REGULATION OF GLUTAMATE DEHYDROGENASES IN *NEUROSPORA CRASSA* AS A RESPONSE TO CARBOHYDRATES AND AMINO ACIDS IN THE MEDIA

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Abstract

Mycelial pads of *N. crassa* grown for 48 hr in minimal medium were harvested, washed, and transferred to test media containing a variety of carbon and nitrogen sources. When some amino acids served as the sole carbon source, NAD-GDH was induced and the activity of NADP-GDH declined. Addition of sucrose depressed or prevented induction of NAD-GDH while NADP-GDH activity was maintained. Internal amino acid concentrations increased when mycelial pads were incubated in amino acids that induced NAD-GDH, but these accumulated amino acids were only oxidized in the absence of sucrose. The rate of amino acid accumulation decreased if sucrose was present in the media. A hypothesis is presented that the induction of NAD-GDH and the activity of NADP-GDH are a function of the ratio of amino acids to sucrose or sucrose metabolites or both. Urea was an excellent inducer of NAD-GDH in the presence or absence of sucrose, although the rate of induction was greater in the absence of sucrose. Incubation of mycelial pads in urea also led to extremely high concentrations of amino acids, thus supporting the ratio hypothesis. Mycelial pads incubated in media containing D-alanine accumulated the amino acid very efficiently, but metabolized it very poorly. Nevertheless, there was strong induction of NAD-GDH, indicating that amino acids *per se* were the compounds involved on one side of the balance. Addition of 58 mM sucrose to the test medium containing D-alanine prevented the induction of NAD-GDH but did not prevent the accumulation of alanine within the mycelium. Incubation of mycelial pads in media containing all combinations of 50 mM NH_4Cl and 58 mM sucrose established that NH_4^+ enhanced the induction of NAD-GDH. NH_4^+ *per se* did not induce NAD-GDH.

I. INTRODUCTION

Wild-type strains of *Neurospora crassa* synthesize two glutamate dehydrogenases; one specific for nicotinamide adenine dinucleotide phosphate [L-glutamate : NADP oxidoreductase (deaminating) E.C.1.4.1.4] (NADP-GDH) and the other specific for nicotinamide adenine dinucleotide [L-glutamate : NAD oxidoreductase (deaminating) E.C.1.4.1.2] (NAD-GDH). NADP-GDH is biosynthetic, while NAD-GDH is oxidative (Sanwal and Lata 1962; Strickland 1969). Incubation of mycelia in media containing L-glutamate or urea resulted in induction of NAD-GDH and this induction was inhibited by the addition of cycloheximide (Strickland 1969). Tuveson, West, and Barratt (1967) also showed induction of NAD-GDH activity in conidia incubated in amino acids. In this paper the regulatory effects of adding amino acids, urea, or various inorganic nitrogen salts to the media were studied and correlated to the free amino acid pools that formed in response to these media. A

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hypothesis is presented that the regulation of the two glutamate dehydrogenases is controlled by a balance between the free amino acid pool and sucrose or sucrose metabolites or both.

II. Materials and Methods

(a) *Strain Used*

Wild-type *N. crassa* strain 74-OR8-1a (derived from STA4) was used in all the experiments. Mycelial pads were obtained by inoculation of approximately 10^6 conidia (5–8 days old) into 100 ml Vogel's medium N (Vogel and Bonner 1956) containing 58 mM sucrose followed by incubation at 30°C on a reciprocating shaker (160 r.p.m.) for 48 hr. These mycelial pads were then used for all the experiments described.

(b) *Chemicals*

NADH and NADPH (enzymatically reduced), α-ketoglutarate, and all amino acids were obtained from Sigma Chemical Company, St. Louis, Missouri. All salts and the urea were reagent grade.

(c) *Culture Media*

Two basic culture media were used (1) Vogel's medium N (Vogel and Bonner 1956) (referred to in the rest of the paper as medium N) and (2) a modified medium N in which ammonium nitrate was omitted (referred to in the rest of the paper as medium M). All other supplements added to either medium N or medium M are indicated in the tables.

