PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Quantitative Examination by the Carbon Balance Sheet Method of the Types of Products Formed from Glucose by Species of Penicillium (Including Citromyces)

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Studies in the Biochemistry of Micro-organisms.

Part IV.—*Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by species of* Penicillium (*including* Citromyces).

By John Howard Birkinshaw, John Henry Victor Charles, Arthur Clement Hetherington *and* Harold Raistrick, *with an Appendix by* Charles Thom.

The work described in Part III of this series, dealing with the preparation of carbon balance sheets for species of *Aspergillus*, was continued on exactly the same lines, using species of *Penicillium* as the organisms for study.

The methods used were exactly the same as those described in Parts II and III, the same medium being used, the same temperature of incubation, 23°–25° C. and the same methods of cultivation and analysis.

The results obtained are given in the following pages and are arranged in groups according to the classification of the *Penicillia* adopted by Thom in his recent book 'The *Penicillia*' (The Williams and Wilkins Co., 1929). The whole of the cultures of *Penicillium*, amounting in all to about a hundred species, were sent to Dr. Thom and were examined by him. In some cases these cultures are described in his book and in all cases Dr. Thom gave his opinion as to the correct names to be applied to the different species. The history of the species used and Dr. Thom's comments on them will be found in an Appendix at the end of this paper. We desire to take this opportunity of thanking Dr. Thom for his very kind co-operation.

Group I.

Division I.—Monoverticillata.

Section I. Monoverticillata-stricta.

Subsection 2. Stricta-floccosa.

The species examined in this group (Ad. 74, Ad. 80, Ad. 79, Ad. 78, Ad. 21, Ad. 29, Ad. 71, Ad. 73) consist of eight strains, all of which belong to the *P.* (*Citromyces*) *Pfefferianum–P. spinulosum* series, and the carbon balance sheets are given in Table I. All these species would formerly have been included in Wehmer's genus *Citromyces*, but, as the modern tendency is to eliminate this genus, the procedure adopted by Dr. Thom is followed in this paper.

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TABLE I.

Species of *Penicillium* :	*P.* (*Citromyces*) *Pfefferianum*–*P. spinulosum* series.							
Catalogue number :	Ad. 74	Ad. 80	Ad. 79	Ad. 78	Ad. 21	Ad. 29	Ad. 71	Ad. 73
Experiment number :	F 55	F 64	F 63	F 59	126	F 11	F 78	F 82
Incubation period in days :	77	72	70	61	37	64	40	70
Carbon Balance Sheet.								
Carbon in solution (start) ... ... gm.	4·944	4·952	4·952	4·944	5·043	4·944	4·952	4·952
Carbon in H_2SO_4 ... ... „	0·002	0·002	0·001	0·002	0·001	—	0·001	0·002
„ in CO_2 ... ... „	—	2·203	1·847	2·235	1·294	2·046	1·416	1·884
„ in mycelium ... ... „	0·887	0·747	0·661	0·834	0·642	0·821	0·551	0·707
„ in solution (end) ... ... „	1·655	1·885	2·423	1·735	2·952	2·004	2·843	2·150
„ accounted for ... ... „	—	4·837	4·932	4·806	4·889	4·871	4·811	4·743
„ accounted for ... ... per cent.	—	97·7	99·6	97·2	97·0	98·5	97·2	95·8
Analysis of Solution.								
Carbon in residual glucose ... ... gm.	0·252	0·577	1·136	0·689	2·014	1·106	1·935	1·217
„ in CO_2 in solution ... ... „	nil	0·002	nil	0·001	0·003	0·007	0·009	0·002
„ in volatile acids ... ... „	0·002	0·009	nil	0·009	0·011	0·027	0·027	0·034
„ in non-volatile acids ... ... „	0·937	0·799	0·862	0·358	0·314	0·318	0·318	0·285
„ in volatile neutral compounds ... „	0·036	0·083	0·032	0·014	nil	0·060	0·060	0·033
„ in synthetic compounds ... ... „	0·212	0·135	0·069	0·085	0·211	0·123	0·170	0·111
Total carbon accounted for ... ... „	1·439	1·605	2·099	1·156	2·553	1·641	2·519	1·682
„ „ in solution ... ... „	1·655	1·885	2·423	1·735	2·952	2·004	2·843	2·150
Carbon unaccounted for (by difference) ... „	0·216	0·280	0·324	0·579	0·399	0·363	0·324	0·468
Residual Glucose.								
Glucose (by polarimeter) ... ... per cent.	0·099	0·287	0·623	0·430	1·035	0·521	1·051	0·583
„ (SHAFFER-HARTMANN) ... ... „	0·126	0·288	0·568	0·345	1·007	0·553	0·968	0·608
„ (WOOD-OST) ... ... „	—	—	0·571	—	1·066	0·571	0·930	—
„ (by alkaline iodine)... ... „	0·144	0·317	0·655	0·463	1·111	0·569	1·049	0·676
Acids.								
Titration (N/1 acid) ... ... c.c.	29·1	21·7	20·3	5·0	5·1	5·7	5·2	4·4
Volatile acids (N/1 acid) ... ... „	0·17	0·83	0·03	0·74	1·51	1·01	1·47	1·43
Barium salts (weight) ... ... gm.	0·029	0·048	0·015	0·041	0·069	0·102	0·110	0·152
Calcium salts (weight) ... ... „	3·474	2·958	2·957	1·327	1·460	1·625	1·250	1·248
Volume of oxygen absorbed ... ... c.c.	—	3865	3289	3460	2059	3406	2326	3272
Respiration coefficient	—	1·06	1·05	1·21	1·18	1·13	1·14	1·08
Mycelium (weight) ... ... gm.	1·726	1·427	1·298	1·707	1·276	1·644	1·069	1·355
„ (carbon) ... ... per cent.	51·4	52·4	50·9	48·8	50·3	49·9	51·6	52·2

The whole of these species form a well-defined group having certain very definite characteristics :—

(i) All give rise to large amounts of non-volatile acids. This is particularly marked in the case of strains Ad. 74, Ad. 80 and Ad. 79, which give larger amounts of titratable acidity and non-volatile acids than any other species of *Penicillium* examined. The nature of this acidity has been investigated, has been proved to be due to citric acid, and is reported in Part XII of this series. These species are also distinguished by the production of a purple-coloured substance, a methoxy-dihydroxy-toluquinone, which is also described in Part XII. The remaining five species give rise to smaller, but still quite considerable, quantities of titratable acidity and non-volatile acids.

(ii) None of the eight strains produces appreciable amounts of alcohol ("carbon in volatile neutral compounds"), though, with the exception of Ad. 21, all of them give rise to detectable amounts. A natural sequence to this is the fact that all these strains have low respiration coefficients.

(iii) All the strains give moderate amounts of "carbon unaccounted for."

Group II.

Division I.—Monoverticillata.

Section I. Monoverticillata-stricta.

Subsection 4. *Velutina.*

This group includes five strains (Ad. 6, Ad. 7, Ad. 67, Ad. 68 and Ad. 69) which fall in the *P.* (*Citromyces*) *glabrum* Wehmer series, and one strain (Ad. 48) which has been diagnosed as *P. aurantio-violaceum* Biourge. The carbon balance sheets for these species are given in Table II.

Formerly the five strains would have been included in Wehmer's genus *Citromyces* as strains of *Citromyces glaber*, but, as explained under Group I, they are now classed as species of *Penicillium*. These five strains form a well-defined group and are of especial interest since all of them give rise to a new biochemical product, which is specific for this group, to which we propose to give the name "citromycetin" and which is described in detail in Part XI of this series. In contradistinction, none of the strains in Group I produces this substance. In addition to this specific product, all the five strains of *P.* (*Citromyces*) *glabrum* also show the following characteristics :—

(i) All give rise to considerable amounts of titratable acidity in the form of non-volatile acids. This has been shown to be citric acid, though, in the case of cultures incubated under full aerobic conditions, *i.e.*, in flasks plugged with cotton-wool, a portion of the acidity is caused by the presence of citromycetin. The last carbon balance sheet in Table II (Expt. No. 143) was prepared from a

TABLE II.

