STUDIES IN THE PHYSIOLOGY OF FUSARIA. THE RESPIRATORY AND FERMENTATIVE MECHANISMS

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INTRODUCTION

Interest in the physiology of the *Fusaria* centers mainly about their ability to produce alcohol and carbon dioxide as practically the only end products of metabolism from a variety of carbon compounds. Early work by Wollenweber (1913) and Sherbakoff (1915) dealt mainly with the morphological differentiation of the organisms. Biochemical studies of this large group of organisms have been carried out by White and Willaman (1928) and by Birkinshaw *et al.* (1931). In the latter work detailed carbon balance sheets of the metabolism of a number of species of the organisms on a *glucose* "synthetic" medium were carried out and it was concluded that the dissimilation of glucose by these organisms led almost exclusively to the production of ethyl alcohol and carbon dioxide. In some cases, a small amount of acid was also produced. The oxidative mechanisms of these organisms and the possible presence of phosphorylating mechanisms in carbohydrate dissimilation have been investigated by Nord (1939). The results of most of the experimentation point to a possible similarity between the alcohol and carbon dioxide-producing mechanisms of *Fusaria* and those found in yeasts. Nord (1939) is of the opinion, however, that the dissimilation of glucose by *Fusaria* need not go by way of phosphorylation. This would appear to offer a point of difference between the metabolism of yeast and *Fusaria*, since MacFarlane (1939) has shown that phosphorylation does occur in living yeast cells. No extensive study has, however, been made of the various mechanisms involved in the dissimilation of glucose by *Fusaria*, especially in comparison with analogous mechanisms in glucolysis by living yeast cells. The present investigation has been planned with a view to such a comparison.

In the present investigation a study has been made of the respiratory

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and fermentative mechanisms of resting cell preparations of *Fusaria*. The availability of a number of carbohydrates and of certain other common compounds for carbon dioxide production by *Fusaria* was investigated, and the presence of a glucose-dissimilating mechanism which is always present and therefore "constitutive" in the sense of Karström (1938) was established, as well as the presence of an "adaptive" mechanism for the dissimilation of galactose, in a sense, similar to galactozymase in yeast (Stephenson and Yudkin, 1936). The effects on *Fusaria* metabolism of selective poisons such as iodoacetate, fluoride, and cyanide have been investigated and striking similarities to the effects on yeasts established. The possibility of more than a single glucolyzing mechanism being involved was explored, as well as the question of the presence of a glycogenetic-like process which may precede glycolysis.

Methods

Description of the Organism

The organism selected for the study is catalogued as *Fusarium* sp. *H* in the M. I. T. pure culture collection. A study of its characteristics indicates that it is closely related to *F. tricothecoides*. It was selected because of the uniformity of growth and biochemical properties.

Uniform preparations of the *Fusarium*, "free" from metabolites, analogous to "resting cell" preparations of bacteria and yeast were prepared. The organism was grown on a modified Czapek-Dox medium of the following composition: glucose, 10 gm.; peptone, 2.5 gm.; $NaNO_3$, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4 . 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; distilled water, 1000 ml. Into 1 liter conical flasks were introduced 100 ml. portions of the medium and these were then sterilized by autoclaving at 15 pounds steam pressure for 20 minutes. Each of the flasks was inoculated with 1 ml. of a concentrated (four agar slant growths washed into 75 ml. of water) spore-mycelium suspension. Incubation was at 28°C. in the dark, unless otherwise noted. The period of incubation varied with the nature of the experiment. At the end of the desired incubation period, the growths were washed with distilled water on a filter cloth until free of extracellular metabolites, as indicated by the complete absence of glucose and ethyl alcohol in the washings. The washed mats were then pressed lightly to remove excess wash water and the moist tissue was introduced into a shaking bottle containing glass beads, and 15–25 ml. of M/20 KH_2PO_4 buffer at pH 4.85 for each mycelial mat used. The flasks were shaken vigorously until a uniform suspension was obtained as indicated by the ability to pipette easily with a 2.5 mm. bore pipette. Such suspensions gave unusually uniform results. Older growths (10 days) are more difficult to disperse unless they are minced finely before shaking. The dry weight of tissue per unit volume of suspension was determined for subsequent calculations.