(d) *Enzyme Extraction and Assay*

These procedures have been described previously (Strickland 1969). However, in this paper all specific activities are given as International milliunits per milligram protein (m.u./mg).

(e) *Analysis of Amino Acids*

Washed, frozen mycelial pads were ground in alumina (1 g per 2 g mycelia) and extracted for 20 min in absolute methanol. Similar results were obtained by extraction in boiling water or 1% picric acid. Extracts were concentrated by vacuum evaporation at room temperature and the amino acids were quantitated on a Beckman 120C amino acid analyser.

III. Results

(a) *Effects of Individual Amino Acids*

Changes in the specific activities of glutamate dehydrogenases in mycelial pads incubated in medium M supplemented individually with L-serine, glycine, or L-aspartate were measured (Table 1). With all these amino acids, there was an

Table 1

Effect of amino acids on the specific activity of NAD-GDH and NADP-GDH in mycelial pads

Time after Transfer (hr)	50 mM L-Serine*		50 mM Glycine*		50 mM L-Aspartic Acid*	
	NAD-GDH (m.u./mg)	NADP-GDH (m.u./mg)	NAD-GDH (m.u./mg)	NADP-GDH (m.u./mg)	NAD-GDH (m.u./mg)	NADP-GDH (m.u./mg)
0	—	—	81	1237	82	1230
8	958	128	645	79	424	213
24	1765	6	1702	13	690	16
36	2388	1	1632	6	929	12
48	2777	2	2375	6	1236	30

* Washed mycelial pads were transferred to medium M supplemented as indicated.

increase in NAD-GDH and a decrease in NADP-GDH activity. Control data, obtained by incubation of mycelia in medium N containing 58 mM sucrose showed no change in NAD-GDH and a 50% decrease in NADP-GDH over a 48-hr period (Strickland 1969). Higher concentrations of glycine (100 mM) produced changes in

TABLE 2

EFFECT OF VARIATION OF ALANINE AND AMMONIUM NITRATE CONCENTRATIONS ON THE SPECIFIC ACTIVITY OF NAD-GDH AND NADP-GDH IN MYCELIAL PADS

Time after Transfer (hr)	50 mM L-Alanine, no NH_4NO_3*		50 mM L-Alanine, 25 mM NH_4NO_3*		50 mM DL-Alanine, no NH_4NO_3*		50 mM DL-Alanine, 25 mM NH_4NO_3*	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH
0	104	1002	—	—	—	—	—	—
4	522	586	1416	884	368	513	903	412
8	810	284	1945	429	815	373	1939	84
12	924	104	2399	127	1130	133	1887	15
24	1708	29	2110	24	1985	19	2380	5
48	1753	13	3122	20	4851	12	4341	10

* Washed mycelial pads were transferred to medium M supplemented as indicated.

the activity of the two enzymes similar to those obtained with 50 mM glycine (data not shown). In contrast, 100 mM L-alanine resulted in a greater increase of NAD-GDH activity after 48 hr, but did not accelerate the decrease of NADP-GDH (Tables 2 and 3).

TABLE 3

EFFECT OF ALANINE AND SUCROSE ON THE SPECIFIC ACTIVITIES OF NAD-GDH AND NADP-GDH IN MYCELIAL PADS

Time after Transfer (hr)	50 mM L-Alanine + 58 mM Sucrose*		100 mM L-Alanine*	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH
0†	77	1218	—	—
4	161	955	353	437
8	146	853	573	238
24	266	394	1091	8
36	1020	24	1590	14
48	1026	7	2858	19

* Washed mycelial pads were transferred to medium M supplemented as indicated.

† Control.