Species of *Penicillium* :	*P. aurantio-violaceum* BIOURGE.	*P.* (*Citromyces*) *glabrum* WEHMER series.					
Catalogue number :	Ad. 48	Ad. 6	Ad. 7	Ad. 67	Ad. 68	Ad. 69	Ad. 7
Experiment number :	F 36	134	F 5	F 74	F 75	F 76	143
Incubation period in days :	68	51	55	19	32	42	10
Carbon Balance Sheet.							
Carbon in solution (start) ... ... gm.	4·944	5·043	5·043	4·952	4·952	4·952	4·944
Carbon in H_2SO_4 ... ... ,,	0·001	0·003	0·003	0·003	0·002	0·001	—
,, in CO_2 ... ... ,,	2·385	2·008	2·277	1·292	1·150	1·524	—
,, in mycelium ... ... ,,	1·280	0·872	1·030	0·692	0·462	0·582	1·339
,, in solution (end) ... ... ,,	1·174	1·973	1·843	2·842	3·258	2·765	1·588
,, accounted for ... ... ,,	4·840	4·856	5·153	4·829	4·872	4·872	—
,, accounted for ... ... per cent.	97·9	96·3	102·2	97·5	98·4	98·4	—
Analysis of Solution.							
Carbon in residual glucose ... ... gm.	0·848	0·663	0·536	1·578	2·348	1·681	0·912
,, in CO_2 in solution ... ... ,,	0·015	0·001	0·004	0·004	0·003	0·002	nil
,, in volatile acids... ... ... ,,	0·015	0·026	0·016	0·025	0·021	0·039	0·001
,, in non-volatile acids ... ... ,,	0·154	0·345	0·402	0·304	0·341	0·315	0·556
,, in volatile neutral compounds ,,	0·006	0·126	0·101	0·208	0·059	0·071	0·013
,, in synthetic compounds ... ,,	0·083	0·191	0·218	0·036	0·093	0·090	0·397
Total carbon accounted for ... ... ,,	1·121	1·352	1·277	2·155	2·865	2·198	1·879
,, ,, in solution... ... ... ,,	1·174	1·973	1·843	2·842	3·258	2·765	1·588
Carbon unaccounted for (by difference) ,,	0·053	0·621	0·566	0·687	0·393	0·567	Surplus of 0·291
Residual Glucose.							
Glucose (by polarimeter) ... per cent.	0·459	0·340	0·301	0·856	1·342	0·969	0·331
,, (SHAFFER-HARTMANN) ,,	0·424	0·331	0·268	0·789	1·174	0·841	0·456
,, (WOOD-OST) ... ... ,,	0·435	—	—	0·815	1·178	0·838	0·454
,, (by alkaline iodine) ... ,,	0·430	0·397	0·339	0·899	1·302	0·926	1·177
Acids.							
Titration (N/1 acid) ... ... c.c.	0·3	8·4	6·6	4·1	7·6	7·9	9·2
Volatile acids (N/1 acid) ... ... ,,	0·89	1·72	1·48	1·48	1·47	1·79	0·17
Barium salts (weight) ... ... gm.	0·101	0·089	0·068	0·140	0·089	0·189	0·014
Calcium salts (weight) ... ... ,,	0·674	1·667	1·597	1·117	1·559	1·280	1·819
Volume of oxygen absorbed ... ... c.c.	4016	3225	3277	1854	1878	2501	—
Respiration coefficient	1·11	1·16	1·30	1·27	1·15	1·14	—
Mycelium (weight) ... ... gm.	2·542	1·754	2·054	1·364	0·919	1·124	2·518
,, (carbon) ... ... per cent.	50·4	49·7	50·2	50·7	50·3	51·7	53·2

culture grown under these conditions and is incorporated here as illustrating the tremendous effect which full aeration produces on the type of metabolic products formed.

(ii) All strains give rise to small but definite amounts of volatile neutral compounds and have in consequence small respiration coefficients.

(iii) All strains give rise to marked amounts of "carbon unaccounted for."

P. aurantio-violaceum Biourge, Ad. 48, gives a carbon balance sheet which is so different in type from the other carbon balance sheets in Table II that, from a biochemical point of view, it cannot logically be placed in this group. Physiological tests also show this species to be quite different from the other strains.

Group III.

Division II.—Asymmetrica.

Section I. Velutina.

Subsection 1. *Velutina-elliptica-magna.*

The species examined in this group (Ad. 52, Ad. 81 and Ad. 102) consists of three strains of *P. digitatum* Saccardo, obtained from different parts of the world. The carbon balance sheets are given in Table III. The most striking feature of these carbon balance sheets, which are all of the same type, is the relatively large figure obtained for "carbon in H_2SO_4." The figure obtained with these three strains is about ten times as large as that obtained with any other species in any genus examined. It is shown in Part XVIII of this series that the substance responsible for this high figure is ethyl acetate, and this species appears to be unique in the production, at any rate in considerable quantities, of this ester. The production of this ester is further indicated by the fact that cultures of this species, grown under the conditions of the metabolism experiments, have a very agreeable ester smell.

Further points of interest in the carbon balance sheets are (*a*) the large amounts of "carbon in volatile neutral compounds," which have been shown to be due to ethyl alcohol (see Part XVIII), and (*b*) the relatively high respiration coefficient.

TABLE III.

Species of *Penicillium*:	*P. digitatum* SACCARDO.			
Catalogue number:	Ad. 52	Ad. 52	Ad. 81	Ad. 102
Experiment number:	F 40	F 60	F 86	F 87
Incubation period in days:	81	58	88	80
Carbon Balance Sheet.				
Carbon in solution (start) ... ... gm.	4·944	4·944	4·719	4·719
,, in H_2SO_4 ... ... ,,	0·177	0·207	0·072	0·082
,, in CO_2 ... ... ,,	2·601	2·080	1·652	2·254
,, in mycelium ... ... ,,	0·530	0·604	0·412	0·436
,, in solution (end) ... ... ,,	1·395	1·917	2·404	1·918
,, accounted for ... ... ,,	4·703	4·808	4·540	4·690
,, accounted for ... ... per cent.	95·1	97·2	96·2	99·4
Analysis of Solution.				
Carbon in residual glucose ... ... gm.	0·038	0·314	0·649	0·030
,, in CO_2 in solution ... ... ,,	0·007	0·010	0·012	0·006
,, in volatile acids ... ... ,,	0·007	0·018	0·029	0·007
,, in non-volatile acids ... ... ,,	0·107	0·098	0·079	0·104
,, in volatile neutral compounds ,,	0·968	0·992	1·043	1·416
,, in synthetic compounds ... ,,	0·066	0·013	0·138	0·026
Total carbon accounted for ... ... ,,	1·193	1·445	1·950	1·589
,, ,, in solution ... ... ,,	1·395	1·917	2·404	1·918
Carbon unaccounted for (by difference) ,,	0·202	0·472	0·454	0·329
Residual Glucose.				
Glucose (by polarimeter) ... per cent.	0·015	0·131	0·393	0·032
,, (SHAFFER-HARTMANN) ,,	0·019	0·157	—	—
,, (WOOD-OST) ... ... ,,	—	—	0·325	0·015
,, (by alkaline iodine) ... ,,	0·070	0·182	0·401	0·082
Acids.				
Titration (N/1 acid) ... ... c.c.	0·7	1·4	3·1	nil
Volatile acids (N/1 acid) ... ... ,,	1·62	0·76	0·08	nil
Barium salts (weight) ... ... gm.	0·152	0·087	0·127	0·077
Calcium salts (weight) ... ... ,,	0·436	0·415	0·390	0·317
Volume of oxygen absorbed ... ... c.c.	3492	2710	1904	2730
Respiration coefficient	1·39	1·44	1·63	1·55
Mycelium (weight) ... ... gm.	1·086	1·262	0·919	0·966
,, (carbon) ... ... per cent.	50·0	47·8	44·8	45·1

Group IV.

Division II.—Asymmetrica.

Section I. Velutina.

Subsection 2. Velutina-divaricata.

Only two species are included in this group, *i.e.*, Ad. 42 (*P. Steckii* Zaleski) and Ad. 23 (*P. citrinum* Thom). The carbon balance sheets for these species, given in Table IV, show the following characteristics :—

(i) Moderate amounts of titratable acidity and "carbon in non-volatile acids."

(ii) Negligible amounts of "carbon in volatile neutral compounds" and hence low respiration coefficients.

(iii) Considerable amounts of "carbon unaccounted for."

Ad. 42 thus gives a very similar carbon balance sheet to Ad. 23 and it has also been shown to have very similar physiological properties, but Ad. 23 (*P. citrinum* Thom) is distinguished from Ad. 42, and indeed from all other species examined, either of *Penicillium* or of any other genus, by the fact that it and two other strains of *P. citrinum* (Ad. 95 and Ad. 114) have been shown to produce a specific biochemical product, to which the name citrinin has been given (see Parts XIV and XV).

Group V.

Division II.—Asymmetrica.

Section I. Velutina.

Subsection 3. Radiata (*P. chrysogenum* series).

Included in this group are six species (Ad. 11, Ad. 24, Ad. 56, Ad. 14, Ad. 35 and Ad. 53) which are all closely related morphologically, biochemically and physiologically. The carbon balance sheets, which are given in Table V, show the following characteristics :—

(i) With one exception, Ad. 24, they all give rise to exceptionally large amounts of "carbon unaccounted for," amounting in one case, Ad. 11, to over 30 per cent. of the glucose metabolized. The nature of the metabolic products of Ad. 11 has been investigated and is reported in detail in Part XVII of this series. Suffice it to say here that the "carbon unaccounted for" consists, in this case, principally of mannitol.

(ii) All the species, again with the exception of Ad. 24, show large amounts of titratable acidity and high figures for "carbon in non-volatile acids."

(iii) None of the species forms alcohol except in very small amounts and in consequence all of them have low respiration coefficients.

The species in this group as a whole seem to stand out from the majority of species of *Penicillium* because of the large amounts of total metabolic products other than CO_2 and alcohol which they form.

TABLE IV.

Species of *Penicillium*:	*P. Steckii* ZALESKI.	*P. citrinum* THOM.
Catalogue number:	Ad. 42	Ad. 23
Experiment number:	F 30	F 23
Incubation period in days:	58	43
Carbon Balance Sheet.		
Carbon in solution (start) ... ... gm	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	0·001	0·001
,, in CO_2 ... ... ,,	1·780	1·443
,, in mycelium ... ... ,,	1·080	0·629
,, in solution (end) ... ... ,,	1·992	2·804
,, accounted for ... ... ,,	4·853	4·877
,, accounted for ... ... per cent.	98·2	98·6
Analysis of Solution.		
Carbon in residual glucose ... ... gm.	0·770	2·026
,, in CO_2 in solution ... ... ,,	0·001	0·009
,, in volatile acids ... ... ,,	0·002	0·024
,, in non-volatile acids ... ... ,,	0·268	0·202
,, in volatile neutral compounds ... ... ,,	0·004	0·048
,, in synthetic compounds ... ... ,,	0·160	0·053
Total carbon accounted for ... ... ,,	1·205	2·362
,, ,, in solution ... ... ,,	1·992	2·804
Carbon unaccounted for (by difference) ... ... ,,	0·787	0·442
Residual Glucose.		
Glucose (by polarimeter) ... ... per cent.	0·516	1·033
,, (SHAFFER-HARTMANN) ... ... ,,	0·385	1·013
,, (WOOD-OST) ... ... ,,	0·386	1·020
,, (by alkaline iodine) ... ... ,,	0·433	1·116
Acids.		
Titration (N/1 acid) ... ... c.c.	3·3	3·0
Volatile acids (N/1 acid) ... ... ,,	nil	1·18
Barium salts (weight) ... ... gm.	0·004	0·112
Calcium salts (weight) ... ... ,,	0·930	0·869
Volume of oxygen absorbed ... ... c.c.	2902	2212
Respiration coefficient	1·15	1·23
Mycelium (weight) ... ... gm.	2·280	1·176
,, (carbon) ... ... per cent.	47·3	53·5

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TABLE V.