Attempts were made to prepare cell-free preparations by grinding with sand in the cold with subsequent centrifugation; by freezing and grinding; and by pressing at very high pressures. In all cases, inactive preparations were obtained. The dried, washed tissue also shows no activity (CO_2 production) after re-suspension in a phosphate-glucose

solution. Boyland *et al.* (1937) showed that the reason for the inactivity of tumor extracts is to be found in the very rapid destruction of cozymase and adenylpyrophosphate which occurs on damage to the cells. Euler *et al.* (1936) found similar changes in acetone preparations of brain. A later communication will deal with the possible rôle of cozymase in *Fusaria* metabolism. In these studies, therefore, freshly prepared, washed suspensions of cells (as described above) were employed.

The respiratory and fermentative activities of the organisms were measured manometrically by means of Barcroft manometers in a water bath controlled to ± 0.02°C. Unless otherwise noted, experiments were carried out at 30°C. The manometers were shaken at 120 oscillations per minute. The cups and contents were adapted to the temperature of the bath for 10 minutes before the manometer taps were shut. Caustic was replaced frequently during long experiments where large volumes of CO_2 were evolved. By the direct, two manometer method (Dixon, 1934), the CO_2 evolved and the oxygen consumed could be estimated and the R.Q.; *i.e.*, CO_2 evolved/O_2 consumed could be determined. An atmosphere of air was used for the aerobic studies. For anaerobic experiments an atmosphere of nitrogen was used. From the dry weight of organism used the $Q_{O_2}^{air}$; *i.e.*, c.mm. of O_2 consumed per hour per milligram of dry weight was calculated. In the same way $Q_{CO_2}^{air}$ and $Q_{CO_2}^{N_2}$ were calculated.

EXPERIMENTAL AND DISCUSSION

Effect of Age of Growth on Endogenous (Respiratory) and Exogenous (Fermentative) Activity of Fusarium sp. H.

A number of flasks of the usual medium were inoculated with the concentrated spore-mycelium suspension and incubated at 28°C. in the dark. At desired intervals suitable numbers of flasks to give sufficient preparation were removed and a suspension was prepared for immediate use as described. When the preparation was not in use during an experiment, it was stored at 0°C. No preparations were stored for more than 8 hours. The experiment was carried out over a period of 7 days and estimations were made of hourly R.Q., $Q_{O_2}^{air}$, and $Q_{CO_2}^{air}$. In order to correlate the production of alcohol with the respiratory and fermentative activity of the organism, analyses of the alcohol content of the metabolism solutions on which the organism had been grown were carried out by the method of Friedemann and Klaas (1936). The results of the experiment are given in Table I.

From the results it is apparent (1) that in very young mats the $Q_{O_2}^{air}$ is extremely high, and with increase in the age of the growth the $Q_{O_2}^{air}$ falls markedly. (2) In the presence of added glucose, the $Q_{CO_2}^{air}$ and the R.Q. of young mats show an immediate marked increase which seems to indicate an immediate attack of the added glucose. With increase in age the response to added glucose is delayed, but definite after incubation with glucose for a short period. The incubation period is increased as the age of the mat increases. (3) In the course of the endogenous metabolism of very young

mats, the $Q_{O_2}^{air}$ and the $Q_{CO_2}^{air}$ fall markedly as the experiment progresses, so that after 4 hours, the $Q_{O_2}^{air}$ has fallen from 39.5 for the 1st hour to 13.1 for the 4th hour. The $Q_{CO_2}^{air}$ falls from 31.9 to 11.0. The exogenous metab-

