Cheng, P. T. ond J. E. Willis. Glucose-6-phosphate dehydrogenow activity of Neurospora grown on media containing molonote ond citrate.

Reports in the literature suggest that N. crassa might serve as a convenient source of glucose-6-phosphate dehydrogenare (G6PDH) for purification and production. Rodhakrishnon (1960 Biochim. Biophyr. Acta 40: 546) found that crude extracts of N. crassa (16117A) contained G6PDH of specific activity 0.31 µmoles TPNH produced/minute/mg. protein (25°C). By comparison, Noltmonn et al. (1961 J. Biol. Chem. 236: 1225) prepared crystalline G6PDH from yeast extracts of initial specific activity 0.27 µmoles TPNH/mg. protein at 30°C. Furthermore, up to 9 times enhancement of G6PDH activity has been reported for extracts of heavily conidioted N. crassa grown on nitrate-sucrose-malonate medium as compared to medium lacking molonote (Turion 1962 N N#2: 15). Thus, it appeared that growth of N. crassa on molonote medium would provide ceils rich in G6PDH. However, in the experiments reported in this communication, no significant enhancement of G6PDH activity was observed in cells grown on malonate as compared to controls.

The microorganism used in this study was wild type N. crassa strain 52970 (ATCC 10816). Cells were grown at 28°C for different periods of time in 100 ml of culture medium. The medium was similar to that described by Beadle (1945 Physiol. Rev. 25: 643), except that sodium nitrate was used in place of the ammonium salt ond tartrate was replaced by citric or malonic acid. All media were adjusted to pH 6.8 with NaOH before sterilization. After the given period of incubation the culture was filtered with suction. The cell moss was washed with three 100-ml portions of water and a portion was token for dry weight determination. The remainder of the sample was placed in the deep freeze for one hour, mixed with powdered Dry Ice and ground with a mortar and pestle for 30 minutes. After sublimation of the Dry Ice, the sample was treated with 5 ml of 0.05 M glycylglycine buffer, pH 8.0, per gram wet weight of cells. The buffer was added slowly over a period of IO minutes with continued grinding. The resulting mixture was stirred for one hour at room temperature and centrifuged at 13,000 x g for 20 minutes to yield the crude extract used in this study. Preliminary experiments showed that growth of the organism for 88 hours on concentrations of molonote from 0.01 M to 0. IO M hod no effect on the specific activity of G6PDH. However, levels of malonate greater than 0.05M retard the growth of this strain of Neurospora.

A level of malonate that did not interfere with growth (0.05 M) was chosen for on experiment in which the effect of time of incubation on growth and on G6PDH activity of malonote-grown cells was compored to that on cells grown on 0.01 M citrate or on control medium (no organic acid). Growth was more ropid on the media containing organic acids thon in the control cultures (Table I). The specific acitivity of G6PDH was greater in the young control cultures, although the total units of enzyme activity were somewhat higher in the young molonote-grown cells. In the period between 112 and 160 hours, the enzyme activity decreased while the dry weight of the organism increased. In contrast with the results of Turian (loc. cit.), there was essentially no difference in the G6PDH activity in extracts of cells grown on molonote or citrate as compared to the control media. This disagreement in results could be due to the use of different strains of Neurospora or to some methodological difference.

Table 1. Effect of incubation time on dry weight and G6PDH activity.

Medium[a]	Dry Weight (g)[b]				Total Activity[c]				Specific Activity[d]			
Time (hrs.)	66	112	160	232	66	112	160	232	66	112	160	232
Control	.10	.47	.74	.91	8.5	19.3	16.0	15.2	.69	.55	.43	.36
Citrate	.24	.62	1.07	.88	14.3	19.3	14.5	9.7	.48	.52	.44	.27
Malonate	.37	.63	.98	.84	19.8	26.8	16.7	9.7	.49	.63	.38	.36

a Control = sucrose-nitrate medium; Citrate = control + 0.01M citrate; Malonate = control + 0.05M molonote.
b Average weight per 100 ml from two 100-ml incubation mixtures.
c Average units per 100 ml from two IOO-ml incubation mixtures.
d µmoles TPNH produced/minute/mg protein in the assay system of Noltmonn et al. (loc. cit.). Protein was determined by the Lowry procedure.

In a separate experiment, Neurospora was grown on 0.1 M molonote or 0.1 M citrate. The G6PDH activity decreased rapidly in older cultures. In fact, no activity was detected in extracts of 10-day cultures grown on 0. 1 M citrate. In all of the above-mentioned experiments, 6-phosphogluconate dehydrogenare activity was assayed, also. The some pattern of activity changes was observed for both enzymes of the hexose monophosphote shunt. It would appear that the shunt moy be more important in young cultures than in older cultures. - - - P-L Biochemicals, Inc., Milwaukee, Wisconsin 53205.