Addition of 25 mM NH_4NO_3 to medium M containing 50 mM L-alanine resulted in a larger increase of NAD-GDH activity than was obtained in medium M containing 50 mM L-alanine alone (Table 2). The decline of NADP-GDH was the same under both experimental conditions. 50 mM DL-alanine gave an even greater increase of

NAD-GDH than 50 mM L-alanine. In this instance addition of 25 mM NH_4NO_3 resulted in a faster rate of induction but the final specific activities after 48 hr were similar (Table 2).

(*b*) *Effect of Sucrose or NH_4^+ or Both*

If 58 mM sucrose was added to medium M containing 50 mM L-alanine, then the increase in NAD-GDH was substantially reduced and the decline of NADP-GDH was retarded (Tables 2, 3). Thus, the presence of sucrose appeared to prevent the increase of NAD-GDH and to maintain the level of NADP-GDH. To test this hypothesis, mycelial pads were incubated for 48 hr in medium N supplemented by different concentrations of sucrose (Table 4). When mycelial pads were incubated in the

TABLE 4

EFFECT OF VARIATION OF THE SUCROSE CONCENTRATION ON THE SPECIFIC ACTIVITIES OF NAD-GDH AND NADP-GDH IN MYCELIAL PADS

Time after Transfer (hr)	No Sucrose*		58 mM Sucrose*		146 mM Sucrose*		232 mM Sucrose*	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP GDH	NAD-GDH	NADP-GDH
0†	—	—	123	939	90	998	—	—
4	—	—	142	654	108	565	127	716
12	355	45	85	1032	77	1704	87	1713
24	318	53	88	498	82	1377	—	1328
36	246	40	77	298	77	1160	103	1477
48	229	44	85	274	129	1211	128	1414

* Washed mycelial pads were transferred to medium N supplemented as indicated.
† Control.

absence of sucrose, there was a quick drop in the level of NADP-GDH and a moderate increase (two to threefold) in NAD-GDH. Addition of 58 mM sucrose prevented the decline of NADP-GDH for at least 12 hr, and there was no increase in NAD-GDH. Addition of 146 mM or greater concentrations of sucrose resulted in the maintenance of NADP-GDH for the full 48 hr period without any change in the activity of NAD-GDH. The initial drop in NADP-GDH activity between 2 and 4 hr occurred at all four concentrations of sucrose and is unexplained.

It has been postulated that NH_4^+ is the actual effector of NAD-GDH induction (Stachow and Sanwal 1967). This hypothesis was tested further by incubation of mycelial pads in medium M and all combinations of 58 mM sucrose and 50 mM NH_4Cl (Table 5). In the absence of NH_4^+, the mycelial pads became stringy and ragged and could not be maintained for longer than 12 hr. In the presence of sucrose, there was no increase in NAD-GDH activity regardless of the presence or absence of 50 mM NH_4Cl (Table 5, columns 6 and 8), but the level of NADP-GDH was maintained at a higher level in the absence of NH_4Cl (Table 5, columns 7 and 9). In the absence of sucrose there was a transient 10-fold increase in NAD-GDH if 50 mM NH_4Cl was added and a

threefold increase in NAD-GDH if 50 mM NH_4Cl was omitted (Table 5, columns 2 and 4). In the absence of sucrose, the decline of NADP-GDH was much more rapid if NH_4Cl was present (Table 5, columns 2 and 4).

TABLE 5

EFFECT OF NH_4^+ AND SUCROSE ON THE SPECIFIC ACTIVITY OF NAD-GDH AND NADP-GDH IN MYCELIAL PADS

Time after Transfer (hr)*	No Sucrose, no NH_4Cl†		No Sucrose, 50 mM NH_4Cl†		58 mM Sucrose, no NH_4Cl†		58 mM Sucrose, 50 mM NH_4Cl†	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH
4	378	1456	867	900	—	—	169	1193
8	354	784	1090	138	171	1580	160	1394
12	347	563	630	23	173	1882	130	810
24	—	—	434	16	—	—	121	653
48	—	—	508	16	—	—	220	236

* Zero time activities were similar to those in Tables 1, 2, and 4.

