,,	(SHAFFER-HARTM	ANN)	•••	•••	1	,,	$1\cdot 247$	0.650	0.263
,,	(Wood-Ost)					,,	$1\cdot 254$	0.648	
,,	(by alkaline iodin	e)	•••	•••		,,	$1 \cdot 342$	0.692	0.319
		Acids.							Decrease o
l Titrati	on (N/1 acid)		•••			c.c.	Nil	1.0	0.8
	e acids (N/1 acid)				•••		1.28	$4 \cdot 37$	0.74
	n salts (weight)		•••			gm.	0.073	0.517	0.077
	m salts (weight)	•••	•••	•••	•••	,,	0.615	0.354	0.797
	e of oxygen absorbe		•••	•••	•••	c.c.	1704	2668	3534
nespir	ation coefficient	•••	•••	•••	•••	•••	$1 \cdot 31$	$1 \cdot 28$	$1 \cdot 26$
Myzaali	um (weight)					Av	0.040	1 000	0.00=
•		•••	•••	•••	•••	gm.	0.849	1.999	$2 \cdot 237$
,,	(carbon)	• • •	•••		per	$\operatorname{cent.}$	$51 \cdot 0$	$53 \cdot 2$	$53 \cdot 3$

Genus 5. Fumago, 2 species.

- (1) Fumago species, Catalogue No. Ag. 63. Isolated at Ardeer as a bench contaminant of beer wort.
- (2) Fumago vagans Pers., Catalogue No. Ag. 92. Purchased from Baarn.

The carbon balance sheets for these two species are given in Table XI. In the case of Fumago vagans, Ag. 92, two balance sheets are given. The first one, B 14, was prepared from a culture grown on the usual CZAPEK-Dox solution containing 0·2 per cent. sodium nitrate as the source of nitrogen. The second balance sheet for this species, B 14 (X), was prepared from a culture grown on a similar CZAPEK-Dox solution in which, however, 0·2 per cent. ammonium nitrate was used as the source of nitrogen in place of the usual 0·2 per cent. sodium nitrate.

Both species of Fumago give balance sheets which are decidedly interesting from a biochemical point of view, although they are slightly different in type, because of the fact that, while Ag. 63 gives appreciable amounts of volatile neutral compounds (0.320 gm.), Ag. 92 gives only a negligible amount of the same type of compound (0.010 gm.). Of particular interest is the large amount of "carbon unaccounted for "given by both species and amounting to 0.512 gm. (15 per cent. of the glucose fermented) for Ag. 63, 0.965 gm. (19.8 per cent.) for Ag. 92 on sodium nitrate, and 0.468 gm. (19.5 per cent.) for Ag. 92 on ammonium nitrate. Another interesting feature is the relatively high titratable acidity and "carbon in non-volatile acids," which amounts to 0.189 gm. (5.4 per cent.) for Ag. 63, 0.407 gm. (8.4 per cent.) for Ag. 92 on sodium nitrate, and 0.268 gm. (11 per cent.) for Ag. 92 on ammonium nitrate. There is also a very marked difference in the percentage of glucose estimated by the polarimeter and Shaffer-Hartmann methods, for, with Ag. 92 on sodium nitrate, the respective figures are 0.344 and 0.043 per cent., while with the same species on ammonium nitrate the figures are 1.709 and 1.300 per cent. It is thus obvious that this species produces some material from glucose which is optically dextrorotatory, but which has no reducing effect on alkaline copper solutions.

Both species of *Fumago*, and particularly *Fumago vagans*, Ag. 92, are thus marked out as species worthy of further investigation. An account of the metabolic products isolated from *Fumago vagans*, Ag. 92, is given in Part XVII.

Genus 6. Clasterosporium, 2 species.

- (1) Clasterosporium species, Catalogue No. Ag. 64. Isolated at Ardeer from rotting cotton pulp at the methane plant. Identified by Mr. F. T. Brooks, of Cambridge.
- (2) Clasterosporium species, Catalogue No. Ag. 15. Isolated by Mr. F. T. Brooks from sea-weed.

Table XI.—Carbon balance sheets for species of Fumago.