TABLE I

Metabolic Activity of Resting Cell Suspensions of Fusarium sp. H. Prepared from Cultures of Varying Age

Time (hrs.)	Without added glucose			With added glucose		
	$Q_{O_2}^{air}$	$Q_{CO_2}^{air}$	R.Q.	$Q_{O_2}^{air}$	$Q_{CO_2}^{air}$	R.Q.
1 day cultures (alcohol content of growth medium 0.08 mg./ml.)						
1	39.5	30.7	0.78	34.4	63.7	1.85
2	30.3	25.7	0.84	38.2	56.3	1.49
3	19.3	15.7	0.81	38.9	55.1	1.42
4	13.1	11.0	0.84	36.1	56.0	1.55
2 day cultures (alcohol content of growth medium 0.55 mg./ml.)						
1	16.1	16.1	1.00	14.7	21.7	1.48
2	15.2	12.1	0.80	13.6	23.0	1.69
3	14.0	12.0	0.86	11.3	22.7	2.05
4	9.9	9.0	0.90	12.3	26.0	2.14
5	8.2	6.9	0.85	11.3	26.0	2.30
3 day cultures (alcohol content of growth medium 0.62 mg./ml.)						
1	13.9	14.0	1.01	14.1	19.2	1.36
2	15.5	14.1	0.91	15.0	22.6	1.50
3	14.3	12.7	0.89	14.3	26.7	1.85
4	12.9	11.9	0.92	13.2	26.1	1.97
5 day cultures (alcohol content of growth medium 0.80 mg./ml.)						
1	7.3	6.7	0.91	6.1	6.1	1.00
2	7.0	5.7	0.81	6.0	7.0	1.17
3	7.0	5.5	0.78	6.8	9.7	1.43
4	6.1	4.7	0.77	5.8	10.2	1.78
7 day cultures (alcohol content of growth medium 0.64 mg./ml.)						
1	6.5	6.0	0.93	5.9	6.2	1.05
2	6.2	5.3	0.86	5.7	6.5	1.14
3	6.2	5.3	0.87	5.7	7.4	1.30

olism (added glucose), however, shows no such decrease, remaining fairly constant throughout the duration of the experiment. This is analogous to the observations on young yeast cultures by Stier and Stannard (1936) where the progressive fall of endogenous activity is attributed to the progressive utilization of "endogenous substrate" and that the dissimilation of

carbohydrate stores is a respiratory process. (4) During the 1st day alcohol production is very low, which would indicate that endogenous metabolism is predominant, since analyses show that alcohol is not produced in endogenous metabolism but is produced during exogenous metabolism (Table II). This is also the case in yeast metabolism. After the first day there is a sudden marked increase in alcohol production which seems to be correlated with the stabilization of the endogenous metabolism, since the $Q_{O_2}^{air}$ (endogenous) during the course of an experiment now remains relatively constant. Again the analogy to the mechanism in living yeast is very pronounced.

TABLE II

Ethyl alcohol production in "nutrient" and "non-nutrient" media by *Fusarium* sp. *H.* resting cell suspensions which correspond to the exogenous and endogenous metabolism, respectively.

Suspension prepared from	Suspended in	Time of incubation and shaking	Alcohol production*
		hrs.	*mg./ml.*
3 day mats	Dextrose-phosphate	6	0.23
3 " "	Phosphate	6	0.00
4 " "	Dextrose-phosphate	5	0.17
4 " "	Phosphate	5	0.00

* Ethyl alcohol as analyzed by the method of Friedemann and Klaas (1936). Experiments were carried out at room temperature and alcohol determinations were made in triplicate on the suspension filtrates.

