JOURNAL OF BACTERIOLOGY, Dec. 1971, p. 1310-1317.

Vol. 108, No. 3
Printed in U.S.A.

Effects of Mutations and Growth Conditions on Lipid Synthesis in *Neurospora crassa*

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Received for publication 27 August 1971

A morphological mutant (*col-2*) of *Neurospora*, which is partially deficient in glucose-6-phosphate dehydrogenase (G-6-PD) activity and has lower levels of reduced nicotinamide adenine dinucleotide phosphate (NADPH), accumulated threefold more triglycerides during log-phase growth than the wild-type strain. Increased lipid deposition was not found in other strains that included slow-growing morphological mutants, NADPH-deficient strains, G-6-PD-deficient mutants, wild-type revertants from *col-2*, and a *cel, col-2* double mutant. The *cel, col-2* strain was supplemented with an exogenous source of fatty acids because it cannot synthesize these lipid moieties. The observed normal lipid content of this strain suggests that the lipid deposition in *col-2* on glucose is due to an overstimulation of fatty acid synthesis and not a deficiency in fatty acid breakdown. The neutral lipid levels in both wild type and *col-2* were decreased to identical levels when grown on glutamate as a carbon source. This effect was not due to changes in glutamic dehydrogenase levels. The omission of citrate from the glutamate medium reduced wild-type neutral lipid levels even further, but had no effect on *col-2*. The variations with time in the neutral lipid levels of *col-2* upon changes in these carbon sources are presented, as well as a discussion of the possible types of regulatory effects unique to the *col-2* mutation which might affect fatty acid synthesis.

Previous studies have shown that the genetic lesion in a morphological mutant of *Neurospora crassa* (*col-2*) produced an altered glucose-6-phosphate dehydrogenase (G-6-PD) (4). Subsequent work showed that this organism had a decreased level of total nicotinamide adenine dinucleotide phosphate (NADPH) (2) and a much lower content of linolenic acid (5) as compared to the wild-type strain. The present work is based on the observation that the *col-2* mutant strain shows a threefold increase above the wild type in the neutral lipid content when grown on glucose as a major carbon source (5). This is an unexpected finding because a decrease in fatty acid synthesis might be expected in an NADPH-deficient strain. The present study is concerned with effects of growth conditions and mutations on the concentration and composition of fatty acids in the phospholipid and neutral lipid fractions of *Neurospora*.

MATERIALS AND METHODS

Neurospora strains and growth conditions. RL3-8A (wild type), *col-2*, *bal*, and *frost* have been described previously (2). The *cel* mutant (FGSC 165) was supplemented with 0.1% Tween 40 (Pierce Biochemicals) because it requires saturated fatty acids for growth (10). The *cel* mutant and the *am*$^-$ strain (FGSC 521) were obtained from the Fungal Genetic Stock Center, Humboldt State College, Arcata, Calif. A number of the experiments were based on liquid shake cultures grown at 25 C on either Vogel's minimal medium (18), called the standard medium, containing 1% glucose or in the same medium with 1% L-glutamic acid replacing D-glucose. The citrate concentration in these media was 0.3%. A rotary shaker at 230 cycles/min was employed. All data are based on 400-ml cultures grown for 2 days in 1-liter Erlenmeyer flasks. Mycelium used for the inoculum was grown for 2 to 4 days in 50-ml cultures on the same medium and under identical conditions as were those used for the final culture. The procedure for preparing the inoculum was similar to that described previously (5). About 8 to 10 mg (dry weight) of the mycelium was sheared in a Waring Blendor for 1 min under sterile conditions and was used to inoculate each 400-ml culture. Sheared mycelium was used for all transfers of *col-2* to liquid media because of the organism's slow initial growth from slant cultures. In the experiments in which cultures were transferred from one medium to a different medium, some data are based on 400-ml cultures grown for 2 days under standard conditions on the standard glucose medium. These were then filtered, transferred to the same volume of glutamate medium minus citrate, and harvested at the indicated times after the second incubation under usual conditions. The reciprocal experiment was performed by employing replicate

400-ml cultures which were grown 2 days under standard conditions on glutamate in the absence of citrate, filtered, and then transferred to the standard glucose medium. These cultures were also harvested at indicated time intervals after the second incubation under usual conditions.