					-	-	
			Species	of Fumago:	Fumago species.	Fumago vagans.	Fumago vagans or NH ₄ NO ₃
			Catalog	gue number:	Ag. 63	Ag. 92	Ag. 92
]	Experim	ent number:	В 3	B 14	B 14 (X)
		Incuba	ation per	riod in days:	77	75	71
	Conton	Balance i	07 4		-		
		Daiance i	Sneet.				
Jarbon	in solution (start)			gm.	5.018	4.952	4.975
Carbon	in H ₂ SO ₄			•••	0.003	0.001	0.001
	in CO ₂			"	$1 \cdot 472$	1.860	0.751
	in mycelium			••• ,,	0.651	1.440	0.617
	in solution (end)			••• ,,	2.780	1.582	3.544
,,	in solution (end)	•••	•• •••	••• ,,	2 100	1 004	9.944
,,	accounted for			,,	4.906	4.883	4.913
,,	accounted for			per cent.	97.8	98.6	97.9
	Analysis	of Solut	ion				
arbon	in residual glucose	-		gm.	1.538	0.087	2.600
, and some	in CO ₂ in solution	•••	••	_	0.008	1	
	in volatile acids			••• ,,		Nil	Nil
			••	*** ,,	0.049	0.004	0.047
	in non-volatile acid		• • • • • • • • • • • • • • • • • • • •	,,	0.189	0.407	0.268
,,	in volatile neutral		nds	••• ,,	0.320	0.010	0.023
,,	in synthetic compo	unds .		••• ,,	0.164	0.109	0.138
Total c	arbon accounted for	•			2.268	0.617	3.076
,,	,, in solution		•• •••	••• ,,	2.780	1.582	3.544
Carbon	unaccounted for (b	v differe	nce)	•••	0.512	0.965	0.468
	-	~	······································	••• • 99 ,			
71		al Glucos	se.				
GIUCOS6	e (by polarimeter)	•••	•••	per cent.	0.794	0.344	1.709
,,	(SHAFFER-HARTMA	NN) .		,,	0.769	0.043	1.300
"	(Wood-Ost)		•••	,,,	0.762		1.370
,,	(by alkaline iodine) .	•••	,,,	0.835	0.117	1.395
		Acids.					
Titrati	on (N/1 acid)	• • • •	•••	c.c.	2.3	7.0	7.6
Volatil	e acids (N/1 acid)	•••			$3 \cdot 20$		$2 \cdot 13$
	n salts (weight)	•••	•••	· ,, · gm.	0.262	0.016	0.260
Calciur	n salts (weight)	•••	•••		0.981	1.363	1.029
Volum	e of oxygen absorbe					-	
	ation coefficient	· · ·	•••		$\begin{array}{c c} 2177 \\ 1\cdot 27 \end{array}$	$\begin{array}{c c} 3144 \\ 1 \cdot 11 \end{array}$	$1220 \\ 1 \cdot 15$
Myceli	um (weight)	• • •		gm.	$1\cdot 224$	$2 \cdot 917$	1.218
,,	(carbon)		•••		53.2		
"	\/ ···	•••	•••	\cdot per cent.	00.7	49.4	50.7

117

The carbon balance sheets for these two species are given in Table XII. They are both biochemically interesting, although they differ in type, for while Ag. 64 gives a very considerable amount of volatile neutral compounds, 0.690 gm. (14 per cent. of glucose fermented), Ag. 15 gives only a negligible amount of the same type of product, 0.006 gm. (0.3 per cent.). Both of them, however, give very appreciable amounts of products in the class "carbon unaccounted for." Under this heading Ag. 64 gives 1.070 gm. of carbon (22 per cent.), while Ag. 15 gives 0.356 gm. (9 per cent.). Neither species shows any other item of biochemical interest. It is obvious that both these species, and particularly Clasterosporium species, Ag. 64, are worthy of further investigation, and an account of an investigation of the metabolic products of Clasterosporium, species Ag. 64, is given in Part XVII.

Genus 7. Rhacodium, 1 species.

(1) Rhacodium cellare Pers., Catalogue No. Ag. 13. Isolated at Ardeer from wine cellar debris.

The carbon balance sheet for this species is given in Table XIII.

The only point of biochemical interest about this carbon balance sheet is the fact that *Rhacodium cellare* appears to form small amounts, 0·158 gm. (6·3 per cent.), of a non-volatile acid which may possibly be optically dextro-rotatory, since there is a considerable difference between the glucose as estimated by the polarimeter (1·512 per cent.) and by the Shaffer-Hartmann method (1·257 per cent.). With the exception of this there is a complete absence of any other features of biochemical interest.