Species of *Penicillium*:	*P. chrysogenum* series.			*P. baculatum* WESTLING.	*P. notatum* WESTLING.	*P. melea-grinum* BIOURGE.	*P. puberulum* BAINIER.	*P. Biourgei-anum* ZALESKI.
Catalogue number:	Ad. 11	Ad. 24	Ad. 56	Ad. 14	Ad. 35	Ad. 53	Ad. 31	Ad. 87
Experiment number:	F 66	F 21	F 44	F 68	F 20	F 43	F 13	F 85
Incubation period in days:	47	64	48	54	69	56	48	43
Carbon Balance Sheet.								
Carbon in solution (start) ... ... gm.	4·952	4·944	4·944	4·952	4·944	4·944	4·944	4·952
Carbon in H_2SO_4 ... ... ,,	0·001	0·001	nil	0·001	0·001	0·001	0·012	0·002
,, in CO_2 ... ... ,,	1·675	2·241	1·563	1·838	1·784	1·790	1·749	1·862
,, in mycelium ... ... ,,	1·024	1·574	1·003	0·923	0·998	0·937	0·696	0·858
,, in solution (end) ... ... ,,	2·176	0·987	2·272	2·107	2·155	2·154	2·397	2·143
,, accounted for ... ... ,,	4·876	4·803	4·838	4·869	4·938	4·882	4·854	4·865
,, accounted for ... ... per cent.	98·5	97·1	97·8	98·3	99·9	98·8	98·2	98·2
Analysis of Solution.								
Carbon in residual glucose ... ... gm.	0·069	0·278	0·774	0·662	0·116	0·076	0·264	0·371
,, in CO_2 in solution ... ... ,,	nil	0·012	nil	0·001	nil	0·002	0·003	nil
,, in volatile acids ... ... ,,	nil	0·018	nil	0·003	0·001	0·008	nil	0·006
,, in non-volatile acids ... ... ,,	0·520	0·247	0·345	0·266	0·816	0·613	0·300	0·423
,, in volatile neutral compounds ... ,,	0·004	0·014	0·001	0·013	0·017	0·013	0·863	0·021
,, in synthetic compounds ... ... ,,	0·089	0·081	0·078	0·235	0·061	0·106	0·076	0·075
Total carbon accounted for ... ... ,,	0·682	0·650	1·198	1·180	1·011	0·818	1·506	0·896
,, ,, in solution ... ... ,,	2·176	0·987	2·272	2·107	2·155	2·154	2·397	2·143
Carbon unaccounted for (by difference) ... ,,	1·494	0·337	1·074	0·927	1·144	1·336	0·891	1·247
Residual Glucose.								
Glucose (by polarimeter) ... ... per cent.	0·060	0·108	0·518	0·406	0·263	0·200	0·222	0·324
,, (SHAFFER-HARTMANN) ... ,,	0·035	0·139	0·387	0·331	0·058	0·038	0·132	0·186
,, (WOOD-OST) ... ... ,,	—	—	0·406	—	—	—	—	—
,, (by alkaline iodine)... ... ,,	0·091	0·270	0·448	0·392	0·082	0·072	0·172	0·202
Acids.								
Titration (N/1 acid) ... ... c.c.	8·2	1·7	5·9	3·1	11·7	11·2	3·5	7·8
Volatile acids (N/1 acid) ... ... ,,	nil	0·85	ni	nil	nil	0·80	nil	1·91
Barium salts (weight) ... ... gm.	0·018	0·143	0·034	0·015	0·067	0·042	0·016	0·044
Calcium salts (weight) ... ... ,,	1·803	1·230	1·118	0·776	2·739	1·963	1·073	1·457
Volume of oxygen absorbed ... ... c.c.	2851	3317	2564	3070	3203	2696	2078	2927
Respiration coefficient	1·10	1·27	1·14	1·12	1·04	1·24	1·57	1·16
Mycelium (weight) ... ... gm.	2·151	2·690	2·116	1·915	1·845	1·891	1·438	1·662
,, (carbon) ... ... per cent.	47·6	58·5	47·4	48·2	54·1	49·5	48·4	51·6

Group VI.

Division II.—Asymmetrica.

Section I. Velutina.

Subsection 4. Velutina-restricta.

Only one species in this subsection was investigated and the carbon balance sheet is given in Table V.

This species, Ad. 31, *P. puberulum* Bainier, is the one from cultures of which Alsberg and Black isolated penicillic acid. The balance sheet shows relatively large amounts of both "carbon in volatile neutral compounds" and "carbon unaccounted for," together with smaller amounts of titratable acidity and "carbon in non-volatile acids." The metabolic products of this species are at present under investigation.

Group VII.

Division II.—Asymmetrica.

Section II. Brevi-compacta.

The only species included in this group which was examined was Ad. 87, *P. Biourgeianum* Zaleski. Its carbon balance sheet is given in Table V and is of the same type as the carbon balance sheets of species in the subsection *Radiata* (*P. chrysogenum* series) also given in Table V. It is characterized by the large amounts of "carbon unaccounted for," the relatively high titratable acidity present in the form of "non-volatile acids" and the absence of appreciable amounts of "volatile neutral compounds."

Group VIII.

Division II.—Asymmetrica.

Section III. Lanata-typica.

Seven species (Ad. 25, Ad. 49, Ad. 34, Ad. 46, Ad. 12, Ad. 33 and Ad. 60) are included in this group and the carbon balance sheets are given in Table VI. Ad. 25 and Ad. 49, which are different strains of *P. caseicolum* Bainier, and Ad. 34, *P. Camemberti* Thom, are species isolated from cheese and form a well-defined sub-group having the following characteristics: the carbon balance sheets are of the same type and in no case is any metabolic product other than CO_2 formed in appreciable amounts. The other four species, Ad. 46 (*P. ochraceum* Thom), Ad. 12 (*P. lanoso-viride* Thom), Ad. 33 (*P. lanosum* Westling) and Ad. 60 (*P. lanoso-cæruleum* Thom), form a sub-group having certain characteristics in common but showing certain obvious differences. Thus, Ad. 46 and Ad. 12 give rise to large amounts of "volatile neutral compounds," of "carbon unaccounted for," have large respiration coefficients, but do not produce appreciable amounts of titratable acidity, either of a volatile or non-volatile nature. Ad. 60 agrees with the first two species in producing considerable amounts of "volatile

TABLE VI.

Species of *Penicillium*:	*P. caseicolum* BAINIER.		*P. Camemberti* THOM.	*P. ochraceum* THOM.	*P. lanoso-viride* THOM.	*P. lanosum* WESTLING.	*P. lanoso-cœruleum* THOM.
Catalogue number:	Ad. 25	Ad. 49	Ad. 34	Ad. 46	Ad. 12	Ad. 33	Ad. 60
Experiment number:	F 16	F 37	F 19	F 34	F 67	F 15	F 48
Incubation period in days:	41	69	61	57	37	50	67
Carbon Balance Sheet.							
Carbon in solution (start) ... ... gm.	4·944	4·944	4·944	4·944	4·952	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	0·001	0·001	0·001	lost	0·020	0·001	0·036
,, in CO_2 ... ... ,,	1·255	2·142	2·414	1·900	1·988	1·540	2·558
,, in mycelium ... ... ,,	0·452	0·576	0·730	0·776	0·575	1·015	1·029
,, in solution (end)... ... ... ,,	3·192	2·165	1·726	2·063	2·357	2·352	1·193
,, accounted for ... ... ,,	4·900	4·884	4·871	4·739	4·940	4·908	4·816
,, accounted for ... ... per cent.	99·1	98·8	98·5	95·8	99·8	99·3	97·4
Analysis of Solution.							
Carbon in residual glucose ... ... gm.	2·914	1·718	1·398	0·048	0·128	1·008	0·012
,, in CO_2 in solution ... ... ,,	0·017	0·005	0·012	0·002	0·003	0·001	0·017
,, in volatile acids ... ... ,,	0·014	nil	0·005	0·003	0·001	nil	0·001
,, in non-volatile acids ... ... ,,	0·109	0·321	0·186	0·299	0·142	0·365	0·168
,, in volatile neutral compounds ,,	0·031	0·007	0·017	1·053	1·301	0·006	0·675
,, in synthetic compounds ... ,,	0·056	0·071	0·078	0·164	0·031	0·078	0·138
Total carbon accounted for ... ... ,,	3·141	2·122	1·696	1·569	1·606	1·458	1·011
,, ,, in solution ... ... ,,	3·192	2·165	1·726	2·063	2·357	2·352	1·193
Carbon unaccounted for (by difference) ,,	0·051	0·043	0·030	0·494	0·751	0·894	0·182
Residual Glucose.							
Glucose (by polarimeter) ... per cent.	1·501	0·929	0·725	0·104	0·114	0·674	—
,, (SHAFFER-HARTMANN) ,,	1·457	0·859	0·699	0·024	0·064	0·504	0·006
,, (WOOD-OST) ... ... ,,	1·479	0·914	0·710	—	—	0·519	—
,, (by alkaline iodine) ... ,,	1·504	0·928	0·731	0·066	0·126	0·527	0·064
Acids.							Decrease of
Titration (N/1 acid) ... ... c.c.	0·2	0·4	0·1	2·0	0·3	4·2	0·7
Volatile acids (N/1 acid) ... ... ,,	0·93	0·28	0·52	nil	0·08	nil	0·77
Barium salts (weight) ... ... gm.	0·060	0·001	0·016	0·044	0·017	0·016	0·047
Calcium salts (weight) ... ... ,,	1·145	1·621	0·767	1·068	0·492	1·231	0·637
Volume of oxygen absorbed ... ... c.c.	2122	3654	4203	2145	2203	2533	3966
Respiration coefficient	1·12	1·12	1·08	1·65	1·69	1·14	1·21
Mycelium (weight) ... ... gm.	0·940	1·205	1·542	1·620	1·229	2·107	2·113
,, (carbon) ... ... per cent.	48·1	46·3	47·3	47·9	46·8	48·2	48·7

neutral compounds" and in the non-formation of acidity. On the other hand, this species gives rise to only small amounts of "carbon unaccounted for." Ad. 33 agrees with Ad. 46 and Ad. 12 in giving a large amount of "carbon unaccounted for," but differs from them in giving a moderate amount of "non-volatile acids," in the complete absence of "volatile neutral compounds" and in having a respiration coefficient approximating to unity. It has also been found that these four species differ materially in certain physiological details. There is thus no evidence that they form a group of species which are closely related biochemically.

