This experiment presented an excellent chance for photorespiration to be exhibited if it existed. However, it was not observed. Also, since the rates of respiration were approximately the same in the light and in the dark, it is extremely unlikely that light inhibits respiration. It seems certain, therefore, that light does not have a direct effect on respiration.

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Enzymic Conversion of Maltose into Unfermentable Carbohydrate

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Pigman (*6*) and Stark (*9*) reported that amylase preparations from molds, pancreas, and saliva are capable of synthesizing unfermentable carbohydrate or carbohydrates from maltose. However, the work of these authors is incomplete, since they did not separate or characterize the synthesized product.

While studying the enzymic hydrolysis of starch in distillery corn mash, by the use of submerged fungal cultures, we found that the secondary conversion of dextrins[1] into fermentable sugars was markedly inhibited by maltose.[2] The question was therefore raised as to whether the observed reduction in the rate of dextrin hydrolysis was actually a result of maltose inhibition or of the conversion of maltose into unfermentable carbohydrate. Conclusive data have since been obtained to show that such an enzymic conversion actually occurs.

The submerged fungal culture was prepared with *Aspergillus niger* NRRL 337 in a distillers' dried solubles-corn meal medium (*1*). One volume of the culture filtrate was mixed with two volumes of a 2.2% maltose[3] solution in 0.3 M acetate buffer (pH 4.4) and incubated at 30° C. Samples were taken periodically and analyzed for glucose, maltose, and unfermentable carbohydrate (dextrins) according to the tripartite method[4] of Stark and Somogyi (*10*). The data given in Fig. 1 show that, while a part of the maltose was hydrolyzed to glucose through maltase activity, another part was simultaneously converted into unfermentable carbohydrate. The presence of unfermentable material at zero hour was ascribed to dextrins contained in the fungal culture and in the maltose. During the reaction period the amount of unfermentable substance increased considerably, reaching a maximum at 8 hr, when 17.6% of the original maltose had been converted to unfermentable carbohydrate. A decrease in unfermentable material was observed after the maltose had been completely utilized (10–12 hr). The amount remaining accounted for only 10.2% of the maltose at the end of 24 hr. It is therefore concluded that the fungal culture must contain some enzyme or enzymes which catalyze the conversion of maltose into unfermentable carbohydrate. In the absence of maltose, this carbohydrate can be converted into glucose.

A similar experiment, in which glucose was used in place of maltose, showed no formation of unfermentable substance; the amount of glucose remained unchanged during the 24-hr reaction period. Apparently the enzyme or enzymes were inactive toward glucose.

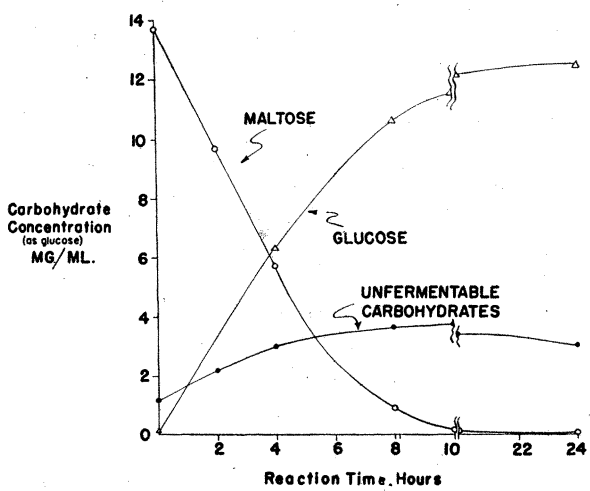

FIG. 1. Action of fungal enzymes upon maltose.

As a further confirmation of the formation of unfermentable substances, a similar experiment was conducted using a 10% maltose solution. Instead of using buffer, the filtrate of fungal culture was adjusted to pH 4.4 before mixing with the maltose solution. Ammonium sulfate, at a concentration of 1.5 g/l, was added as nutrient for subsequent yeast fermentation. After the fungal enzymes had been in contact with the maltose for 24 hr at 30° C, the mixture was sterilized and inoculated with an actively growing culture of distiller's yeast which had been grown in a yeast extract-glucose medium. At the end of fermentation (3 days), the medium was found to contain 40.7 mg of unfermentable carbohydrate per ml, equivalent to 41.5% of the maltose used (Table 1). The same table shows that the reaction mixture, which was sterilized immediately after mixing the maltose solution and the filtrate of the fungal culture, con-

[1] Like barley malt, the fungal culture enzymes hydrolyze starch primarily into fermentable sugars and dextrins.