The Induction Period

It is apparent that for the study of growths older than 1 day an incubation period with glucose or any other substrate to be studied is necessary to make apparent any excess (exogenous) carbon dioxide production. Similar precautions must be taken with yeast where it is believed that either a reserve material must be built up (Willstätter and Rohdewald, 1937) or, the organism must be brought to a certain anabolic phase or level (Stier and Stannard, 1936). The occurrence of an induction period in the fermentation of glucose by living yeast has been observed by Willstätter and Rohdewald (1937) who found a marked increase in glycogen during this period and suggested that glucose is first transformed to glycogen before fermentation. Goda (1938) also observed the formation of glycogen in young yeast but observed that in old yeast there was a rapid fermentation of added glucose without a parallel formation of glycogen.

From the data in a typical experiment (Table III) it is evident that no appreciable *fermentable* reserve material is built up. It might be suggested that during the period of incubation the glucose is fragmented to smaller,

more available intermediates which are in solution and which then give rise to carbon dioxide. That this is not the case is evident from experiments where suspensions were incubated with glucose until the R.Q. was well above the endogenous R.Q., then thoroughly washed until free of any metabolites contained in the medium, and re-suspended in a fresh glucose-phosphate buffer solution. The R.Q. obtained after re-suspension was approximately equal to the R.Q. before washing free from the medium. When incubation is carried out in the presence of iodoacetate, CO_2 evolution and O_2 uptake are markedly inhibited due to long exposure to the poison.

TABLE III

Experiments Indicating the Direct Attack of Glucose by Fusarium sp. H.

	Experiment (1)	Experiment (2)
1. R.Q. without added substrate immediately after preparation of suspension	0.87	1.08
2. R.Q. with added glucose (0.07 M) immediately after preparation of suspension	0.92	1.06
3. R.Q. after shaking with glucose (0.07 M) for 3 hrs.	1.88	1.66
4. R.Q. after shaking with glucose (0.07 M) for 3 hrs. and then washing from glucose	1.02	—
5. R.Q. after shaking with glucose (0.07 M) for 3 hrs., washing away the metabolism solution, and replacing with glucose (0.07 M)	1.67	—
6. R.Q. after shaking with glucose (0.07 M) and 0.004 M iodoacetate for 3 hrs.	—	1.11
7. R.Q. after shaking with glucose (0.07 M) and 0.004 M iodoacetate for 3 hrs., then washing, and suspending in glucose-free buffer	—	1.00
8. R.Q. after treating as (7) except suspended in glucose after washing	—	1.03

The fermentative system is, however, completely inhibited. From the data in experiment 1 in Table III, it can be seen that there is a slight rise in R.Q. from 0.87 for the endogenous rate to a new endogenous value of R.Q. = 1.02 after shaking with glucose and then washing away the suspending medium. This might be accounted for by the presence of either a small amount of intracellular glucose or by other intracellular intermediate products of the breakdown of glucose. It is apparent therefore that no unlimited accumulation of intermediate products occurs but that there may be a constant and perhaps extremely low working level of such substances. That the excess carbon dioxide does not arise from a reserve material seems quite evident.

It has been found that under certain conditions of growth, *i.e.* when the flasks were sown with dilute spore-mycelium suspensions and incubated in

the light at 22–24°C. rather different growths were obtained which were characterized by the fact that the suspensions of old organisms (10–11 days) could attack glucose with formation of CO_2 directly (without an incubation period). Direct attack of added glucose by this type of growth as compared to the delayed attack by the usual growths must be due to differences in mat permeability rather than the necessity for building up cellular glycogen-like substances since in the absence of glucose the usual low endogenous R.Q. is obtained.

The Nature of the Endogenous and Exogenous Metabolism

The endogenous and exogenous mechanisms in *Fusaria* metabolism were investigated and shown to be similar to the mechanisms in yeast. These methods of study were employed: (1) carbon dioxide evolved aerobically and anaerobically with and without glucose, and (2) the effects of poisons such as sodium iodoacetate, potassium fluoride, and cyanide on the exogenous and endogenous mechanisms.