Fatty acid analyses. The procedures used to determine the component fatty acids in the neutral lipids and phospholipids were described in detail in a previous communication (5). The total lipids were extracted from fresh samples of mycelium with chloroform-methanol (2:1) and separated into neutral lipids and phospholipids on chromatographic columns of silicic acid. The fatty acids moieties in these two lipid fractions were converted to the corresponding methyl esters by acid-catalyzed transesterification and analyzed in a Loenco model 15B gas chromatograph. A Technicon integrator was used to determine the peak areas of plots derived from the gas chromatograph. The relative amount of each individual fatty acid is given as the mole per cent of the total, and the total fatty acid content of a lipid fraction is expressed in terms of the micromoles per gram of the dry mycelium from which that fraction was derived. The separation of the neutral lipids into subclasses was on Florisil columns by the procedure of Carroll (6).

RESULTS

Effect of mutations on neutral lipid levels. A previous report from these laboratories indicated that *col-2* had a threefold higher level of fatty acids in the neutral lipid fractions (~7.0% of the dry weight) when compared to the wild-type strain grown under identical conditions (5). This property was not due to the 10-fold slower growth rate of this morphological mutant because other slow-growing colonial mutants (*col-3* and *rg*) did not show this accumulation (5). Other G-6-PD mutants (*balloon* and *frost*) also did not show a large accumulation of neutral lipids (Table 1). The genetic background of the *col-2* strain appeared to play no role in this phenomenon since many different isolates of *col-2* from backcrosses to different wild-type strains had similar high lipid accumulation, and, furthermore, wild-type-like revertants of this mutant (*col-2b*$_2$, *inos S1* and *col-2b*$_2$, *inos R11*) had normal neutral lipid levels (5). Therefore, the lipid deposition appears to be uniquely associated with the mutation at the *col-2* locus.

The steady-state level of neutral lipids in *Neurospora* should be a function of the relative rates of fatty acid synthesis and degradation. To test whether the accumulation of neutral lipid was due to a defect in degradation or an increase in synthesis, the double mutant *col-2*, *cel* was isolated. The *cel* mutation prevents normal fatty acid syntheses and makes an organism with this lesion dependent on an exogenous source of fatty acids (10) for which Tween 40 may be used. The *col-2*, *cel* double mutant, when grown on glucose medium with Tween 40 as a source of fatty acids, contained only 60 μmoles of total fatty acids per g of dry mold in the neutral lipids, compared to a value of 100 μmoles per g obtained for the single mutant (*cel*) when grown on the same medium. Apparently, accumulation of neutral lipids in *col-2* reflects increased synthesis rather than impaired degradation of these lipids.

Effect of variation in the growth media. Systematic variations in the composition of the media were attempted to try to determine the nature of the metabolic imbalance in *col-2* which leads to neutral lipid accumulation. The data in Table 2 compare the fatty acid compositions of the neutral lipids and phospholipids of the wild-type and *col-2* strains when grown on media containing either glucose or glutamate as the major carbon source. When grown on the standard medium with glucose, the *col-2* strain contains more than three times the amount of fatty acids in its neutral lipids than does the normal strain. When glucose was replaced by glutamate, the fatty acids of the neutral lipids dropped twofold in wild type and sixfold in *col-2*, and the resulting basal levels in both strains were identical. The wild type and *col-2* grew slower on glutamate than on glucose in shake flasks. The NADPH content of both wild type and *col-2* was about 20% higher on glutamate than with glucose (S. Brody, *unpublished data*). In addition,

TABLE 1. *Lipid composition of the balloon and frost strains*[a]