Table XII.—Carbon balance sheets for species of Clasterosporium.

		Spe	cies of	Claste	rospor	ium:	C. species.	C. species.
			Ca	atalogu	e nun	aber:	Ag. 64	Ag. 15
			Exp	erimen	t nun	nber:	B 8	B 10
		Inc	ubatio	n perio	d in d	lays:	39	66
Carbo	on Ba	lance S	heet.					
Carbon in solution (start)	•••	•••	•••	•••		gm.	$5 \cdot 043$	$5 \cdot 043$
Carbon in H ₂ SO ₄		•••	•••	•••		,,	0.008	0.001
,, in CO ₂	•••	•••		•••		,,	$1 \cdot 723$	1.945
,, in mycelium		•••	•••	•••	•••	,,	0.857	$1 \cdot 302$
,, in solution (end)	•••	•••	•••	•••	•••	,,	$2 \cdot 237$	1.535
" accounted for	•••				•••	,,	$4 \cdot 825$	4.783
" accounted for	•			•••	per	cent.	95.7	94.9
Anal	ysis o	f Soluti	on.					
Carbon in residual glucose	• • •	•••				gm.	0.176	0.928
" in CO ₂ in solution	•••	•••		•••			0.019	0.043
" in volatile acids	•••	•••	•••	•••	•••	,,	0.025	0.019
" in non-volatile acid		•••		•••		"	0.117	0.143
in relatile neutral					•••	,,	0.690	0.006
,, in synthetic compo		•••	•••	•••	•••	,,	0.140	0.040
Total carbon accounted for							1.167	1.179
,, ,, in solution	•••	•••	•••	•••	•••	. ,,	$2\cdot 237$	1.535
Carbon unaccounted for (b)	v diffe	erence)	•••			-	1.070	0.356
		glucose.						
Glucose (by polarimeter)	•••	•••	•••	•••	per	cent.	0.067	0.468
,, (Shaffer-Hartma	,	•••	•••	•••		,,	0.088	0.464
" (Wood-Ost) …	•••	•••	•••	•••		,,		0.470
,, (by alkaline iodine)	• • • • • • • • • • • • • • • • • • • •	•••	•••	•••		,,	0.158	0.481
	Aci	ds.						Decrease of
Titration (N/1 acid)	•••	•••		••••	•••	c.c.	$0\cdot 4$	0.9
Volatile acids $(N/1 \text{ acid})$	•••	•••	•••	•••	•••	,,	$1 \cdot 05$	0.69
Barium salts (weight)			•••	•••	•••	gm.	0.100	0.083
Calcium salts (weight)	•••	•••	•••	•••	•••	,,	0.624	0.808
Volume of oxygen absorbed	1	•••		•••		c.c.	1869	2846
Respiration coefficient	•••	•••	•••		•••		$1 \cdot 74$	1.30
Mycelium (weight)	•••	•••	•••			gm.	1.608	2.338
,, (carbon)	•••	•••		•••		cent.	53.3	55.6

Table XIII.—Carbon balance sheet for a species of Rhacodium.