[2] A report including this finding has been prepared for publication.

[3] Maltose (cp Fisher) recrystallized in the laboratory.

[4] Carbohydrate contents of samples either before or after treatment with baker's yeast were all determined as glucose by Somogyi's method (*8*) after complete acid hydrolysis in 0.69 *N* HCl solution for 2.5 hr in boiling water.

tained no such product; neither did one in which glucose was used in place of maltose.

TABLE 1

ENZYMIC CONVERSION OF MALTOSE TO UNFERMENTABLE CARBOHYDRATES

Glucose used in reaction mixture mg/ml	Maltose used in reaction mixture mg/ml	Reaction period total hr	Unfermentable carbohydrates after yeast fermentation mg/ml
97.0	...	0	2.72
97.0	...	24	3.04
...	98.1	0	3.38
...	98.1	24	40.7

In order to obtain data on the nature of the unfermentable carbohydrate, it was separated from the fermented medium by the following procedure. Extraneous material was removed from the fermented medium by precipitation with barium hydroxide, followed by ammonium carbonate. The filtrate was evaporated to dryness on a steam bath, and the residue taken up in a small amount of water. The addition of methanol resulted in a precipitate which had a carbohydrate content equivalent to 5% of the total carbohydrate in the original fermented medium. Acetone, added to the methanol solution, produced a brown, syrupy precipitate. This precipitate, after washing with acetone and drying under vacuum, analyzed 88.5% carbohydrate (as glucose, after acid hydrolysis), equivalent to 74.8% of the unfermentable carbohydrates in the original fermented medium. This was considered the crude product of the unfermentable carbohydrate produced enzymatically from maltose.

TABLE 2

REDUCING POWERS OF THE UNFERMENTABLE CARBOHYDRATES

Samples	Glucose content* after acid hydrolysis (A)	Glucose content calculated from direct reducing power: Toward Cu-tartrate-phosphate reagent (B)	Glucose content calculated from direct reducing power: Toward NaIO† (C)	Ratio of direct reducing power to total reducing power after acid hydrolysis: (B) : (A)	Ratio of direct reducing power to total reducing power after acid hydrolysis: (C) : (A)
	%	%	%		
Original fermented medium	4.07	1.71	...	1 : 2.38	.....
Crude product	88.5	35.4	36.3	1 : 2.49	1 : 2.43
Purified product	92.8	38.9	33.5	1 : 2.38	1 : 2.76
Maltose monohydrate	99.5	55.7	48.7	1 : 1.78	1 : 2.04

* Determined from reducing power toward Somogyi's Cu-tartrate-phosphate reagent.

† By the method of Caldwell *et al.* (2), which was found to be less sensitive toward impurities contained in the fungal culture than the original method of Willstätter and Schudel (Green [3]).

The crude product was purified further after dissolving in water to make a 4% solution. This solution was alternately treated with ion-exchange resins Amberlite IR-100 and IR-4B, and the resulting solution was then completely decolorized with Darco activated carbon. The solution was then evaporated to a thick syrup. Traces of insoluble solids were removed from the syrup by centrifugation. The addition of five volumes of methanol to the syrup produced a small quantity of precipitate with a carbohydrate content equivalent to 4% of the total amount present. The methanol solution was poured into ten times that volume of acetone, and a white, curdy precipitate was obtained. This precipitate was filtered with suction, dried overnight at 56° C under 28–29 in. Hg vacuum, and kept in a desiccator. The dried product was highly hygroscopic and analyzed 92.8% carbohydrate. A total of 4.5 g of this product was obtained from 9.5 g of the crude product.

TABLE 3

RECONVERSION OF UNFERMENTABLE CARBOHYDRATES TO FERMENTABLE SUGARS BY FUNGAL ENZYMES

Carbohydrate substrate	Yeast inoculum added	Fungal culture added % (by vol)	Initial carbohydrates mg/ml	Unfermentable carbohydrates after 3-day incubation mg/ml
Crude product	+	10	20.5	4.0
Crude product	+	0	22.0	21.4
Purified product	+	10	10.2	3.2
Purified product	+	0	10.2	10.1

Reducing powers (calculated as glucose), determined before and after acid hydrolysis of the unfermentable carbohydrate, are listed in Table 2. For comparison, the reducing powers of the recrystallized maltose monohydrate are also given. The last two columns give the ratios of these reducing powers—an indication of the number of glucose units per molecule or chain length (*4*). The values in these columns show that the unfermentable carbohydrate must have an average molecular size somewhat greater than maltose, but less than three glucose units per molecule.