Fatty acids in lipid fractions[b]	*Balloon*		*Frost*	
	1[c]	2	1	2
Neutral lipids				
Palmitic	31.8	31.2	31.2	35.5
Palmitoleic	0.9	0.7	0.6	1.0
Stearic	3.5	3.6	4.6	3.8
Oleic	21.2	22.1	18.0	12.8
Linoleic	38.2	38.3	42.0	44.0
Linolenic	4.4	4.1	3.6	2.9
Total[d]	111	101	111	99
Phospholipids				
Palmitic	33.0	33.0	29.6	28.1
Palmitoleic	1.3	0.9	1.1	1.4
Stearic	1.0	1.1	1.5	1.0
Oleic	8.3	7.2	6.7	6.0
Linoleic	53.4	53.9	56.6	59.7
Linolenic	3.0	3.9	4.4	4.0
Total[d]	36	29	45	45

[a] Grown on the standard medium with 1% glucose.
[b] Fatty acid values are given as mole per cent.
[c] Duplicate cultures.
[d] Values given in micromoles of total fatty acids in lipid fraction derived from 1 g of dry mycelium.

TABLE 2. *Effect of carbon source on the lipid compositions of the wild-type and col-2 strains*

Fatty acids in lipid fractions[a]	Primary carbon source			
	Glucose[b]		Glutamate[b]	
	Wild type	*Col-2*	Wild type	*Col-2*
Neutral lipids				
Palmitic	27.0 ± 1.05[c]	33.2 ± 0.73[d]	16.2 ± 0.68[c]	14.4 ± 0.91[c]
Palmitoleic	2.4 ± 0.28	1.4 ± 0.14	1.3 ± 0.10	0.8 ± 0.12
Stearic	4.5 ± 0.66	5.8 ± 0.27	3.8 ± 0.39	5.6 ± 0.31
Oleic	18.0 ± 1.30	18.0 ± 0.82	11.2 ± 0.37	12.1 ± 0.84
Linoleic	38.7 ± 1.87	39.4 ± 0.64	60.1 ± 0.71	63.1 ± 1.13
Linolenic	9.4 ± 0.84	2.2 ± 0.27	7.4 ± 0.75	4.0 ± 0.40
Total[e]	66.2 ± 1.10	218.5 ± 8.0	34.7 ± 2.00	35.1 ± 1.82
Phospholipids				
Palmitic	26.8 ± 0.80	27.7 ± 0.68	25.2 ± 0.49	26.7 ± 0.16
Palmitoleic	1.7 ± 0.19	1.4 ± 0.18	1.0 ± 0.07	0.8 ± 0.08
Stearic	2.0 ± 0.28	2.3 ± 0.20	1.2 ± 0.14	1.3 ± 0.24
Oleic	9.3 ± 0.61	6.7 ± 0.72	6.0 ± 0.39	4.0 ± 0.25
Linoleic	48.7 ± 1.64	59.5 ± 1.10	60.6 ± 1.74	65.1 ± 0.81
Linolenic	11.5 ± 1.12	1.7 ± 0.29	6.0 ± 0.46	2.1 ± 0.33
Total[e]	33.6 ± 1.74	31.0 ± 1.0	38.5 ± 1.47	34.7 ± 1.66

[a] Values for fatty acids expressed as in Table 1.
[b] Standard medium with either 1% glucose or 1% glutamic acid.
[c] Mean of seven cultures ± standard error.
[d] Mean of 15 cultures ± standard error.
[e] Values given in micromoles per gram of dry mycelium.

the glutamate-grown mycelia of both strains showed large changes in the relative molar ratios of saturated to unsaturated fatty acids in the neutral lipids. In the wild type, the mole per cent of the saturated fatty acids, palmitate and stearate, decreased from 31.5 on glucose medium to 20.0% on the glutamate medium, and in *col-2*, from 39.0 saturated fatty acids to 20%.

In contrast to the large variations observed in the fatty acid content of the neutral lipids of the mold as a result of either the *col-2* mutation or a change in the culture medium, the amount of the total fatty acids in the phospholipids of these cultures remained relatively unchanged (Table 2). The qualitative changes in the fatty acids were also relatively small in the phospholipids.