		Specie	es of R	hacodi	um:	$Rhacodium\ cellare.$	
	Catalogue number:						
Experiment number:						B1	
	In	cubatio	n perio	d in d	lays:	60	
Carbon	Balance	e Sheet.					
Carbon in solution (start)				•••	gm.	5.018	
Carbon in H ₂ SO ₄			•••		,,	0.001	
$,, \text{in CO}_2 \dots \dots$	•••	•••	•••		,,	$1 \cdot 048$	
,, in mycelium	•••		•••		,,	0.995	
,, in solution (end)	•••	•••	•••	•••	,,	2.865	
" accounted for	•••	•••			,,	4.909	
" accounted for	•••	•••	• • • •	\mathbf{per}	cent.	97.8	
Analysi	s of So	lution.					
Carbon in residual glucose	•••	•••			gm.	$2 \cdot 514$	
,, in CO ₂ in solution		• • •	•••	• • •	,,	0.004	
,, in volatile acids	•••	•••			,,	0.002	
,, in non-volatile acid			•••	•••	,,	0.158	
,, in volatile neutral of		nds	•••		,,	0.007	
,, in synthetic compo	unds	•••	•••	•••	,,	0.071	
Total carbon accounted for	• •••	•••			٠,	$2 \cdot 756$	
" " in solution	. •••	•••	•••	•••	,,	$2 \cdot 865$	
Carbon unaccounted for (b	y differ	ence)	•••	•••	,,	0.109	
Resid	ual Glu	cose.					
Glucose (by polarimeter)			•••	per	cent.	$1 \cdot 512$	
,, (Shaffer-Hartma	NN)		•••		,,	$1\cdot 257$	
,, (Wood-Ost)	•••				,,	$1 \cdot 230$	
,, (by alkaline iodine)	• • •	•••	•	,,	1 · 267	
	Acids.					Decrease of	
Titration (N/1 acid)					c.c.	$0\cdot 4$	
Volatile acids (N/1 acid)	•••	•••				0.35	
Barium salts (weight)				• • • •	gm.	0.029	
Calcium salts (weight)	•••	•••	•••		,,	0.571	
Volume of oxygen absorbed	d		•••	•••	c.c.	1914	
Respiration coefficient		•••	•••	•••	•••	1.03	
Mycelium (weight)	•••			•••	gm.	$1 \cdot 972$	
				• • •	D-11.	- U	

FUNGI IMPERFECTI.

Order: HYPHOMYCETALES.

Family: STILBACEÆ.

Genus 1. Stysanus, 1 species.

(1) Stysanus species, Catalogue No. Ag. 51. Isolated at Ardeer from rotting pulped cotton.

The carbon balance sheet for this species, which is given in Table XIV, shows one item of outstanding biochemical interest. This is the figure for "carbon unaccounted for," $0.520 \, \mathrm{gm}$. (16.7 per cent. of the glucose fermented). It is obvious that this species should be further investigated with a view to elucidating the nature of the products included under this heading.

There are no other items of biochemical interest in this particular balance sheet.

FUNGI IMPERFECTI.

Order: HYPHOMYCETALES.

Family: Tuberculariaceæ.

Genus 1. Fusarium (see Part V).

Genus 2. Epicoccum, 2 species.

- (1) Epicoccum species, Catalogue No. Ag. 57. Isolated at Ardeer as a bench contaminant of CZAPEK-Dox agar.
- (2) Epicoccum species, Catalogue No. Ag. 32. Isolated at Ardeer from the grain of an infected wheat ear.

The carbon balance sheets for these two species of *Epicoccum* are given in Table XV. They are both very similar in character to the carbon balance sheets given by a certain type of *Fusarium*. Both species give appreciable amounts of volatile neutral compounds, and Ag. 57, at any rate, has a relatively high respiration coefficient, 1·64. They are most closely related to the type of *Fusarium* species given in Table I of Part V in the group including *Fusarium solani* and *Fusarium lini*. The characteristics which these *Epicoccum* species have in common with this group of *Fusaria*—and this applies more particularly to *Epicoccum* species, Ag. 32, than to the species Ag. 57—are (1) a moderate amount of "carbon unaccounted for" and (2) a relatively high value for "carbon in volatile acids." Thus the carbon in volatile acids for *Epicoccum* species, Ag. 32, which is 0·108 gm., is, while small in itself, very much larger than any other figure for the same type of compound given by any other species of fungus described in this paper.

Table XIV.—Carbon balance sheet for a species of Stysanus.