† Washed mycelial pads were transferred to medium M supplemented as indicated.

(c) *Effect of Urea*

It has long been known that addition of urea to medium N containing sucrose resulted in a very large induction of NAD-GDH (Sanwal and Lata 1962; Stachow and Sanwal 1967). Further experiments were done to test the effect of urea alone and in the presence of sucrose with or without NH_4^+ (Table 6). Incubation of pads in medium

TABLE 6

EFFECT OF 50 mM UREA ON THE SPECIFIC ACTIVITIES OF NAD-GDH AND NADP-GDH

Time after Transfer (hr)	No Supplement*		58 mM Sucrose*		58 mM Sucrose + 25 mM NH_4NO_3*	
	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH	NAD-GDH	NADP-GDH
0	80	1180	81	1237	—	—
8	532	18	378	535	—	—
12	666	11	1048	128	267	361
24	799	23	2601	10	912	65
48	—	—	3415	8	2989	52

* Washed mycelial pads were transferred to medium M containing 50 mM urea supplemented as indicated.

M plus 50 mM urea produced approximately an eightfold increase in NAD-GDH and the usual decline in NADP-GDH. Under these experimental conditions the mycelia could not be maintained longer than 24 hr. Addition of 58 mM sucrose to this medium resulted in an initial slower rate of increase of NAD-GDH, but a final increase of 35-fold after 48 hr. Furthermore, the rate of decline of NADP-GDH was slowed

substantially for at least 12 hr. Addition of 58 mM sucrose and 25 mM NH_4NO_3 resulted in an even slower initial rate of increase of NAD-GDH and a final increase of only 30-fold. These experimental conditions also resulted in the slowest decline of NADP-GDH.

(*d*) *Amino Acid Analysis*

Strickland (1969) showed that NAD-GDH was induced if glutamate was used as the sole carbon and nitrogen source. Addition of sucrose to the medium decreased the uptake of glutamate and there was no induction of the enzyme. Mycelial pads were therefore incubated under various conditions and analysed for free amino acids both within the mycelia and in the culture media (Table 7). Mycelial pads incubated in

TABLE 7

FREE AMINO ACIDS AND AMMONIA IN MYCELIAL PADS OF *N. CRASSA* INCUBATED IN VARIOUS MEDIA

Values are expressed as μmoles of free amino acid or ammonia per 100 mg dry weight of mycelial pads. n.d., not detectable (< 0·05 μmole)

Time after Transfer (hr)	Free Amino Acids					NH_4^+*
	Ser	Ala	Glu+Gln	Other	Total	
			Medium N+58 mM sucrose†			
0 (pad)	0·3	6·4	4·2	3·7	14·6	0·6
8 (pad)	—	—	—	—	—	—
8 (medium)	—	—	—	—	—	—
12 (pad)	0·3	6·0	2·3	3·7	12·3	0·6
12 (medium)	n.d.	n.d.	n.d.	n.d.	n.d.	—
24 (pad)	0·5	2·8	6·9	3·6	13·8	0·1
24 (medium)	n.d.	n.d.	n.d.	n.d.	n.d.	—
48 (pad)	0·5	3·3	4·4	4·9	13·1	0·2
48 (medium)	n.d.	0·4	1·1	0·2	1·7	—
			Medium N+58 mM sucrose+50 mM urea†			
0 (pad)	—	—	—	—	—	—
8 (pad)	0·9	16·2	10·9	4·7	32·7	0·8
8 (medium)	n.d.	2·8	n.d.	n.d.	2·8	—
12 (pad)	0·7	20·9	10·4	5·4	37·4	0·5
12 (medium)	n.d.	6·3	1·6	0·4	8·3	—
24 (pad)	0·8	36·5	17·6	7·1	62·0	1·6
24 (medium)	1·8	29·8	11·0	8·7	51·3	—
48 (pad)	1·1	25·3	25·8	10·7	62·9	1·5
48 (medium)	3·7	45·4	37·9	34·5	121·5	—

* NH_4^+ in media not measured.