(*a*) *Aerobic and Anaerobic CO_2 Production*

From the results shown in Table IV, it is apparent that in the absence of substrate there is no significant anaerobic production of CO_2. However, with added glucose there is definite anaerobic CO_2 production which is equal to about 70 per cent of the added CO_2 produced aerobically in the presence of added glucose. This would indicate that the mechanisms are different, and also that a part of the increase in CO_2 production due to added glucose may involve an aerobic mechanism.

Experiments were carried out in the usual manner and varying concentrations of inhibitor added in M/20 phosphate to give the desired final concentration. In some instances the concentrations were increased as the experiments progressed. The results are indicated in Table V. It is apparent that both iodoacetate and fluoride in relatively low concentrations affect the exogenous metabolism without affecting the endogenous metabolism. Higher concentrations do affect the endogenous metabolism without disturbing the R.Q. Similar observations have been made on yeast cells by Stier and Stannard (1936). With certain concentrations of iodoacetate, the exogenous metabolism has been completely inhibited with no simultaneous effect on the endogenous metabolism. A series of experiments with fluoride and iodoacetate on the anaerobic CO_2 production shows a very marked decrease on the addition of these specific inhibitors (Table IV). Cyanide, on the other hand, markedly affects the respiratory mechanism but only slightly affected fermentation.

From these experiments it can be concluded that the exogenous and endogenous CO_2 producing-mechanisms are distinct, and in general, the situation in *Fusaria* is analogous to that in yeast.

TABLE IV

The Nature of the Anaerobic Carbon Dioxide-Producing Mechanism of Resting Cell Suspensions of Fusarium sp. H

1. Anaerobic production of CO_2 with and without dextrose

Experiment	Medium	CO_2 evolved c. mm./hr. (N_2 atmosphere)	$Q_{CO_2}^{N_2}$	$Q_{CO_2}^{air}$
1	Phosphate	15	Negligible	34.2
	Dextrose-phosphate	257	27.8	63.7
2	Phosphate	0	0.0	30.7
	Dextrose-phosphate	224	22.0	54.1

2. Effect of iodoacetate on the anaerobic (nitrogen atmosphere) production of CO_2

Molarity of IAA	c. mm. CO_2 evolved/hr.	Per cent inhibition
Control	224	—
0.0040	67	70
0.0065	26	88

3. Effect of fluoride on the anaerobic (nitrogen atmosphere) production of CO_2

Molarity of KF	c. mm. CO_2 evolved/hr.	Per cent inhibition
Control	265	—
0.0040	133	50
0.0400	31	87

Fermentation of Various Carbon Sources by Fusarium sp. H.

A study has been made to determine the ability of the organism to attack a variety of common carbon sources with the production of exogenous CO_2, (1) when grown on glucose and suspended in a medium containing a carbon source to be studied or, (2) when grown on a carbon source other than glucose and suspended in a medium containing that carbon compound or another. The organisms were grown in the usual manner on 1 per cent solutions of glucose or another carbon source, and suspensions were prepared as described previously. The results of the experiments are summarized in Table VI. It is evident that (1) the glucose-dissimilating mechanism is residual in all growths, no matter what the source of carbon for growth has been, and may be called constitutive. When grown on non-hexose sources of carbon, the residual glucose-dissimilating power is very small; but when grown on any of the hexoses investigated, the glucose-