Morphological details of Ad. 12 are given in THOM's '*Penicillia*' on p. 314, and of Ad. 33 on p. 317.

GROUP IX.

Division II.—Asymmetrica.

Section III. Lanata-typica.

Subsection 2. *Lanata-zonata.*

This group includes two species, Ad. 82 (*P. commune* THOM) and Ad. 19 (*P. fusco-glaucum* BIOURGE), the carbon balance sheets for which are given in Table VII. These carbon balance sheets, which are very similar in characteristics, are of the same type as those given in Table VI for Ad. 46 and Ad. 12. This is, in fact, so much the case, that it is difficult to distinguish between the balance sheets of Ad. 19 and Ad. 12 and from this point of view these two species might quite well be included biochemically in the same group as Ad. 46 and Ad. 12.

Morphological details of Ad. 19 are given in THOM's '*Penicillia*' on p. 326.

GROUP X.

Division II.—Asymmetrica.

Section IV. Lanata-divaricata.

Two different strains of *P. lilacinum* THOM, obtained from different parts of the world, are included in this group, their carbon balance sheets being given in Table VII. Morphologically and physiologically these two strains agree well with each other, but consideration of the two carbon balance sheets shows that these are of different types and it appears that these two strains show the same biochemical differences as has previously been noted in Part III with strains of *Aspergillus nidulans* (p. 35). Thus the carbon balance sheet of the strain Ad. 32 is of the same general type as that of the four strains *A. nidulans*, Ac. 67, Ac. 78, Ac. 84 and Ac. 85, while the strain Ad. 37 is of the type represented by *A. nidulans*, Ac. 80 (see Part III, Table V). The main difference between these two strains is the production by Ad. 32 of considerable amounts of "volatile neutral compounds," while Ad. 37 produces only negligible amounts.

TABLE VII.

Species of *Penicillium* :	*P. commune* THOM.	*P. fusco-glaucum* BIOURGE.	*P. lilacinum* THOM.		*P. Daleæ* ZALESKI.	*P. Godlewskii* ZALESKI.
Catalogue number :	Ad. 82	Ad. 19	Ad. 32	Ad. 37	Ad. 55	Ad. 62
Experiment number :	F 79	F 72	F 14	F 25	F 42	F 50
Incubation period in days :	49	36	53	57	35	71
Carbon Balance Sheet.						
Carbon in solution (start) ... gm.	4·952	4·952	4·944	4·944	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	0·012	0·028	0·009	nil	0·019	0·002
,, in CO_2 ... ... ,,	2·196	2·216	2·049	1·256	1·866	2·058
,, in mycelium ... ... ,,	0·662	0·677	0·994	0·901	0·436	1·149
,, in solution (end) ... ... ,,	1·944	1·933	1·784	2·693	2·494	1·602
,, accounted for ... ... ,,	4·814	4·854	4·836	4·850	4·815	4·811
,, accounted for ... per cent.	97·2	98·0	97·8	98·1	97·4	97·3
Analysis of Solution.						
Carbon in residual glucose ... gm.	0·287	0·004	0·444	2·414	0·100	0·626
,, in CO_2 in solution ... ,,	0·001	0·009	0·017	0·016	0·010	nil
,, in volatile acids ... ... ,,	0·016	0·007	0·049	0·009	0·013	0·017
,, in non-volatile acids ... ,,	0·136	0·134	0·189	0·207	0·125	0·348
,, in volatile neutral compounds ,,	0·799	1·295	0·641	0·020	1·819	0·014
,, in synthetic compounds ,,	0·067	0·045	0·102	0·089	0·066	0·240
Total carbon accounted for ... ,,	1·306	1·494	1·442	2·755	2·133	1·245
,, ,, in solution ... ... ,,	1·944	1·933	1·784	2·693	2·494	1·602
Carbon unaccounted for (by difference) ... ... ,,	0·638	0·439	0·342	Surplus of 0·062	0·361	0·357
Residual Glucose.						
Glucose (by polarimeter) ... per cent.	0·199	0·046	0·195	1·123	0·071	0·297
,, (SHAFFER-HARTMANN) ,,	0·143	0·002	0·222	1·207	0·050	0·313
,, (WOOD-OST) ... ,,	—	—	—	1·210	—	0·332
,, (by alkaline iodine) ,,	0·226	0·052	0·261	1·226	0·113	0·362
Acids.	Decrease of	Decrease of				
Titration (N/1 acid) ... ... c.c.	0·1	0·5	1·9	1·2	1·1	3·5
Volatile acids (N/1 acid) ... ... ,,	0·78	1·59	2·44	0·32	1·40	1·09
Barium salts (weight) ... ... gm.	0·066	0·115	0·252	0·027	0·136	0·066
Calcium salts (weight) ... ... ,,	0·673	0·419	0·881	1·141	0·589	1·287
Volume of oxygen absorbed ... c.c.	2910	2475	2452	1698	1815	3353
Respiration coefficient	1·41	1·68	1·57	1·40	1·93	1·14
Mycelium (weight) ... ... gm.	1·295	1·387	1·659	1·495	0·901	2·342
,, (carbon) ... per cent.	51·2	48·8	59·9	60·2	48·4	49·5

These two strains agree in the unusually large percentage of carbon in the mycelium, 59·5 per cent. and 60·2 per cent., being very similar in this respect to three strains of *Scopulariopsis* given in Table XIII, which give figures of 62·9 per cent., 62·2 per cent. and 60·3 per cent.

Group XI.

Division II.—Asymmetrica.

Section V. Asymmetrica-funiculosa.

Subsection 1. *Funiculosa-divaricata.*

Two species are included in this group, Ad. 55 (*P. Daleæ* Zaleski) and Ad. 62 (*P. Godlewskii* Zaleski). The carbon balance sheets which are given in Table VII are of different types since, while Ad. 55 produces large amounts of "volatile neutral compounds" and has a high respiration coefficient, Ad. 62 produces little if any of this type of compound and has a correspondingly low respiration coefficient. The carbon balance sheet of Ad. 55 is of the same type as those of the strains included in the *P. terrestre* Jensen series given in Table VIII. It is also of interest to note that in their physiological reactions Ad. 55 and Ad. 62 show considerable differences and are obviously not closely related species from a biochemical point of view. Moreover, another strain of *P. Daleæ* Zaleski, Ad. 116, for which unfortunately no carbon balance sheet was prepared but which is referred to in Part VII of this series, differs from the strain Ad. 55, since Ad. 116 produces considerable amounts of kojic acid from glucose, while Ad. 55, on the other hand, does not give rise even to traces of this interesting biochemical product.

Group XII.

Division II.—Asymmetrica.

Section V. Asymmetrica-funiculosa.

Subsection 2. *Funiculosa-typica.*

Eight different strains, obtained from various sources, belonging to the large series *P. terrestre* Jensen, were examined (Ad. 8, Ad. 4, Ad. 5, Ad. 54, Ad. 18, Ad. 50, Ad. 59 and Ad. 9). Their carbon balance sheets are given in Table VIII. These eight strains, with the exception of Ad. 9, represent a well-defined group having the following characteristics:—

(i) They all have the same type of carbon balance sheet.

(ii) They all give moderate or negligible amounts of titratable acidity and "carbon in non-volatile acids."

(iii) They all give large amounts of "volatile neutral compounds" and have correspondingly high respiration coefficients.

(iv) They all give moderate amounts of "carbon unaccounted for."

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Table VIII.