† Washed mycelial pads were transferred to the media indicated.

medium N containing 58 mM sucrose (i.e. control media) had a total amino acid content within the mycelia ranging from 12 to 15 μmoles/100 mg dry weight. Amino acid levels in the culture media were negligible. The internal NH_4^+ concentration ranged from 0·1 to 0·6 μmole/100 mg dry weight. In contrast, pads incubated in the same medium supplemented by 50 mM urea had very high internal concentrations of

amino acids. The majority of these amino acids occurred as alanine, glutamic acid, and glutamine. Furthermore, the amino acid concentration after 48 hr in the medium was double the concentration within the mycelium. NH_4^+ concentrations within the mycelia increased four to fivefold as compared with the concentrations found in the absence of urea. It should be remembered that medium N plus sucrose gave no increase in NAD-GDH, whereas the addition of 50 mM urea gave a very high induction.

Free amino acid concentrations in mycelia incubated in medium M supplemented with 50 mM L-glutamate and in medium M supplemented with 50 mM L-glutamate and 58 mM sucrose were measured (Table 8). The accumulation of

TABLE 8

FREE AMINO ACIDS AND AMMONIA IN MYCELIAL PADS OF *N. CRASSA* INCUBATED IN MEDIA CONTAINING L-GLUTAMATE AND L-GLUTAMATE PLUS SUCROSE

Values are expressed as μmoles of free amino acid or ammonia per 100 mg dry weight of mycelial pad

Time after Transfer (hr)	Ala	Glu+Gln	NH_4^+	Ala	Glu+Gln	NH_4^+
	50 mM L-Glutamate*			50 mM L-Glutamate+ 58 mM sucrose*		
4	3·9	24·6	1·3	—	—	—
8	5·8	18·8	0·7	9·8	10·9	0·1
12	7·6	17·0	0·5	8·2	10·3	—
24	6·3	15·5	4·9	12·0	20·8	0·4
36	7·6	12·6	49·3	7·8	15·0	0·5
48	4·5	10·8	41·2	7·9	14·2	0·5

* Washed mycelial pads were transferred to medium M supplemented as indicated.

glutamate plus glutamine was much faster in the absence of sucrose, but by 48 hr the concentrations of glutamate plus glutamine and also of alanine were higher in the presence of sucrose. The decline in the concentration of these amino acids in the absence of sucrose combined with the steep increase of NH_4^+ suggested that extensive deamination was occurring. Addition of 58 mM sucrose to the media did not prevent the accumulation of a high internal amino acid concentration, but it did prevent deamination of those amino acids. In this paper it has already been shown that there was strong induction of NAD-GDH when mycelia were incubated on 50 mM L-alanine and that this induction was largely prevented by addition of 58 mM sucrose to the medium. Strickland (1969) previously showed that there was moderate induction of NAD-GDH when mycelia were incubated on medium M plus 50 mM L-glutamate, but that addition of 58 mM sucrose to this medium prevented the induction.

Free amino acid concentrations within mycelia incubated in medium M plus various combinations of 50 mM L-alanine or 50 mM DL-alanine and 25 mM NH_4NO_3 were also measured (Table 9). Internal amino acid concentrations were high under all experimental conditions and NH_4^+ increased suggesting that amino acids were being deaminated (and therefore oxidized). Incubation of mycelia on medium M containing 50 mM L-alanine and 25 mM NH_4NO_3 resulted in a lesser internal concentration of

alanine, glutamate, and glutamine. At the same time internal NH_4^+ concentration was higher and the increase of NAD-GDH was greater (Table 2), suggesting that an inorganic nitrogen source (in addition to alanine) was necessary for maximum metabolism. The same conclusion could be reached for the experiments using 50 mM DL-alanine in the presence or absence of an inorganic nitrogen source. However, the most outstanding difference in the results obtained with L-alanine and DL-alanine was the large internal accumulation of alanine when mycelia were incubated in DL-alanine, particularly in the absence of NH_4NO_3. Medium M containing 50 mM DL-alanine also produced the greatest increase of NAD-GDH ever obtained (Table 2). The conclusion was that D-alanine was being accumulated within the mycelium but was not being metabolized. This accumulation *per se* was then responsible for the increase in NAD-GDH.