TABLE V

*Effect of Cyanide on the Respiratory Activity**

O_2 uptake			CO_2 evolution		
Inhibitor	c. mm./hr.	Inhibition	c. mm./hr.	Inhibition	R.Q.
		per cent		*per cent*	
Control	247	—	270	—	1.09
0.00025 M KCN	217	16.4	251	7	1.16
0.0005	191	22.7	242	10.4	1.27
0.0020	156	36.9	216	19.6	1.38
0.0040	137	44.5	167	38.1	1.22
0.0080	123	50.0	153	43.3	1.24
0.0120	60	75.8	77	71.4	1.28
Effect of cyanide on the fermentative activity†					
Control	165	—	412	—	2.49
0.00025 M KCN	131	20.6	440	Accel.	3.38
0.0005	88	46.7	394	4.4	4.47
0.0010	90	46.5	369	10.4	4.10
0.0020	94	43.1	301	26.9	3.20
0.0040	73	55.7	235	43.0	3.22
Effect of potassium fluoride on the respiratory activity					
Control	148	—	136	—	0.93
0.0012 M KF	148	0	140	0	0.95
0.0018	148	0	138	0	0.94
0.0024	148	0	133	0	0.90
0.0048	158	0	140	0	0.89
0.0056	148	0	132	0	0.89
0.0090	127	14.2	117	14.0	0.92
0.0180	111	25.0	116	14.0	1.04
Effect of fluoride on the fermentative activity					
Control	284	—	625	—	2.20
0.0012 M KF	290	0	502	19.7	1.73
0.0024	288	0	400	36.0	1.39
0.0036	255	10.2	372	40.5	1.46
0.0048	245	13.7	333	46.7	1.36
Effect of sodium iodoacetate on the respiratory activity					
Control	195	—	190	—	0.97
0.0010 M IAA	190	0	192	0	1.01
0.0020	187	0	187	0	1.00
0.0030	197	0	190	0	0.96
0.0040	189	0	188	0	1.00
0.0060	167	14.3	160	15.8	0.96
0.0070	144	26.2	137	27.9	0.95
Effect of iodoacetate on the fermentative activity					
Control	180	—	324	—	1.80
0.0010 M IAA	182	0	260	19.8	1.43
0.0020	187	0	214	34.0	1.15
0.0040	179	0	171	53.0	0.95

* No added glucose.

† 1 per cent glucose.

dissimilating power is equal to that of the growths when grown on glucose itself. This is analogous to the observations on bacteria by Hegarty (1939) and Karström (1938). (2) Growths on glucose will attack glucose, mannose, fructose, but not galactose. Fructose is always attacked least vig-

TABLE VI

Composite Table of R.Q. of Fusarium sp. H. on Various Substrates

Tested on	Grown on							
	Glucose*	Glucose	Galactose*	Galactose + glucose	*l*-Xylose	*l*-Arabinose	Glycerol	Lactate
No substrate	0.95	0.88	1.58	1.20	1.00	0.87	0.92	0.91
Glucose	1.75	1.84	2.52	2.30	1.20	1.17	1.24	1.30
α—Glucose	1.60	—	—	2.25	—	—	—	—
Mannose	1.71	1.67	2.39	2.20	—	—	—	—
Galactose	0.95	0.90	2.33	2.00	0.97	0.88	0.90	0.88
Fructose	1.33	1.37	1.67	1.41	1.02	0.96	0.93	0.95
Xylose	0.95	0.87	—	1.16	0.98	0.88	0.89	0.92
Arabinose	0.93	0.89	—	—	0.89	0.89	0.88	0.96
Lactose	0.83	0.89	1.64	1.26	—	—	—	—
Sucrose	1.14	1.09	1.60	—	—	—	—	—
Maltose	0.93	0.89	—	1.19	—	—	—	—
Mannitol	0.89	0.90	—	—	—	—	—	—
Glucose+mannose	1.70	—	—	2.24	—	—	—	—
Glucose+galactose	1.75	1.80	2.55	2.30	—	—	—	—
Glycerol	0.61	0.59	—	—	0.50	0.21	0.64	0.62
α—glycerol PO_4	0.51	0.61	—	0.53	—	—	—	—
Hexose diphosphate	—	0.95	—	—	0.93	0.90	—	—
Pyruvate	1.35	1.44	1.88	—	1.38	1.43	1.31	1.47
Pyruvate+glucose	2.08	2.17	2.80	—	—	—	—	—
Succinate	0.96	0.89	1.61	—	1.00	0.92	0.87	0.88
Lactate	0.84	0.78	—	—	0.68	0.72	0.77	0.68
Acetate	0.96	0.86	—	—	—	—	0.89	0.89
Ethyl alcohol	0.87	0.84	—	—	—	—	—	—
Salacin	0.94	0.87	—	—	—	—	—	—
Citrate	0.96	0.92	—	—	—	—	—	—
Phosphoglyceric acid	—	0.89	—	—	—	—	—	—