Species of *Penicillium*:	*P. terrestre* Jensen series.							
Catalogue number:	Ad. 8	Ad. 4	Ad. 5	Ad. 54	Ad. 18	Ad. 50	Ad. 59	Ad. 9
Experiment number:	F 18	F 3	F 4	F 41	F 71	F 38	F 47	F 9
Incubation period in days:	28	24	35	34	40	49	33	74
Carbon Balance Sheet.								
Carbon in solution (start) ... ... gm.	4·944	5·043	5·043	4·944	4·952	4·944	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	0·013	0·017	0·020	0·021	0·012	0·024	0·028	0·001
,, in CO_2 ... ... ,,	1·562	1·626	1·924	1·994	1·930	2·257	1·988	2·282
,, in mycelium ... ... ,,	0·396	0·416	0·547	0·455	0·479	0·718	0·501	0·922
,, in solution (end) ... ... ,,	2·865	2·848	2·405	2·393	2·412	1·767	2·321	1·621
,, accounted for ... ... ,,	4·836	4·907	4·896	4·863	4·833	4·766	4·838	4·826
,, accounted for ... ... per cent.	97·8	97·3	97·1	98·4	97·6	96·4	97·8	97·6
Analysis of Solution.								
Carbon in residual glucose ... ... gm.	0·786	0·343	0·123	0·054	0·879	0·012	0·036	0·576
,, in CO_2 in solution ... ... ,,	0·006	0·005	0·008	0·008	0·006	0·015	0·010	0·014
,, in volatile acids ... ... ,,	0·007	0·006	0·011	0·016	0·004	0·005	0·010	0·033
,, in non-volatile acids ... ... ,,	0·291	0·343	0·273	0·133	0·157	0·098	0·087	0·164
,, in volatile neutral compounds ... ,,	1·142	1·478	1·407	1·783	0·952	1·372	1·797	0·093
,, in synthetic compounds ... ... ,,	0·200	0·053	0·133	0·189	0·065	0·076	0·075	0·066
Total carbon accounted for ... ... ,,	2·432	2·228	1·955	2·183	2·063	1·578	2·015	0·946
,, ,, in solution ... ... ,,	2·865	2·848	2·405	2·393	2·412	1·767	2·321	1·621
Carbon unaccounted for (by difference) ... ,,	0·433	0·620	0·450	0·210	0·349	0·189	0·306	0·675
Residual Glucose.								
Glucose (by polarimeter) ... ... per cent.	0·469	0·234	0·134	0·055	0·484	0·018	0·025	0·316
,, (Shaffer-Hartmann) ... ,,	0·393	0·173	0·062	0·027	0·440	0·006	0·018	0·283
,, (Wood-Ost) ... ... ,,	0·408	—	—	—	0·443	—	—	—
,, (by alkaline iodine)... ... ,,	0·443	0·231	0·124	0·097	0·516	0·043	0·082	0·317
Acids.								
Titration (N/1 acid) ... ... c.c.	4·8	4·3	3·9	1·4	0·5	nil	Decrease of 0·7	2·9
Volatile acids (N/1 acid) ... ... ,,	0·80	0·23	0·19	1·13	0·84	1·06	1·60	1·54
Barium salts (weight) ... ... gm.	0·023	0·019	0·053	0·109	0·082	0·100	0·119	0·173
Calcium salts (weight) ... ... ,,	1·084	1·360	1·051	0·573	0·632	0·432	0·342	0·801
Volume of oxygen absorbed ... ... c.c.	1612	1381	2002	1789	2421	2598	1646	3616
Respiration coefficient	1·82	2·20	1·80	2·08	1·49	1·63	2·26	1·19
Mycelium (weight) ... ... gm.	0·785	0·831	1·096	0·929	0·991	1·497	1·056	1·684
,, (carbon) ... ... per cent.	50·4	50·1	49·9	49·0	48·3	48·0	47·5	54·8

Ad. 9 seems to be an atypical member of this series in that it only produces negligible amounts of " volatile neutral compounds " and has a low respiration coefficient.

It is of interest to note that all the strains included in this group show closely agreeing physiological characteristics.

Morphological details of Ad. 8, Ad. 9 and Ad. 18 are given in THOM's '*Penicillia*' on p. 372.

GROUP XIII.

Division II.—Asymmetrica.

Section VI. Fasciculata.

Subsection 1. *Sclerotigena.*

One strain of *P. gladioli* MACHACEK (Ad. 65) and two different strains of *P. italicum* WEHMER (Ad. 84 and Ad. 85) are included in this group, the carbon balance sheets for which are given in Table IX. The three carbon balance sheets are all of the same type, showing moderate amounts of " carbon in volatile neutral compounds " with medium respiration coefficients, negligible amounts of titratable acidity and moderate amounts of " carbon unaccounted for." The positive characteristic of the cultures included in this group, particularly of Ad. 65, is the high percentage of " carbon in mycelium " (62·3 per cent. in Ad. 65). Colour reactions typical of strains of *P. italicum* WEHMER are described in detail in Part XVIII.

GROUP XIV.

Division II.—Asymmetrica.

Section VI. Fasciculata.

Subsection 3. *Viridicata.*

Included in this group are three strains belonging to the series *P. viridicatum* WESTLING (Ad. 76, Ad. 77 and Ad. 83), together with *P. psittacinum* THOM (Ad. 22) and *P. verrucosum* DIERCKX (Ad. 15). The carbon balance sheets for these five cultures are given in Table X. Ad. 22 is included in this group since its carbon balance sheet is very similar in type to those of Ad. 76 and Ad. 77 although, in THOM's morphological classification, it occurs among the *Funiculosa-typica* as subsection 2 of the *Asymmetrica-funiculosa*. In this connection it is of interest to note the following quotation from p. 392 of THOM's book on the *Penicillia*: " Among bright green species placed elsewhere *P. psittacinum* THOM might from its colour and habit be placed here," *i.e.*, in the *P. viridicatum* series.

These five cultures taken together do not form a group agreeing in biochemical characteristics, as indicated by their carbon balance sheets. The four cultures, Ad. 76, Ad. 77, Ad. 83 and Ad. 22, of which the first three are almost indistinguishable in their physiological reactions and morphological characteristics, have carbon balance

Table IX.

Species of *Penicillium*:	*P. gladioli* Machacek.	*P. italicum* Wehmer.	
Catalogue number:	Ad. 65	Ad. 84	Ad. 85
Experiment number:	F 53	F 83	F 84
Incubation period in days:	64	62	56
Carbon Balance Sheet.			
Carbon in solution (start) ... ... gm.	4·944	4·952	4·952
Carbon in H_2SO_4 ... ... ,,	0·029	0·006	0·002
,, in CO_2 ... ... ,,	2·643	2·350	1·834
,, in mycelium ... ... ,,	0·868	0·715	0·623
,, in solution (end) ... ... ,,	1·261	1·750	2·345
,, accounted for ... ... ,,	4·801	4·821	4·804
,, accounted for ... ... per cent.	97·1	97·4	97·0
Analysis of Solution.			
Carbon in residual glucose ... ... gm.	0·018	0·600	1·477
,, in CO_2 in solution... ... ... ,,	0·010	0·007	0·005
,, in volatile acids ... ... ,,	0·001	nil	0·012
,, in non-volatile acids ... ... ,,	0·167	0·169	0·048
,, in volatile neutral compounds ... ,,	0·562	0·631	0·392
,, in synthetic compounds ... ... ,,	0·099	0·086	0·065
Total carbon accounted for ... ... ,,	0·857	1·493	1·999
,, ,, in solution ... ... ,,	1·261	1·750	2·345
Carbon unaccounted for (by difference) ... ,,	0·404	0·257	0·346
Residual Glucose.			
Glucose (by polarimeter) ... ... per cent.	0·028	0·335	0·744
,, (Shaffer-Hartmann) ... ,,	0·009	0·300	0·738
,, (Wood-Ost) ... ... ,,	—	0·302	0·758
,, (by alkaline iodine) ... ,,	0·071	0·347	0·825
Acids.			
Titration (N/1 acid) ... ... c.c.	0·3	0·4	0·7
Volatile acids (N/1 acid) ... ... ,,	1·04	nil	0·87
Barium salts (weight) ... ... gm.	0·054	0·026	0·079
Calcium salts (weight) ... ... ,,	0·721	0·842	0·226
Volume of oxygen absorbed ... ... c.c.	3818	3328	2738
Respiration coefficient	1·30	1·32	1·25
Mycelium (weight) ... ... gm.	1·392	1·367	1·175
,, (carbon) ... ... per cent.	62·3	52·3	53·0

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TABLE X.

Species of *Penicillium* :	*P. viridicatum* WESTLING.			*P. psittacinum* THOM.	*P. verrucosum* DIERCKX.	*P. crustosum* THOM.	*P. expansum* (LINK) THOM series.		*P. Schneggii* BOAS.	*P. corymbiferum* WESTLING.	
Catalogue number :	Ad. 76	Ad. 77	Ad. 83	Ad. 22	Ad. 15	Ad. 16	Ad. 1	Ad. 58	Ad. 51	Ad. 61	Ad. 64
Experiment number :	F 57	F 58	F 80	F 22	F 70	F 61	F 1	F 46	F 39	F 49	F 52
Incubation period in days :	36	52	70	49	31	47	37	79	48	41	46
Carbon Balance Sheet.											
Carbon in solution (start) ... ... gm.	4·944	4·944	4·952	4·944	4·952	4·944	5·043	4·944	4·944	4·944	4·944
Carbon in H_2SO_4 ... ... „	0·004	0·014	0·002	0·009	0·022	0·029	0·006	0·042	0·009	0·024	0·023
„ in CO_2 ... ... „	1·361	2·019	1·978	1·063	1·947	2·313	1·824	2·957	2·035	2·276	2·097
„ in mycelium ... ... „	0·654	0·971	1·102	0·437	0·542	0·627	0·671	0·896	0·694	0·585	0·634
„ in solution (end) ... ... „	2·796	1·769	1·694	3·342	2·335	1·840	2·354	0·904	2·102	1·931	1·956
„ accounted for ... ... „	4·815	4·773	4·776	4·851	4·846	4·809	4·855	4·799	4·840	4·816	4·710
„ accounted for ... per cent.	97·4	96·5	96·4	98·1	97·9	97·3	96·3	97·1	97·9	97·4	95·3
Analysis of Solution.											
Carbon in residual glucose ... ... gm.	0·868	0·088	0·067	1·428	0·070	0·006	0·599	0·008	0·134	0·040	0·032
„ in CO_2 in solution ... ... „	nil	nil	nil	nil	0·006	0·006	0·009	0·021	0·009	0·009	0·004
„ in volatile acids ... ... „	0·002	0·001	0·002	0·012	0·004	0·010	0·013	0·015	0·016	0·009	0·014
„ in non-volatile acids ... „	0·576	0·364	0·718	0·453	0·126	0·133	0·233	0·126	0·178	0·135	0·162
„ in volatile neutral compounds „	0·115	0·409	0·004	0·540	1·582	1·258	0·875	0·533	0·669	1·426	1·268
„ in synthetic compounds ... „	0·097	0·168	0·074	0·084	0·026	0·045	0·101	0·068	0·110	0·085	0·126
Total carbon accounted for ... „	1·658	1·030	0·865	2·517	1·814	1·458	1·830	0·771	1·116	1·704	1·606
„ „ in solution ... ... „	2·796	1·769	1·694	3·342	2·335	1·840	2·354	0·904	2·102	1·931	1·956
Carbon unaccounted for (by difference) „	1·138	0·739	0·829	0·825	0·521	0·382	0·524	0·133	0·986	0·227	0·350
Residual Glucose.											
Glucose (by polarimeter) ... per cent.	0·635	0·088	0·239	0·879	0·075	0·041	0·390	nil	0·099	0·025	0·028
„ (SHAFFER-HARTMANN) „	0·434	0·044	0·034	0·714	0·035	0·003	0·299	0·004	0·067	0·020	0·016
„ (WOOD-OST) ... „	0·457	—	—	0·716	—	—	0·280	—	—	—	—
„ (by alkaline iodine) „	0·515	0·058	0·046	0·735	0·088	0·082	0·336	0·072	0·269	0·078	0·121
Acids.						Decrease of		Decrease of			
Titration (N/1 acid) ... ... c.c.	10·1	5·4	8·3	8·7	0·2	0·3	2·0	0·8	1·6	0·7	1·9
Volatile acids (N/1 acid) ... ... „	0·37	0·35	0·06	0·33	0·83	1·45	0·54	1·47	1·52	0·73	0·57
Barium salts (weight) ... ... gm.	0·012	0·066	0·015	0·045	0·028	0·040	0·042	0·098	0·103	0·078	0·063
Calcium salts (weight) ... ... „	1·691	1·159	2·410	1·554	0·452	0·482	0·995	0·637	0·802	0·616	0·540
Volume of oxygen absorbed ... c.c.	2124	2990	3486	1421	1825	2638	2206	4574	2630	2394	2419
Respiration coefficient	1·19	1·26	1·06	1·40	2·00	1·64	1·55	1·22	1·45	1·78	1·62
Mycelium (weight) ... ... gm.	1·445	2·071	2·311	0·928	1·168	1·330	1·341	1·804	1·297	1·225	1·259
„ (carbon) ... ... per cent.	45·3	46·9	47·7	47·1	46·6	47·2	50·0	49·6	53·5	47·7	50·4