TABLE 9

FREE AMINO ACIDS AND AMMONIA IN MYCELIAL PADS OF *N. CRASSA* INCUBATED IN MEDIUM CONTAINING EITHER L-ALANINE OR DL-ALANINE, WITH OR WITHOUT AMMONIUM NITRATE

Values are expressed as μmoles of free amino acid or ammonia per 100 g dry weight of mycelial pad

Time after Transfer (hr)	Ala	Glu+Gln	NH_4^+	Ala	Glu+Gln	NH_4^+
	50 mM L-Alanine, no NH_4NO_3*			50 mM L-Alanine, 25 mM NH_4NO_3*		
1	23·1	10·6	2·5	4·1	4·0	1·2
4	20·7	9·8	0·6	11·3	6·7	0·5
8	19·7	10·3	1·5	17·9	6·9	0·5
12	27·1	15·2	1·5	3·4	5·5	2·9
24	12·5	13·9	5·9	9·4	4·1	1·0
48	3·2	10·5	12·7	2·0	4·5	17·1
	50 mM DL-Alanine, no NH_4NO_3*			50 mM DL-Alanine, 25 mM NH_4NO_3*		
1	—	—	—	7·9	6·4	—
4	—	—	—	37·6	21·5	0·7
8	—	—	—	26·2	7·1	1·1
12	38·6	24·2	0·2	22·4	7·6	2·7
24	115·5	10·3	1·0	30·8	2·3	2·9
48	140·0	7·9	12·7	45·8	2·8	16·2

* Washed mycelial pads were transferred to medium M supplemented as indicated.

(e) *Effect of Incubation on* D-*Alanine*

Therefore, mycelial pads were incubated in medium M containing 50 mM D-alanine (Table 10). The pads could not be maintained for more than 24 hr, but during that time internal concentrations of alanine had reached 60–80 μmoles/100 mg dry weight. The low internal NH_4^+ concentration suggested that little deamination of D-alanine was occurring and that D-alanine was a poor carbon source. Nevertheless, NAD-GDH specific activity of mycelial pads incubated on 50 mM D-alanine

was higher than the specific activity obtained when mycelial pads were incubated on 50 mM L-alanine (Tables 2, 10). Addition of 58 mM sucrose to medium M containing 50 mM D-alanine prevented the increase of NAD-GDH activity while maintaining that of NADP-GDH (Table 10). However, this addition of sucrose did not prevent the accumulation of alanine within the mycelia.

TABLE 10

SPECIFIC ACTIVITIES OF NAD-GDH AND NADP-GDH AND AMOUNTS OF FREE AMINO ACIDS AND AMMONIA IN MYCELIAL PADS OF *N. CRASSA* INCUBATED IN MEDIA CONTAINING D-ALANINE AND D-ALANINE PLUS SUCROSE

Values for free amino acids and ammonia expressed as μmoles per 100 mg dry weight of mycelial pad