* Cultures attacked glucose directly.

orously. When grown on galactose, the organism can attack the usual carbohydrates and galactose. Glucose, however, always seems to be more vigorously attacked than galactose. Similar studies on galactose adaptation have been made with yeast by Stephenson and Yudkin (1936) and on bacteria by Stephenson (1939). It is also interesting to note that when the organism is grown on galactose or a mixture of galactose-glucose the endogenous R.Q. is considerably higher. This is consistent with the concept that certain sugars are "growth" sugars rather than "fermentable" sugars.

Of the common intermediary metabolites investigated only pyruvate is attacked to give added CO_2 evolution. This is due to the presence of a carboxylase system, which will not be discussed in this communication, and suggests that pyruvic acid is perhaps an intermediate in the dissimilation of glucose by *Fusaria*. It would be expected that pyruvic acid would be attacked at least as vigorously as glucose, but that this does not appear to be the case, from the R.Q. values for 1 hour, may be due to the toxic effect of accumulated acetaldehyde since in the first few minutes the rate of CO_2 evolution from pyruvic acid is extremely high and falls markedly as the reaction progresses. It is also apparent that when the organisms are grown on non-hexose sources of carbon they could not attack any substrate but the hexoses or pyruvic acid with direct evolution of CO_2 under these experimental conditions.

The Nature of the Hexose-Dissimilating Mechanisms

The identity of the mechanisms involved in the direct attack of glucose, mannose, and galactose has been confirmed by addition experiments where the dissimilation of the substrates was investigated separately and then when present together. No additive effects were observed as is indicated from the data in Table VI. It would appear, therefore, that a single dissimilating mechanism is involved for these substrates and that the mechanism is adaptive for galactose dissimilation.

SUMMARY

1. *Fusarium tricothecoides* was selected for a study of the respiratory and fermentative activities of *Fusaria*. "Resting cell" suspensions were investigated by the Barcroft manometric technique.

2. The results of the investigation indicate clearly that the mechanism of endogenous metabolism (respiration) is distinct from the exogenous mechanism (fermentation). Anaerobically no significant CO_2 production is apparent without added substrate. In the presence of glucose the anaerobic CO_2 evolution is practically equal to the added CO_2 evolved aerobically in the presence of added glucose.

Low concentrations of iodoacetate or fluoride selectively poison the exogenous mechanism but do not affect the endogenous mechanism.

Alcohol is not produced in the course of endogenous metabolism, but is produced in the presence of added glucose.

3. A study of the metabolism of the organism throughout its entire growth phase from 1 to 7 days has been made.

4. The ability of suspensions of *Fusarium* sp. *H.*, obtained by growth on a variety of common substrates, to attack a large number of carbon

sources with the production of exogenous CO_2 was determined. It is found that organisms grown on glucose will attack only glucose, mannose, and fructose, but none of the common intermediary metabolites except pyruvic acid. Organisms grown on galactose attack galactose, as well as the other hexoses, indicating an adaptive mechanism.

5. An identical mechanism for the dissimilation of glucose, mannose, and galactose is indicated since no additive effects with these substrates were observed. Growths on non-hexose carbon sources attack glucose slightly under the experimental conditions with the evolution of CO_2, but do not attack any other substrate. This would indicate a residual glucose-dissimilating mechanism in all growths investigated.

6. Striking similarities between the general metabolism of resting suspensions of *Fusarium* sp. *H.* and resting suspensions of yeast cells are apparent.

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