sheets showing the following characteristics: they all give large amounts of "carbon unaccounted for," high titratable acidity present entirely in the form of non-volatile acids, but Ad. 76, Ad. 77 and Ad. 22 give moderate amounts of "carbon in volatile neutral compounds" with medium respiration coefficients, while, on the other hand, Ad. 83 shows a complete absence of this type of metabolic product and has a respiration coefficient approximating to unity. Ad. 15, however, has a carbon balance sheet which, while in other respects very similar to those of Ad. 76, Ad. 77 and Ad. 22, is differentiated by the fact that it does not produce any appreciable amount of titratable acidity.

Group XV.

Division II.—Asymmetrica.

Section VI. Fasciculata.

Subsection 4. *Glauca.*

This subsection is divided by Thom into a number of series. The following species, which are arranged in their appropriate series, have been examined and their carbon balance sheets are given in Table X.

Series *Crustaceum.* Ad. 16, *P. crustosum* Thom.
Series *Restrictum.* None.
Series *Expansum.* Ad. 1, Ad. 58, strains of *P. expansum* (Link) Thom.
Series *Italicum.* Ad. 84, Ad. 85. *P. italicum* Wehmer. The carbon balance sheets for these two strains have already been given in Table IX.
Series —— . Ad. 51, *P. Schneggii* Boas.
Series *Urticæ-patulum.* None.

The species included in this group have carbon balance sheets of similar types, which have no very interesting characteristics except that all the species form considerable amounts of "volatile neutral compounds" and have correspondingly high respiration coefficients. Ad. 51 and, to a smaller extent, Ad. 1 further give rise to considerable amounts of "carbon unaccounted for."

A morphological description of Ad. 16 is given in Thom's '*Penicillia*' on p. 399, and of Ad. 51 on p. 417.

Group XVI.

Division II.—Asymmetrica.

Section VI.—Fasciculata.

Subsection 5. *Coremiella.*

Included in this group are two different strains of *P. corymbiferum* Westling (Ad. 61 and Ad. 64). Their carbon balance sheets, which are given in Table X, have only one outstanding feature, namely the very large figure for "carbon in volatile neutral

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compounds" and the correspondingly high respiration coefficients. The titratable acidity, the "carbon in non-volatile acids," and the "carbon unaccounted for" are all low. The balance sheets are very similar in type and this biochemical similarity is further substantiated by the similarity in their physiological characteristics.

A morphological description of Ad. 64 is given in THOM's '*Penicillia*' on p. 424.

GROUP XVII.

Division III.—Biverticillata-symmetrica.

Section I. Ascogena.

Three species are included in this section, Ad. 39, *P. avellaneum* THOM and TURESSON; Ad. 20, *P. Kiliense* WEIDEMANN; and Ad. 101, *P. spiculisporum* LEHMAN.

Section II. Coremigena.

Two different strains of *P. Duclauxi* DELACROIX (Ad. 63 and Ad. 75) are included in this section.

Section III. Luteo-virida.

Subsection IIIa. Funiculosa.

(i) *Luteo-viride-pinophilum* series. One strain of *P. pinophilum* HEDGCOCK, Ad. 41, is included in this series.

(ii) *P. funiculosum* series. None.

(iii) *P. Herquei* series. One strain of *P. Herquei* BAINIER and SARTORY, Ad. 43, is included in this series.

Subsection IIIb. Luteo-purpurogena.

(i) *P. rugulosum* series. One strain of *P. rugulosum* THOM, Ad. 27, is included in this series.

(ii) *P. purpurogenum* series. One strain of *P. purpurogenum* STOLL, Ad. 36, is included in this series.

(iii) *P. luteum* series (non-ascosporic). The non-ascosporic strain of *P. luteum* ZUKAL, Ad. 30, is included in this series.

The carbon balance sheets for all the above species are given in Table XI. They are, however, of such different types that it is impossible to draw any general conclusions as to the biochemical grouping of these species. Thus, while of the three species included in Section I, *Ascogena*, Ad. 39 and Ad. 20 give only small amounts of any metabolic product other than CO_2, Ad. 101 gives the largest amount of "volatile neutral compounds" and has the highest respiration coefficient of any species of *Penicillium* examined. The production by this species of an apparently specific biochemical product, *i.e.*, the lactone of γ-hydroxy-βδ-dicarboxypentadecoic acid, is

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Species of *Penicillium*:	*P. avellaneum* Thom and Turesson.	*P. Kiliense* Weidemann.	*P. spiculisporum* Lehman.	*P. Duclauxi* Delacroix.		*P. pinophilum* Hedgcock.	*P. Herquei* Bainier and Sartory.	*P. rugulosum* Thom.	*P. purpurogenum* Stoll.	*P. luteum* Zukal.
Catalogue number:	Ad. 39	Ad. 20	Ad. 101	Ad. 63	Ad. 75	Ad. 41	Ad. 43	Ad. 27	Ad. 36	Ad. 30
Experiment number:	F 26	F 116	F 88	F 51	F 56	F 29	F 31	F 10	F 24	F 12
Incubation period in days:	81	70	24	21	42	82	69	55	35	68
Carbon Balance Sheet.										
Carbon in solution (start) ... ... gm.	4·944	4·901	4·807	4·944	4·944	4·944	4·944	4·944	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	0·001	nil	0·022	0·019	0·036	0·001	0·005	0·001	0·024	0·001
,, in CO_2 ... ... ,,	2·555	1·610	1·638	1·739	2·318	1·807	2·129	1·935	2·042	2·017
,, in mycelium ... ... ,,	0·605	0·807	0·181	0·365	0·460	0·546	0·655	0·686	0·620	1·252
,, in solution (end) ... ... ,,	1·717	2·278	2·757	2·719	2·009	2·516	2·114	2·249	2·173	1·505
,, accounted for ... ... ,,	4·878	4·695	4·598	4·842	4·823	4·870	4·903	4·871	4·859	4·775
,, accounted for ... ... per cent.	98·7	95·8	95·7	97·9	97·5	98·5	99·2	98·5	98·3	96·6
Analysis of Solution.										
Carbon in residual glucose ... ... gm.	1·268	1·781	0·134	0·054	0·028	2·038	0·274	1·488	0·022	0·774
,, in CO_2 in solution ... ... ,,	0·011	0·006	0·020	0·013	0·015	0·004	0·005	0·007	nil	0·007
,, in volatile acids ... ... ,,	0·028	0·008	nil	0·037	nil	0·038	0·001	nil	0·002	0·001
,, in non-volatile acids ... ... ,,	0·206	0·146	0·094	0·100	0·132	0·288	0·336	0·359	0·220	0·477
,, in volatile neutral compounds ,,	0·015	0·006	2·381	1·916	1·621	0·017	0·232	0·008	1·423	0·002
,, in synthetic compounds ... ,,	0·090	0·226	0·003	0·040	0·084	0·088	0·050	0·048	0·188	0·265
Total carbon accounted for ... ... ,,	1·618	2·173	2·632	2·560	1·880	2·473	0·898	1·910	1·855	1·526
,, ,, in solution ... ... ,,	1·717	2·278	2·757	2·719	2·009	2·516	2·114	2·249	2·173	1·505
Carbon unaccounted for (by difference) ,,	0·099	0·105	0·125	0·159	0·129	0·043	1·216	0·339	0·318	Surplus of 0·021
Residual Glucose.										
Glucose (by polarimeter) ... per cent.	0·588	0·924	0·060	0·217	nil	1·061	0·173	0·738	0·063	0·275
,, (Shaffer-Hartmann) ,,	0·634	0·890	—	0·227	0·014	1·019	0·137	0·744	0·011	0·387
,, (Wood-Ost) ... ... ,,	0·634	0·924	0·067	—	—	1·040	—	0·788	—	0·270
,, (by alkaline iodine) ... ,,	0·711	0·915	0·146	0·268	0·122	1·017	0·172	0·791	0·048	0·454
Acids.		Decrease of			Decrease of					
Titration (N/1 acid) ... ... c.c.	1·1	0·6	1·5	1·4	0·8	3·0	5·2	1·4	2·0	0·6
Volatile acids (N/1 acid) ... ... ,,	1·10	0·77	nil	2·66	0·56	2·04	nil	nil	0·59	0·42
Barium salts (weight) ... ... gm.	0·133	0·045	0·064	0·262	0·013	0·205	0·009	0·011	0·038	—
Calcium salts (weight) ... ... ,,	0·781	1·260	0·398	0·568	0·581	1·106	—	0·740	0·866	1·918
Volume of oxygen absorbed ... ... c.c.	4279	2881	769	1157	2435	3065	3415	3255	2151	3269
Respiration coefficient	1·12	1·05	4·03	2·82	1·79	1·10	1·17	1·11	1·77	1·04
Mycelium (weight) ... ... gm.	1·152	1·555	0·392	0·748	0·886	0·990	1·335	1·342	1·212	2·397
,, (carbon) ... ... per cent.	52·2	51·9	46·2	48·8	51·9	55·1	49·1	51·1	51·2	52·2