Time after Transfer (hr)	Specific Activities		Free Amino Acids				NH_4^+
	NAD-GDH	NADP-GDH	Ser	Ala	Glu+Gln	Other	
Mycelial pads incubated in medium M containing 50 mM D-alanine							
0*	104	1002	0·3	6·4	4·2	3·7	0·6
4	340	759	0·6	80·8	1·2	3·2	0·7
8	575	227	1·0	69·3	2·9	6·0	1·3
12	1388	53	1·1	81·9	1·6	7·9	1·7
24	1959	29	0·7	64·9	1·3	7·6	2·7
Mycelial pads incubated in medium M containing 50 mM D-alanine+58 mM sucrose							
0*	104	1002	0·3	6·4	4·2	3·7	0·6
4	161	472	0·6	70·4	3·3	3·3	0·5
8	151	451	0·8	93·1	0·8	4·6	0·8
12	197	530	0·9	93·2	1·0	4·8	0·5
24	192	831	0·6	78·9	0·8	5·2	0·4

* Control.

IV. DISCUSSION

In the absence of a carbohydrate, addition of L-glutamate as the sole carbon source resulted in NAD-GDH induction (Strickland 1969). Different amino acids were not equally effective in producing this induction (Sanwal and Lata 1962). In the present paper the order of decreasing effectiveness was DL-alanine = D-alanine > L-serine > glycine > L-alanine > L-aspartic (Tables 1, 2, 10). D-Amino acids were more effective than L-amino acids and the hypothesis is proposed that uptake of D- or L-amino acids was equally efficient, but that D-amino acids were more effective inducers of NAD-GDH because they accumulated internally due to poor metabolism.

In all experiments, the level of NADP-GDH after 48 hr incubation had declined to 1–2% of the zero time value. These experiments did not determine whether the decline of NADP-GDH was due to inactivation or degradation. Stachow and Sanwal (1967) have shown that NADP-GDH was not converted to NAD-GDH during induction. Incubation of mycelia containing valine, isoleucine, or leucine produced no change in the specific activity of either enzyme (data not shown).

Addition of sucrose to medium M containing either L-alanine (Tables 2, 3) or L-glutamate (Strickland 1969) depressed NAD-GDH induction and slowed the

decrease of NADP-GDH. These experimental conditions also depressed the rate of uptake of the amino acids. Kapoor and Grover (1970) found that in *N. crassa* NAD-GDH was subject to repression by sucrose and glucose, while NADP-GDH was induced by increasing concentrations of catabolites. These catabolite effects were overcome when glutamate was added to the medium. In the present experiments, specific activity of NADP-GDH could be controlled by variation of the sucrose concentration in the media (Table 4).

If the internal concentration of amino acids *per se* was responsible for the induction of NAD-GDH, then a correlation between the internal amino acid pool and the level of induction might exist. When mycelia were grown in medium M containing L-glutamate, internal concentrations of glutamate plus glutamine were high in the presence or absence of sucrose. However, the metabolism of these amino acids by deamination (estimated by the increase of NH_4^+) only occurred in the absence of sucrose (Tables 7, 8). These results gave the first indication that the induction of NAD-GDH was not only a function of the internal concentration of amino acids, but was rather dependent on some critical ratio between internal amino acid concentration and internal sucrose or sucrose metabolites or both.

Internal accumulation of amino acids other than glutamate and glutamine also resulted in NAD-GDH induction. When induction of NAD-GDH was carried out using medium M plus 50 mM L-alanine or 50 mM DL-alanine, the levels of induction (Table 2) could be related to the concentration of the alanine pool rather than to the concentration of the glutamate plus glutamine pool (Table 9). Some preliminary experiments showed that if induction was carried out on medium M plus 50 mM L-serine, internal amino acid concentrations per 100 mg dry weight after 24 hr incubation were 68·7 μmoles serine; 2·1 μmoles alanine, and 10·6 μmoles glutamate plus glutamine. L-Serine was also an effective inducer of NAD-GDH (Table 1).

The internal alanine pool was much greater if DL-alanine rather than L-alanine was used for the induction (Table 9). Similarly, internal accumulation of alanine was much greater on D-alanine (in the presence or absence of sucrose) than on L-alanine (Tables 9, 10). At the same time, concentrations of glutamate plus glutamine and NH_4^+ were lower on D-alanine than on control media (Tables 7, 10), indicating that D-alanine was accumulated internally but was not metabolized. It was therefore concluded that D-alanine itself was the inducer of NAD-GDH but acted only if the critical ratio of amino acids : sucrose or sucrose metabolites or both had been reached.