Citrate omission from the glucose-containing media causes minor changes in the neutral lipid levels of wild type and *col-2* (Table 3). The decreased neutral lipid levels induced by glutamate in *col-2* were not affected by the omission of citrate from the medium (Table 3), although this omission greatly affected the neutral lipid level of the wild-type strain when grown on glutamate. Additional experiments concerning other components in the standard medium indicated that neither increased biotin concentration nor the substitution of potassium nitrate for ammonium nitrate in the medium had any significant effects on these results.

Subclasses of neutral lipids. Fractionation of the neutral lipid fraction from either wild type or *col-2* on Florisil columns indicated that most of this fraction was composed of triglycerides (Table 4). In addition, analysis of this fraction from *col-2* grown on glutamate indicated a decrease in triglycerides roughly proportional to the decrease in total neutral lipids. The fatty acid composition of the triglyceride fraction, as well as the other four column fractions, has been determined, but is not presented here for the sake of brevity. These analyses also confirmed the earlier observation that wild-type *Neurospora* cultures contain small amounts of unesterified fatty acids (9).

Effect of hexose monophosphate shunt intermediates. The primary biochemical deficiency of *col-2* involves an alteration in G-6-PD. Thus, a decreased flow of glucose through the phosphogluconate oxidative pathway (hexose monophosphate shunt) is expected in this colonial strain and has been detected by radiorespirometry measurements (3). The increased fatty acid levels found in the neutral lipids of *col-2* could be caused by the decreased concentration of one or more intermediates in the hexose monophosphate shunt if one of these substances had a regulatory role in the syntheses of fatty acid. To test this

TABLE 3. *Effect of citrate omission on lipid levels*[a]

Fatty acids in lipid fraction	Sole carbon source							
	Glucose[b]				Glutamate[c]			
	Wild type		*Col-2*		Wild type		*Col-2*	
	1[d]	2	1	2	1	2	1	2
Neutral lipids								
Palmitic	28.2	33.8	34.1	30.4	15.8	14.5	13.8	16.7
Palmitoleic	1.6	1.2	2.8	1.5	1.1	0.9	0.5	1.1
Stearic	4.2	5.0	4.8	4.7	4.1	3.4	6.2	5.5
Oleic	18.7	15.3	17.6	12.3	6.7	7.3	8.8	8.7
Linoleic	36.4	36.5	38.1	49.4	60.9	62.3	65.8	64.4
Linolenic	10.9	8.2	2.6	1.7	11.4	11.6	4.9	3.6
Total[e]	87	72	183	170	6	9	33	33
Phospholipids								
Palmitic	30.2	29.7	27.4	26.5	25.9	26.6	30.6	29.4
Palmitoleic	1.5	1.2	3.0	2.1	0.9	0.7	0.5	0.8
Stearic	1.9	0.8	1.4	1.5	0.6	1.1	1.5	1.3
Oleic	8.9	8.1	8.0	5.8	3.5	3.0	2.6	1.9
Linoleic	47.2	52.0	58.6	62.7	61.6	60.4	62.7	64.7
Linolenic	10.3	8.3	1.6	1.4	7.4	8.2	2.1	1.9
Total[e]	36	38	30	29	31	38	34	31

[a] Format follows that of Table 2.
[b] Citrate-free medium with 1% glucose.
[c] Citrate-free medium with 1% glutamic acid.
[d] Duplicate cultures.
[e] Values given in micromoles per gram of dry mycelium.