Fermentability of the crude as well as the purified product was tested by inoculating media containing these two products (plus 1.5% yeast extract) with distiller's yeast. Little or no yeast growth and no diminution of the carbohydrate content were observed after 3 days' incubation at 30° C. When 10% (by volume) of submerged fungal culture and yeast was added to these media, fermentation (gas evolution) took place and the carbohydrate disappeared. These data, which are summarized in Table 3, show that the product can be recon-

verted into fermentable sugars if they are continuously removed by yeast fermentation.

The data reported here confirm the report by Pigman (*6*) that a mold enzyme or enzymes can convert maltose to some unfermentable carbohydrate or carbohydrates. The possible occurrence of such enzyme or enzymes in amylolytic preparations undoubtedly explains the apparent inhibition of amylase activity by maltose as reported by Schwimmer (*7*), and the reversibility of enzymic hydrolysis of dextrins as observed by Stark (*9*). The small molecular size of this unfermentable carbohydrate suggests that it might consist partly or entirely of isomaltose (6-[α-D-glucopyranosyl]-D-glucose), which has been isolated by Montgomery *et al.* (*5*) as one of the end products of prolonged starch hydrolysis by taka-amylase, although these authors obtained no isomaltose from maltose. Studies to elucidate the chemical nature of the product, as well as details of the enzymic reaction, are in progress.

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Activation of Arginase *in Vitro* by Mouse Carcass Extract and the Cobalt Ion

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While pursuing another phase of work on the enzyme arginase, it was observed that there might be a sex-linked difference in tissue activity in addition to general tissue variables observed by Greenstein (*1*). Further, it appeared possible that a sex-linked difference could be due to the presence in tissue of an activator other than cobalt.

In this study, the arginase activity was determined according to the method of Mohamed and Greenberg (*3*), using cobalt as metal activator, at a temperature of 40° C, for a period of 30 min. All values are the result of triplicate determinations. As seen in Table 1, the average value for 9 standard preparations of young female bovine livers, with cobalt as the activator, amounts to 377 arginase units; the average of 6 young castrated male bovine livers (cobalt-activated) amounts to 242 arginase units.

TABLE 1

Type of enzyme preparation	Type of activator	Avg arginase units* (*1*) (±2)	Percentage original units†
Young ♀ bovine liver	Co^{++}	377	100
	♂ extract	193	51
	♀ extract	168	45
	none	35	9
Young castrated ♂ bovine liver	Co^{++}	242	100
	♂ exract	176	73
	♀ extract	113	47
	none	35	14

* Per unit of standard preparation. Nine ♀ bovine livers and 6 ♂ bovine livers were used to obtain these averages.
† Ratios for both enzyme preparations with respect to each type of activator.

It appeared possible that the difference noted might be sex-linked. To test this possibility, arginase activity was determined on each of the skinned and eviscerated carcasses of 20 female and 20 male Swiss strain mice. Approximately 1 g of each mouse carcass was ground in a Waring Blendor with 2.5 ml of pH 7.0 phosphate buffer. In Table 2 there is observed approximately a fourfold difference between male and female mouse carcasses in arginase activity when cobalt is used as an activator. In the absence of cobalt there is practically no activity and no difference between male and female mouse carcasses.

In Table 1 it can be seen that a mixture of mouse carcass extract (male or female) and bovine liver arginase, in the absence of added cobalt, shows a value many times higher than either tissue preparation alone. A substance or substances in one of the tissue preparations (liver or carcass) serve as an activator for the enzyme in the other preparation. Since the standard liver preparations represent a partially purified solution

TABLE 2

Type of enzyme preparation	Type of activator	Avg* arginase units per gram of mouse carcass (±2)
♂ Mouse extract	Co^{++}	330
♀ Mouse extract	Co^{++}	90
♂ Mouse extract	none	11
♀ Mouse extract	none	12

* Average computed from determinations made on 20 ♂ and 20 ♀ Swiss-strain mice.

of the enzyme, it seems reasonable, for the present, to attribute the role of activator to the unpurified carcass preparations. Why such a carcass activator does not activate the carcass arginase cannot be stated at this time.

It seems probable that there is present in tissue an activator (or deactivator of an inhibitor) of arginase that may control the rate or degree of activation *in vivo*. Perhaps such an activator may play a role in the control