Urea is a most effective inducer of NAD-GDH (Sanwal and Lata 1962; Stachow and Sanwal 1967) and, contrary to the situation with amino acids, results in induction of NAD-GDH in the presence or absence of sucrose (Table 6). If the hypothesis of a critical ratio is correct, then the internal concentrations of amino acids of mycelia incubated in urea plus sucrose must be extremely high in order to reach this critical ratio. This was indeed found to be correct (Table 7) and the amino acid concentration was so high that free amino acids were excreted in large quantities into the media.

NH_4^+ ions have been found to repress the synthesis of NAD-GDH in yeasts and to induce synthesis of NADP-GDH (Hierholzer and Holzer 1963; Westphal and Holzer 1963). In contrast, Barratt (1963) proposed that NH_4^+ or some other compound in the nitrogen pool caused repression of NADP-GDH in *Neurospora*. Stachow and Sanwal (1967) hypothesized that NH_4^+ *per se* induced NAD-GDH and repressed NADP-GDH.

In the absence of sucrose, addition of NH_4^+ did indeed increase the induction of NAD-GDH (Table 5). If this induction was due to the presence of NH_4^+ rather than to the absence of sucrose, then a similar difference should be found in the presence of sucrose. As can be seen, addition of sucrose prevented the induction of NAD-GDH regardless of the presence or absence of NH_4^+. These experiments also showed that addition of sucrose in the presence or absence of NH_4Cl prevented the decline of NADP-GDH in agreement with the hypothesis that sucrose or its metabolites or both maintained the activity of NADP-GDH.

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VI. References

Barratt, R. W. (1963).—Effect of environmental conditions on the NADP-specific glutamic acid dehydrogenase in *Neurospora crassa*. *J. gen. Microbiol.* **33**, 33–42.

Fincham, J. R. S. (1950).—Mutant strains of *Neurospora* deficient in aminating ability. *J. biol. Chem.* **182**, 61–73.

Hierholzer, G., and Holzer, H. (1963).—Repression of the synthesis of DPN-dependent glutamic acid dehydrogenase in *Saccharomyces cerevisiae* by ammonium ions. *Biochem. Z.* **339**, 175–82.

Kapoor, M., and Grover, A. K. (1970).—Catabolite controlled regulation of glutamate dehydrogenases of *Neurospora crassa*. *Can. J. Microbiol.* **16**, 33–40.

Sanwal, B. D., and Lata, M. (1962).—Concurrent regulation of glutamic acid dehydrogenases of *Neurospora*. *Archs Biochem. Biophys.* **97**, 582–8.

Stachow, C. S., and Sanwal, B. D. (1967).—Regulation, purification and some properties of the NAD-specific glutamate dehydrogenase of *Neurospora*. *Biochim. Biophys. Acta* **139**, 294–307.

Strickland, W. N. (1969).—Induction of NAD-specific glutamate dehydrogenase in *Neurospora crassa* by addition of glutamate to the media. *Aust. J. biol. Sci.* **22**, 425–32.

Tuveson, R. W., West, D. J., and Barratt, R. W. (1967).—Glutamic acid dehydrogenases in quiescent and germinating conidia of *Neurospora crassa*. *J. gen. Microbiol.* **48**, 235–48.

Vogel, H. J., and Bonner, D. M. (1956).—A convenient growth medium for *Neurospora*. *Microb. Genet. Bull.* **13**, 42–3.

Westphal, H., and Holzer, H. (1963).—Synthesis of NAD-dependent glutamate dehydrogenase in protoplasts of *Saccharomyces carlsbergensis*. *Biochim. Biophys. Acta* **89**, 42–6.