TABLE 4. *Distribution of fatty acids in the subclasses of the neutral lipids*

Lipids	Total fatty acids[a]		
	Wild type on glucose[b]	*Col-2* on glucose[b]	*Col-2* on glutamate[b]
Sterol esters	2.8	6.4	2.5
Triglycerides	73.7	211.2	35.1
Diglycerides	9.6	5.3	2.7
Monoglycerides	1.0	2.4	1.0
Free fatty acids	0.4	2.8	0.5

[a] Values are given in micromoles per gram of dry weight.
[b] Major carbon source.

possibility, *col-2* was grown on medium in which D-xylose replaced glucose. Xylose can serve as a source of intermediates in the hexose monophosphate shunt, with the possible exception of 6-phosphogluconate, by its known conversion to D-xylulose-5-phosphate (7), and in this manner can bypass the genetic block inherent in the *col-2* strain. The data in Table 5 show that the fatty acid composition of this mutant strain when grown on D-xylose resembles that obtained with glucose-containing medium. The neutral lipid fraction again shows a high fatty acid content, in one experiment approaching 12% of the dry weight, and the relative molar ratios of the individual fatty acids are similar to those obtained with glucose as the major carbon source. These data do not support the involvement of one or more intermediates in the hexose monophosphate shunt as metabolites whose concentration can determine the amount of fatty acids retained in the neutral lipids of this organism.

Effects of variations in glutamic acid dehydrogenases. The changes produced by glutamate as a carbon source on neutral lipid levels in wild type and *col-2* could be due to one or many different effects of glutamate. It has been reported that the addition of glutamate to the standard medium causes a large decrease in the specific activity of the NADP-specific glutamic acid dehydrogenase (NADP-GDH) and an increase in the nicotinamide adenine dinucleotide (NAD)-specific glutamic acid dehydrogenase (NAD-GDH) of *Neurospora* (15). Therefore, three types of experiments were performed to determine if GDH levels were correlated with neutral lipid levels. The data in Table 5 show the results obtained when *col-2* was grown on the standard medium containing *both* glucose and glutamic acid, a medium known to lower NADP-GDH and to increase NAD-GDH. The qualitative

fatty acid patterns in both neutral lipids and phospholipids correspond to those obtained with only glucose as the primary carbon source. The addition of glutamate lowers the fatty acid content of the neutral lipids only 30% in *col-2* when glucose is available as an alternate carbon source. The two other types of experiments were based on the fact that the specific activities of the two GDH can also be altered by regulation with urea (16) or by the introduction of a mutation in the *am*$^-$ locus, the structural gene for NADP-GDH (8). Data are given in Table 6 for the fatty acid composition of *col-2* when grown on the standard medium with 0.1 M urea replacing NH_4NO_3. The urea in this medium causes a regulation of the two GDH in a manner similar to that produced by glutamate (16). The level of fatty acids in the neutral lipids of *col-2* is still twice that observed in the wild-type strain under normal conditions. In another experiment, the *col-2*, *am*$^-$ double mutant of *Neurospora* had essentially the same neutral lipid levels as did *col-2* (Table 6). The levels of the GDH in *col-2*, *am*$^-$ strains grown without glutamate are similar to those of the wild type grown in the presence of glutamate, i.e., little or no NADP-GDH and increased levels of NAD-GDH (S. Brody, *unpublished data*). Thus, the regulation of the two GDH may account for only a small part of the changes observed in the neutral lipid level of *col-2* when glutamate replaces glucose as a primary carbon source.

Kinetics of neutral lipid synthesis and breakdown. When *col-2* is grown for 2 days on the standard glucose medium and then transferred to medium with glutamate as the sole carbon source, the changes in the fatty acids of the neutral lipids follow an expected pattern (Fig. 1). The concentration of fatty acids in the neutral lipids decreases 40% in the interval between 30 and 50 hr after transfer. The data concerning the total recoveries of fatty acids from cultures show a measurable loss of these lipid moieties during the incubation, indicating that degradation exceeded their rate of syntheses at some time between 30 and 50 hr after transfer to the second medium. The most noteworthy qualitative

TABLE 5. *Effects of other carbon sources on lipid levels in col-2*[a]

Fatty acids in lipid fractions	Primary carbon source			
	D-xylose[b]		Glucose and glutamate[c]	
	1[d]	2	1	2
Neutral lipids				
Palmitic	31.3	29.9	28.5	28.7
Palmitoleic	1.4	1.3	2.1	2.7
Stearic	5.5	6.5	2.7	2.2
Oleic	20.1	20.2	20.0	17.0
Linoleic	40.3	41.2	42.8	45.9
Linolenic	1.5	0.9	3.9	3.5
Total[e]	255	355	155	150
Phospholipids				
Palmitic	27.1	28.6	27.2	27.8
Palmitoleic	1.4	1.1	2.6	2.0
Stearic	1.4	2.4	0.6	1.5
Oleic	6.4	6.4	9.3	7.2
Linoleic	62.5	60.9	57.4	59.8
Linolenic	1.2	0.8	2.9	1.1
Total[e]	27	33	36	37

[a] Format follows that of Table 2 except as is indicated in following footnotes.