dealt with in Part XVI. This difference in biochemical activities is readily explicable when it is remembered that the grouping of these species is admitted by THOM to be largely an arbitrary one. Thus, on p. 445 of his book, THOM says, " In making this aggregate, *P. avellaneum* THOM and TURESSON, and *P. spiculisporum* LEHMAN, *whose affinities are doubtful*, have been included with a truly homogeneous series including *P. luteum* and its allies."

In Section II, *Coremigena*, it is interesting to note that the two different strains of *P. Duclauxi*, Ad. 63 and Ad. 75, give balance sheets which are very similar in type, having as their only outstanding characteristic the production of large amounts of " volatile neutral compounds " and correspondingly high respiration coefficients.

The two species included in subsection IIIa, *P. pinophilum*, Ad. 41, and *P. Herquei*, Ad. 43, have also carbon balance sheets of different types, since, while Ad. 43 gives a very large figure for " carbon unaccounted for," Ad. 41 produces only negligible amounts of products of this type. In other respects the two carbon balance sheets are not very dissimilar.

The three species included in subsection IIIb, *P. rugulosum*, Ad. 27, *P. purpurogenum*, Ad. 36, and *P. luteum*, Ad. 30, also have different types of carbon balance sheets. Thus, while Ad. 27 and Ad. 30 produce practically no " volatile neutral compounds " and have respiration coefficients approaching unity, Ad. 36 gives a large yield of this type of product and has a very high respiration coefficient.

The carbon balance sheet for *P. luteum* (non-ascosporic form), Ad. 30, has several interesting characteristics. Of outstanding interest is the fact that while the titratable acidity is only 0·6 c.c., the " carbon in non-volatile acids " gives the relatively high figure of 0·477 gm., corresponding to about 11 per cent. of some compound precipitated as a calcium salt in 80 per cent. alcohol. The explanation for this will be found in Part XIII, in which are described the preparation and properties of a mucilaginous material which is a complex built up of units of a malonyl polyglucose. It titrates as an acid having a combining weight of between 400 and 500, and is precipitable as a calcium salt from 80 per cent. alcohol. The same types of figures are also given by *P. rugulosum*, Ad. 27, in which the titratable acidity is 1·4 c.c. and the " carbon in non-volatile acids " 0·359 gm, and by *P. tardum*, Ad. 45 (see Table XII), in which the figures are 0·6 c.c. and 0·392 gm. respectively. The nature of the products responsible for these figures in the case of Ad. 27 and Ad. 45 has not yet been investigated.

GROUP XVIII.

Division III. Biverticillata-symmetrica.

Section IV. Miscellanea.

Two strains of *P. tardum* THOM, Ad. 45 and Ad. 47, are included in this group. Their carbon balance sheets, which are given in Table XII, are so different in type as to lead to the belief that, from a biochemical point of view, these two strains are widely different.

Table XII.

Species of *Penicillium*:	*P. tardum* Thom.	
Catalogue number:	Ad. 45	Ad. 47
Experiment number:	F 33	F 35
Incubation period in days:	70	45
Carbon Balance Sheet.		
Carbon in solution (start) ... ... gm.	4·944	4·944
Carbon in H_2SO_4 ... ... ,,	nil	0·015
,, in CO_2... ... ... ,,	2·107	2·037
,, in mycelium ... ... ,,	0·940	0·449
,, in solution (end) ... ... ,,	1·743	2·352
,, accounted for ... ... ,,	4·790	4·853
,, accounted for ... ... per cent.	96·9	98·2
Analysis of Solution.		
Carbon in residual glucose ... ... gm.	1·090	0·576
,, in CO_2 in solution ... ... ,,	0·009	0·008
,, in volatile acids ... ... ,,	0·005	0·026
,, in non-volatile acids... ... ... ,,	0·392	0·102
,, in volatile neutral compounds ... ... ,,	0·002	1·065
,, in synthetic compounds ... ... ,,	0·058	0·045
Total carbon accounted for ... ... ,,	1·556	1·822
,, ,, in solution ... ... ,,	1·743	2·352
Carbon unaccounted for (by difference) ... ... ,,	0·187	0·530
Residual Glucose.		
Glucose (by polarimeter) ... ... per cent.	0·482	0·276
,, (Shaffer-Hartmann) ... ... ,,	0·545	0·288
,, (Wood-Ost) ... ... ,,	0·544	—
,, (by alkaline iodine) ... ... ,,	0·562	0·402
Acids.		
Titration (N/1 acid) ... ... c.c.	0·6	1·2
Volatile acids (N/1 acid) ... ... ,,	0·01	1·59
Barium salts (weight) ... ... gm.	0·004	0·157
Calcium salts (weight) ... ... ,,	1·353	0·584
Volume of oxygen absorbed ... ... c.c.	3655	2440
Respiration coefficient	1·05	1·56
Mycelium (weight) ... ... gm.	1·893	0·916
,, (carbon) ... ... per cent.	49·7	49·0

Group XIX.

Genus *Gliocladium* Corda.

Genus *Scopulariopsis* Bainier.

Genus *Pœcilomyces* Bainier.

Unnamed species of *Penicillium*.

Included in this group are seven species which are grouped together more for the sake of convenience than for any morphological or biochemical relationships. In doing this, the arrangement adopted by Thom in his book has been followed. The species included are :—

(i) Ad. 38—belonging to the genus *Gliocladium* and probably a strain of *Gliocladium roseum* Bainier.

(ii) Ad. 40, Ad. 70 and Ad. 72—three different strains of the species formerly known as *P. brevicaule* Saccardo, but now transferred to the genus *Scopulariopsis* and hence becoming strains of *Scopulariopsis brevicaulis* (Saccardo) Bainier.

(iii) Ad. 44—formerly known as *P. divaricatum* Thom and now transferred to the genus *Pœcilomyces* and becoming a strain of *Pœcilomyces varioti* Bainier.

(iv) Ad. 3 and Ad. 57—two very similar unnamed species of *Penicillium*, the history of which is given in the Appendix.

The carbon balance sheets for these species are given in Table XIII and the only points of outstanding interest in them are the following :—

(i) The three strains of *Scopulariopsis* give balance sheets which are all of the same type and indicate that these strains do not produce, in appreciable quantity, any product other than CO_2. In this connection the following quotation from p. 511 of Thom's book is of interest: "The species (*Scopulariopsis*) appear as agents of decomposition after the usual green Penicillia have ceased to be active; that is in the later stages of decay processes."

(ii) The carbon balance sheets of Ad. 3 and Ad. 57 are so similar in type that these two species are obviously closely related biochemically. Both species give rise to large amounts of "volatile neutral compounds," have high respiration coefficients and moderate amounts of "carbon unaccounted for."

Discussion of Results.

The carbon balance sheets submitted for a large number of different species of *Penicillium* show very wide variations in type, exactly as was noticed with species of *Aspergillus*. The species and their carbon balance sheets have been arranged in the same order as is followed in Thom's '*Penicillia*,' but the close agreement between

TABLE XIII.