			Spec	ies of	ius :	Stysanus species.		
	Catalogue number :					ber :	Ag. 51 B 13	
		Experiment number :						
			Inc	ubation	perio	d in da	ays:	60
	Ca	rbon Bo	lance	Sheet.				
arbon in s	olution (sta	rt)	•••	•••	•••		gm.	$4 \cdot 952$
Carbon in I	H ₂ SO ₄	•••		•••			,,	0.002
	O ₂			•	•••		,,	1.530
	nycelium	•••		•••	• • •		,,	0.685
	olution (en		•••	•••	•••		,,	$2 \cdot 676$
,, acc	ounted for			•••			,,	4.893
,, acc	ounted for	•••		•••	•••	per	cent.	96 • 6
	Ai	nalysis	of Sol	ution.				
larbon in 1	esidual glu	-					gm.	$1 \cdot 842$
in (CO ₂ in solut		•••	•••	•••	•••	_	0.007
in .	volatile acid		•••	•••	•••	•••	,,	0.062
in	non-volatile						,,	0.132
	volatile neu				•••		"	0.079
	synthetic co			•••	•••	•	,,	0.034
otal carbo	on accounte	d for				•••	,,	2.156
,, ,,	in solutio	on	•••	•••	•••	•••	,, ·	$2 \cdot 676$
arbon una	accounted f	or (by	liffere	ence)	•••	•••	,,	0.520
		Residua	ıl Glu	cose.				
Hucose (b	y polarimet	er)			• • •	per	cent.	0.927
	HAFFER-HA					_	,,	0.921
	OOD-OST)	•••	,		•••		,,	0.924
,, (b	y alkaliné i	odine)	• • •	•••	•••		,,	1.023
		A	cids.					Decrease of
Citration (N/1 acid)		• • •	•••	•••		c.c.	$0\cdot 3$
	ids (N/1 ac)				•••		,,	0.23
Barium sal	ts (weight)	•••		•••			gm.	0.032
Calcium sa	lts (weight)		•••	•••	•••	•••	,,	0.454
Volume of	oxygen abs	sorbed		•••			c.c.	2376
	n coefficient		•••	•••	•••	•••		$1 \cdot 21$
Mycelium	(weight)	•••			•••		gm.	$1 \cdot 279$
mycomum						per	~	$53 \cdot 6$

Table XV.—Carbon balance sheets for species of Epicoccum.

			Species of <i>Epicoccum</i> :				Epicoccum species.	$\begin{array}{ c c c }\hline Epicoccum & \text{species}\\ \text{from wheat.}\\ \end{array}$	
			Ca	atalogu	ie nun	aber:	Ag. 57	Ag. 32	
			Exp	erimer	nt nun	nber:	A 2	A 8	
		Inc	ubatio	n perio	od in d	lays:	44	57	
Car	bon Ba	lance Si	heet.						
Carbon in solution (start)	•••	•••	•••	•••		gm.	$4 \cdot 834$	$5 \cdot 018$	
Carbon in H ₂ SO ₄	•••	•••	•••	•••	•••	,,	0.006	0.011	
$,, \text{in CO}_2 \dots$	•••	•••		•••		,,	$1 \cdot 333$	1.587	
" in mycelium …		•••				,,	0.547	$0\cdot 264$	
" in solution (end)	•••	•••	•••	•••	•••	,,	2.857	3.081	
" accounted for		•••		•••	•••	,,	$4 \cdot 743$	4.943	
" accounted for	•••	•••	•••	•••	per	cent.	98.1	98.5	
An	alysis o	f Soluti	on.						
Carbon in residual glucose		•••	•••		•••	gm.	$1\cdot 642$	$2 \cdot 206$	
,, in CO ₂ in solution			•••			,,	0.017	0.002	
,, in volatile acids			•••	•••	•••	1	0.037	0.108	
,, in non-volatile aci	ds		•••	•••		,,	0.088	0.149	
,, in volatile neutral		ounds	•••			,,	0.613	$ 0.\overline{297} $	
,, in synthetic comp	ounds	•••	•••	•••	•••	,,	0.063	0.154	
Total carbon accounted fo	or	•••	• •.•	•••	•••	,,	2 · 460	2.916	
" " in solution	•••	•••	•••	•••	•••	,,	2.857	3.081	
Carbon unaccounted for (by diffe	erence)	•••	•••	•••	,,	0.397	0.165	
R	esidual	Glucose	e.						
Glucose (by polarimeter)	•••	•••	•••		per	cent.	0.825	$1 \cdot 119$	
,, (Shaffer-Hartm	ANN)	•••	• • •	•••		,	0.821	$1 \cdot 103$	
" (Wood-Ost)	•••	•••	•••	•••		,	0.832	$1 \cdot 100$	
,, (by alkaline iodin	.e)	•••	•••	•••		,,	0.870	1.139	
	Ac	ids.							
Titration (N/1 acid)	•••	•••	•••	•••	•••	c.c.	1.1	3.7	
Volatile acids (N/1 acid)	•••	•••	•••	•••	•••	,,	1.69	$3 \cdot 74$	
Barium salts (weight)	•••	•••	•••	•••	•••	gm.	0.176	0.417	
Calcium salts (weight)	•••	•••	•••	•••	•••	,,	0.412	0.606	
Volume of oxygen absorb	ed	•••	•••	•••	•••	c.c.	1537	2558	
Respiration coefficient	•••	•••	•••	•••	•••	• • • •	$1 \cdot 64$	1.16	
Mycelium (weight)		•••				gm.	0.995	0.519	
,, (carbon)	•••	•••	•••	•••		cent.	$55 \cdot 0$	50.9	

123

Discussion of results obtained.