[b] Standard medium with 1% D-xylose replacing glucose.

[c] Standard medium with both 1% glutamate and 1% glucose as primary carbon sources.

[d] Duplicate cultures.

[e] Values given in micromoles per gram of dry mycelium.

TABLE 6. *Fatty acid composition of lipids from col-2 grown on glucose in the presence of urea and from col-2 am*$^-$ *grown on glucose*[a]

Fatty acids in lipid fractions	Primary carbon and nitrogen sources			
	Glucose and urea[b]		Glucose and NH_4NO_3[c]	
	col-2		*col-2 am*$^-$[d]	
	1[e]	2	1	2
Neutral lipids				
Palmitic	25.1	25.9	32.2	30.7
Palmitoleic	1.4	2.2	1.4	1.8
Stearic	3.1	2.9	5.1	5.7
Oleic	22.6	18.4	22.0	20.9
Linoleic	39.3	42.9	38.2	38.9
Linolenic	8.5	7.6	1.1	2.0
Total[f]	132	146	246	233
Phospholipids				
Palmitic	26.5	24.8	26.5	27.5
Palmitoleic	1.3	1.4	1.1	0.8
Stearic	1.5	1.0	2.0	1.8
Oleic	9.3	9.6	7.7	7.0
Linoleic	55.1	58.2	62.1	61.2
Linolenic	6.3	4.9	0.6	1.7
Total[f]	44	39	36	35

[a] Format follows that of Table 2 except as is indicated in following footnotes.

[b] Standard medium with 1% glucose and 0.1 M urea replacing NH_4NO_3.

[c] Standard medium with 1% glucose.

[d] Double mutant of *col-2* with deficiency of nicotinamide adenine dinucleotide phosphate-specific glutamic acid dehydrogenase as a result of *am*$^-$ lesion.

[e] Duplicate cultures.

[f] Values expressed as micromoles per gram of dry mycelium.

change in the neutral lipids was an increase in linoleate from 40.9 to 55.9 moles per cent during the second incubation. Figure 2 shows the results of the reciprocal experiment, in which *col-2* was transferred from glutamate to glucose. There was a rapid fivefold increase in the fatty acid concentration of the neutral lipids within 30 hr after transfer with concomitant qualitative changes, especially with respect to the relative amounts of palmitate and linoleate. The palmitate increased from 16.7 to 35.2 moles per cent and the linoleate dropped from 64.4 to 47.6 moles per cent during the 70-hr period after transfer. The concentration and the total amount of fatty acids in the neutral lipids of the cultures increased manyfold during the incubation. The relatively small changes observed in the fatty acids of the phospholipids did not indicate any particular trend in the above two experiments.

Similar studies with wild type on the effects of changes in carbon source on the fatty acid composition yielded only one noteworthy result. Wild-type cultures, pregrown for 2 days on either glutamate or glucose, had the expected normal fatty acid composition 30 hr after transfer to glucose medium, at which time the cultures attained near maximum dry weight. Incubation beyond 30 hr showed a rapid loss in the total amount of fatty acids in the mold, with fatty acids in the neutral lipids falling to about 30% of the normal value during the interval between 30 and 50 hr after transfer. This indicated a rapid degradation of fatty acids in the early stages of autolysis that follows cessation of log-phase growth. *Col-2* did not reach this stage of growth during the experimental period because of its slower growth rate.