	Glio-cladium roseum BAINIER.	*Scopulariopsis brevicaulis* (SACCARDO) BAINIER.			*Pœcilo-myces varioti* BAINIER.	*Penicillium* species unnamed.	
Catalogue number :	Ad. 38	Ad. 40	Ad. 70	Ad. 72	Ad. 44	Ad. 3	Ad. 57
Experiment number :	F 28	F 27	F 77	F 81	F 32	F 2	F 45
Incubation period in days :	63	49	60	63	63	39	59
Carbon Balance Sheet.							
Carbon in solution (start) ... ... gm.	4·944	4·944	4·952	4·952	4·944	5·043	4·944
Carbon in H_2SO_4 ... ... ,,	0·013	0·001	0·001	nil	0·022	0·014	0·028
,, in CO_2 ... ... ,,	2·109	2·099	1·913	1·824	2·557	2·012	1·954
,, in mycelium ... ... ,,	0·565	1·284	1·361	1·113	0·862	0·724	0·509
,, in solution (end)... ... ... ,,	2·160	1·522	1·595	1·879	1·395	2·088	2·335
,, accounted for ... ... ,,	4·847	4·906	4·870	4·816	4·836	4·838	4·826
,, accounted for ... ... per cent.	98·0	99·2	98·3	97·5	97·8	95·9	97·6
Analysis of Solution.							
Carbon in residual glucose ... ... gm.	0·628	1·054	1·022	1·490	0·014	0·064	0·346
,, in CO_2 in solution ... ... ,,	0·007	0·022	0·004	0·008	0·003	0·007	0·002
,, in volatile acids ... ... ,,	0·063	0·033	0·025	0·036	nil	0·006	0·005
,, in non-volatile acids ... ... ,,	0·186	0·122	0·118	0·113	0·198	0·253	0·195
,, in volatile neutral compounds ... ,,	0·893	0·007	0·004	nil	0·555	1·074	1·168
,, in synthetic compounds ... ,,	0·017	0·074	0·034	0·028	0·118	0·179	0·083
Total carbon accounted for ... ... ,,	1·794	1·312	1·207	1·675	0·888	1·583	1·799
,, ,, in solution ... ... ,,	2·160	1·522	1·595	1·879	1·395	2·088	2·335
Carbon unaccounted for (by difference) ,,	0·366	0·210	0·388	0·204	0·507	0·505	0·536
Residual Glucose.							
Glucose (by polarimeter) ... per cent.	0·336	0·527	0·521	0·678	0·050	0·101	0·257
,, (SHAFFER-HARTMANN) ,,	0·314	0·527	0·511	0·745	0·007	0·032	0·173
,, (WOOD-OST) ... ... ,,	0·310	0·533	0·464	0·742	—	—	—
,, (by alkaline iodine) ... ,,	0·393	0·571	0·595	0·793	0·083	0·090	0·223
Acids.		Decrease of	Decrease of				
Titration (N/1 acid) ... ... c.c.	1·9	0·2	0·3	0·2	2·6	1·9	1·0
Volatile acids (N/1 acid) ... ... ,,	1·62	2·25	1·46	1·46	0·15	nil	1·30
Barium salts (weight) ... ... gm.	0·314	0·158	0·103	0·185	0·062	0·047	0·075
Calcium salts (weight) ... ... ,,	0·723	0·541	0·410	0·511	0·850	0·855	0·778
Volume of oxygen absorbed ... ... c.c.	2753	2973	2712	2853	3651	2379	2189
Respiration coefficient	1·44	1·33	1·27	1·20	1·37	1·58	1·67
Mycelium (weight) ... ... gm.	1·056	2·040	2·190	1·845	1·562	1·469	1·034
,, (carbon) ... ... per cent.	53·5	62·9	62·2	60·3	55·2	49·3	49·3

the morphological classification and the biochemical classification that was found with species of *Aspergillus*, is not so apparent with species of *Penicillium*, although the majority of the groups of *Penicillium* do agree morphologically and biochemically. In some cases, however, wide divergences have been found where species grouped together morphologically give carbon balance sheets of entirely different types and are obviously not very closely related biochemically. In these cases it is possible that subsequent alteration in the morphological classification will bring our findings more into line with THOM's classification since he himself admits that some species of *Penicillium* are grouped together in his book on somewhat arbitrary lines. We have, indeed, found that certain strains of *Penicillium*, admittedly of the same species, may give rise to large amounts of alcohol, while other strains of the same species produce none of this metabolic product. An example of this is given in Group X, where of two strains of *P. lilacinum* THOM, obtained from different parts of the world, one strain gives 12 per cent. of alcohol and the other strain gives scarcely any. In other respects their carbon balance sheets are very similar. Another instance is given in Part III for different strains of *A. nidulans* EIDAM. We have at present no explanation to offer for what is undoubtedly a very definite biochemical fact, since it must be remembered that in all of these experiments identical cultural conditions were followed. On the other hand there are several instances in which different strains of the same species of *Penicillium*, obtained from different parts of the world, give carbon balance sheets which are almost indistinguishable from one another. Notable examples of these are to be found in Table II for five different strains of *P.* (*Citromyces*) *glabrum* WEHMER, and in Table XIII for three different strains of *Scopulariopsis brevicaulis* (SACC.) BAINIER.

It may be well to summarize the results given in this paper by dealing in turn with the outstanding items in the carbon balance sheets.

I. *Growth.*—It may be said that in general the majority of the species of *Penicillium* investigated grow reasonably well on the medium used and under the cultural conditions followed. An outstanding exception is *P. digitatum* SACCARDO, which only begins to show signs of germination on this medium after two to three weeks' incubation but, having once germinated, growth is ultimately quite good. This was found to be the case for three different strains of this species and confirms the observation, previously made by THOM, that *P. digitatum* can only use sodium nitrate as a source of nitrogen with considerable difficulty.

II. *Carbon in Sulphuric Acid Bubbler.*—The class of carbon compounds included under this heading consists of any very volatile organic substance which is absorbed by concentrated sulphuric acid. It is a well-known fact that many species of *Penicillium* under certain cultural conditions have very marked odours, but, under the cultural conditions followed in this paper, only one species of *Penicillium*, *P. digitatum*, gives rise to any marked amount of bodies of this type. The substance responsible has been identified as ethyl acetate and has been shown to be produced by each of the three

different strains of *P. digitatum* investigated. The results obtained are described in detail in Part XVIII.

III. *Carbon in Volatile Acids.*—Consideration of the figures obtained for all species for "carbon in volatile acids" enables one to make an important biochemical generalization. In no case did any species of *Penicillium* produce more than small yields of volatile acids and in many cases this type of body was entirely absent. In fact, the maximum yield from any species of *Penicillium* is that given by *P. lilacinum*, Ad. 32 = 0·049 gm. (Table VII), corresponding to a yield of approximately 1 per cent. A similar absence of the production of volatile acids has already been noticed with species of *Aspergillus* (Part III) and hence this biochemical characteristic of these two great genera of the lower fungi is in very marked contrast to the well-known biochemical characteristic of many of the bacteria of producing large yields of this class of bodies.

IV. *Carbon in Non-volatile Acids.*—All our results go to show that, as with the *Aspergilli* so with the *Penicillia*, the main acids formed are of the non-volatile type, giving calcium salts which are insoluble in 80 per cent. alcohol, the acids themselves being usually polybasic and often hydroxy-acids. The following outstanding examples may be noticed. All the species included in the *P.* (*Citromyces*) *Pfefferianum* series (Table I) produce considerable, and in some cases very large, amounts of citric acid, strains Ad. 74, Ad. 80 and Ad. 79 being particularly marked in this respect and giving yields at least as high as any species of *Aspergillus*. Certain other species, particularly those belonging to the *P. chrysogenum* series (Table V), give large yields of gluconic acid, an observation which has been reported in detail in Part XVII for *P. chrysogenum*, Ad. 11. Presumptive evidence of the production of gluconic acid by Ad. 56, Ad. 14, Ad. 35 and Ad. 53, belonging to the same series, is also furnished in Table V by the large differences between glucose as estimated by the polarimeter and by the Shaffer-Hartmann method.

V. *Carbon in Volatile Neutral Compounds and Respiration Coefficients.*—The results presented give perfectly definite evidence for the view previously expressed in Part III that even under conditions of restricted aeration many species of *Penicillium* and *Aspergillus* do not produce alcohol, since, while certain large groups, *e.g.*, the *P. terrestre* Jensen series given in Table VIII, give yields of alcohol approximating to 40 per cent. and have respiration coefficients as high as 2·26, other groups, *e.g.*, strains of the cheese moulds, *P. caseicolum* and *P. camemberti* (Table VI), all the strains of *P. chrysogenum* (Table V) and many others give yields of alcohol which never exceed 0·2–0·3 per cent. and have respiration coefficients approximating to unity.

VI. *Carbon Unaccounted for.*—Several species of *Penicillium* give large yields of "carbon unaccounted for." The group which is outstanding in this respect is Group V, *i.e.*, the *P. chrysogenum* series. Of six strains included in this group, five give yields of "carbon unaccounted for" varying between 20 and 30 per cent. of the glucose metabolized. The metabolic products of one of these strains, Ad. 11, are described in Part XVII. The *P. viridicatum* series given in Table X, together with *P. puberulum*,

Table V, and *P. Schneggii*, Table X, give yields approximating to 20 per cent., many other species giving somewhat smaller yields. Obviously any of these species would repay investigation from the point of view of obtaining good yields of some metabolic product which may or may not, of course, be new. On the other hand, there are groups, represented particularly by the cheese moulds, *P. caseicolum* and *P. camemberti* (Table VI), and strains of *Scopulariopsis* (Table XIII) which do not produce from glucose in appreciable amounts any metabolic product other than carbon dioxide and hence, from this point of view, may be disregarded for future work.

VII. *Carbon in Mycelium.*—The majority of the species of *Penicillium* examined have a carbon content in their mycelium of approximately 50 per cent. There are, however, some exceptions to this rule and it is interesting to note that these fall into certain groups. Thus, of two strains of *P. lilacinum* Thom examined (Table VII), the mycelium of one strain, Ad. 32, contains 59·5 per cent. of carbon, while that of the other strain, Ad. 37, contains 60·2 per cent.; and the three strains of *Scopulariopsis* (Table XIII) have carbon contents of 62·9 per cent., 62·2 per cent. and 60·3 per cent. respectively. These figures are quite definite and unmistakable, though their significance is somewhat obscure.

Summary.

A quantitative examination has been made, by the carbon balance sheet method described in Part II, of the types of products formed from glucose by a large number of different species of *Penicillium*. These carbon balance sheets are collected in a number of groups arranged according to the morphological classification of species of *Penicillium* followed in Thom's book 'The *Penicillia*.' Many of these groups have their own peculiar biochemical characteristics, though the agreement between the morphological classification and the biochemical classification is not so close as was found to be the case with species of *Aspergillus*.

By means of the carbon balance sheets a choice has been made of species suitable for intensive examination with a view to isolating and identifying their metabolic products. The genus *Penicillium* seems to be unusually rich in this type of organism.

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