Only a relatively small proportion of the carbon balance sheets presented in this paper, from a large variety of fungi, show no biochemically interesting features at all, though in most cases it is impossible to generalize because the number of species investigated in any particular genus is often small. However, of the species examined it is possible to say that none of the following gives an appreciable amount of any metabolic product from glucose other than CO_2 , and they are thus biochemically uninteresting and not promising for further intensive examination.

Class: ASCOMYCETES.

Genus 2. The only species of Chætomium investigated.

Genus 3. The only species of *Sclerotinia* investigated.

Class: BASIDIOMYCETES.

Genus 1. Both species of *Ustilago* investigated.

Order: HYPHOMYCETALES.

Family: Moniliaceæ.

Genus 2. Both species of Sporotrichum investigated.

Genus 4. The only species of Cephalothecium investigated.

Family: Dematiaceæ.

Genus 1. All 5 species of *Cladosporium* investigated.

The only item of interest from a biochemical point of view in the carbon balance sheets of a certain number of other species is the production of varying amounts of "carbon in volatile neutral compounds" (probably ethyl alcohol), and correspondingly high respiration coefficients. These species also are not very attractive for further investigation.

Class: ASCOMYCETES.

Genus 1. The only species of Sordaria investigated.

Order: HYPHOMYCETALES.

Family: Moniliaceæ.

Genus 3. Both species of *Trichoderma* investigated.

Family: Dematiaceæ.

Genus 2. Four species of *Helminthosporium* out of six investigated.

Genus 4. All three species of Alternaria investigated.

Family: Tuberculariace Æ.

Genus 2. Both species of *Epicoccum* investigated.

These two species have other items of minor interest, e.g., moderately high "carbon in volatile acids" but, like the Fusaria (see Part V), they are not generally interesting.

The remainder of the species examined have some points of particular biochemical interest, and most of them give promise of repaying further intensive examination. These species include the following:—

Class: ASCOMYCETES. None.

Class: BASIDIOMYCETES. None.

Order: HYPHOMYCETALES.

Family: Moniliaceæ.

Genus 1. Both species of *Eidamia* investigated.

These two species show characteristics strongly reminiscent of some of the Aspergillus niger group, i.e., high "carbon in volatile neutral compounds," moderate titratable acidity, "carbon in non-volatile acids" and "carbon unaccounted for." They might repay further examination, but are not so promising as some of the other species dealt with in this paper.

Family: Dematiaceæ.

- Genus 2. *Helminthosporium*. One species of six investigated gives a high figure for "carbon unaccounted for."
- Genus 3. Heterosporium. One species of two investigated gives a moderate figure for "carbon unaccounted for."
- Genus 5. Fumago. Both species investigated give-
 - (a) a high figure for "carbon unaccounted for."
 - (b) high titratable acidity and "carbon in non-volatile acids."
- Genus 6. Clasterosporium. Both species investigated give good figures for "carbon unaccounted for." One species, Ag. 64, gives a very high figure.
- Genus 7. Rhacodium. The only species investigated produces a quantity of an optically active compound, possibly a non-volatile acid.

This family includes the largest proportion of biochemically interesting genera and species of any investigated in this paper, and if a renewal of the collection of carbon balance sheets is contemplated, it seems advisable to collect a number of species of this family, and of the specified genera, for investigation.

Family: STILBACEÆ.

Genus 1. Stysanus. The only species investigated gives a fairly high figure for "carbon unaccounted for."

125

It is evident from the above facts that the biochemically interesting fungi are by no means confined to the commoner genera and species—such as species of Aspergillus and Penicillium—but that investigation of some of the rarer species, many of which grow well on purely synthetic media, is bound to lead to results of interest and to the isolation of interesting, and possibly new, types of metabolic products.

Summary.

Carbon balance sheets are given for a wide variety of species of fungi belonging to different orders, families and genera. A number of these species are evidently of biochemical interest and should be further investigated. These are dealt with in some detail in the discussion of results obtained.