DISCUSSION

The *col-2* strain of *Neurospora* is a morphological mutant altered in one of the different protein subunits of G-6-PD (17). This enzyme initiates the first step in the phosphogluconate oxidative pathway (hexose monophosphate shunt) that appears to be a principal source of NADPH for *Neurospora*. It is known from previous studies that *col-2* has decreased levels of NADPH when compared to the wild type (2). Because NADPH is generally considered to be required for fatty acid synthesis, it was unexpected to find that *col-2* had threefold *more* fatty acids in its neutral lipids than the wild type when grown on a glucose-containing medium (Table 2). The NADPH deficiency in itself does not lead to fatty acid deposition since other NADPH-deficient mutants did not overproduce fatty acids. One might assume that the nature of the mutations which affect G-6-PD is probably the determining factor.

Recent work by Scott and Tatum (17) has indicated that the G-6-PD in *Neurospora* is a multimeric molecule composed of several protein subunits, with three known unlinked structural genes apparently responsible for the synthesis of these subunits. The aberrant *col-2* gene is one of

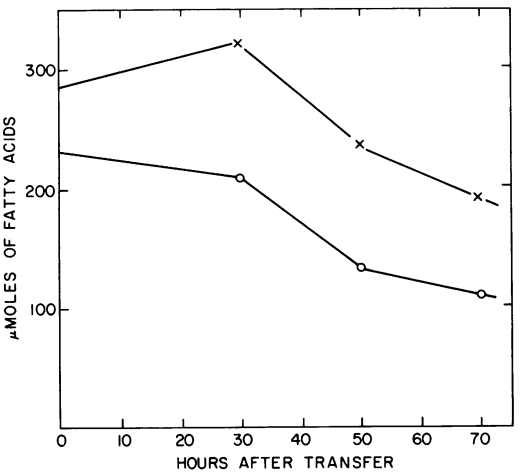

FIG. 1. *Kinetics of fatty acid decrease in col-2 upon transfer from glucose to glutamate media.* (O) *Micromoles of total fatty acids in the neutral lipids per gram (dry weight);* (×) *the total fatty acids in both neutral lipids and phospholipids from each 400-ml culture. Dry weights of cultures at 0, 30, 50, and 70 hr after transfer were 1.07, 1.32, 1.47, and 1.46 g, respectively. See Materials and Methods for details of the procedure.*

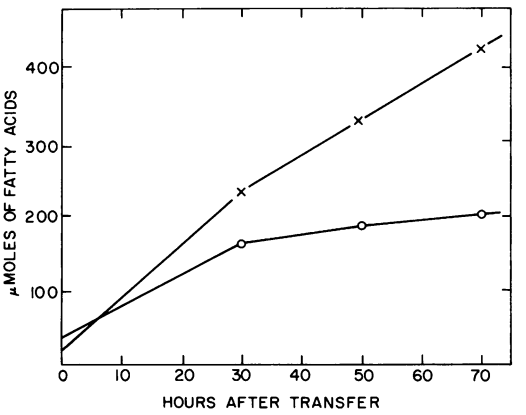

FIG. 2. *Kinetics of fatty acid increase in col-2 upon transfer from glutamate to glucose media.* (O) *Micromoles of total fatty acids in the neutral lipids per gram (dry weight);* (×) *the total fatty acids in both neutral lipids and phospholipids from individual 400-ml cultures. Dry weights of cultures at 0, 30, 50, and 70 hr after transfer were 0.27, 1.19, 1.52, and 1.94 g, respectively. See Materials and Methods for details of the procedure.*

these structural genes for the G-6-PD (17). Mutant strains (*balloon* and *frost*) are also known which appear to involve structural genes for two other protein subunits of the same enzyme (17). Mutations concerning any one of the three subunits of G-6-PD (*col-2*, *balloon*, or *frost*) can produce strains of *Neurospora* with abnormal morphologies, lowered NADPH levels, G-6-PD variants with decreased G-6-P affinities, and lower levels of linolenic acid (2, 17). In addition to the different morphologies, the only other distinction detected so far between these mutations has been that only the *col-2* mutation affects the K_m for NADP of the G-6-PD (4, 17). The present work indicates another difference between these stains in that the *balloon* and *frost* mutations do not cause the extreme increase of the fatty acid concentration noted in *col-2* (Tables 1 and 2). Although the significance of these observations is not understood at this time, it is possible that the *col-2* mutation affects a G-6-PD subunit which is also found in an enzyme involved in fatty acid biosynthesis.

The accumulation of neutral lipids in *col-2* is taken as a reflection of an increased rate of fatty acid synthesis, rather than a defect in a fatty acid degradative system. This conclusion is based on various observations. One, the fatty acid-requiring double mutant *cel*, *col-2* did not accumulate excessive amounts of neutral lipids when supplemented with a source of fatty acids. If there had been a serious defect in a degradative system, one might have expected a large buildup of fatty acids in the triglycerides since an excess of fatty acids was available in the media. Secondly, it is clear that *col-2* has a system for the degradation of fatty acids, as indicated by the drop in fatty acid content per flask in growing mycelia after a change in carbon source (Fig. 1). Finally, the amount of fatty acids found in the phospholipid fraction was quite constant. This observation suggests that the amount of membranes per gram (dry weight) of an organism remains fairly constant and that the percentage of lipids in a membrane is also fairly constant. Therefore, any increase in the rate of fatty acid synthesis would be reflected only in neutral lipid accumulation, up to the point where the induction of fatty acid degradation would limit this deposition. It should also be pointed out that this increased rate of fatty acid synthesis appears to increase the level of all of the subclasses of neutral lipids even though triglycerides make up the bulk of the neutral lipids (Table 4).

The accumulation of neutral lipids in *col-2* is a function of the carbon source since this phenomenon was not observed when glutamate was the primary carbon source. This lack of accumulation on glutamate is due apparently to a decreased rate of fatty acid synthesis rather than an increased rate of degradation since the addition of a source of fatty acids (Tween 40) to the glutamate media led to a high level of neutral lipid (containing 200 μmoles/g of fatty acids) in *col-2* and wild type. A decreased rate of fatty acid synthesis on glutamate could reflect changes in the level or activity of a rate-limiting enzyme or altered availability of either substrates or cofactors. The present data can rule out only the NADPH levels and the levels of the two GDH as factors responsible for the effect of glutamate.

The study concerning the response of *col-2* to a change in carbon source (Fig. 1) shows a decrease in the relative rate of fatty acid synthesis to degradation after 30 hr following transfer to glutamate. This delayed response suggests that an immediate product of glutamate is probably not involved in a feedback mechanism controlling fatty acid synthesis. It is more likely that a decreased supply of acetyl-CoA is limiting this synthesis after the transfer to glutamate. This might be expected if the pathway of glutamate conversion to acetyl-CoA via citrate (11) is utilized for fatty acid synthesis in *Neurospora* grown on glutamate since this is a reversal of the sequence normally encountered with glucose as a carbon source.

The omission of citrate from the glutamate medium caused a large decrease in the neutral lipids of wild type. This observation points to acetyl-CoA carboxylase as a possible regulatory site for fatty acid synthesis in *Neurospora* since activation of this enzyme by citrate *in vitro* is known from studies with other species. Discussions of the regulatory aspects of this enzyme have been published recently (12, 14). The role of acetyl-CoA carboxylase in determining either the lipid aberration of *col-2* or the low fatty acid composition of cultures grown on glutamate is a possibility that will require further study. Another possible pleiotropic effect of the *col-2* mutation might be on those enzymes that regulate the concentration of intermediates in the tricarboxylic acid cycle. In any event, the fact that experimental manipulation of growth conditions and genotype can produce a 35-fold variation in the neutral lipid level of *Neurospora* indicates that this organism could be an excellent one to study the regulation of fatty acid synthesis.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the assistance of Aniko Meenan and Stanley Martins during these studies. This investigation was supported by Public Health Service research grant GM-10935 from the National Institute of General Medical Sciences and National Science Foundation research grant GB-21227.

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