INCORPORATION OF ^{14}C LABELED GLYCINE INTO YEAST PROTEIN*, **

by

ARTHUR H. WEBB, FELIX FRIEDBERG,
AND LAWRENCE M. MARSHALL

Departments of Bacteriology and Biochemistry,
Howard University Medical School,
Washington 1, *D. C. (U.S.A.)*

It has been reported previously that *Torula utilis* cells incorporate radioglycine into trichloroacetic acid precipitable fractions by a process that is oxidative and enzymatic[1]. Glycine seems an ideal carrier in following uptake of radioactive carbon in this system, in that its conversion to other intermediates is at a minimum. According to EHRENSVÄRD it appears preferentially in cell protein hydrolysates and substrate glycine exchanges rapidly with glycine bound in cell protein in *Torula*[2].

Incorporation of ^{14}C from labeled glycine into the protein fraction of *Torula* cells can be markedly altered by deletion of substrate constituents, anaerobiosis, and by addition of enzyme poisons. In order to learn more about the mechanism of uptake, the following experiments were undertaken to explore effects of various physical and chemical factors on the rate of turnover of labeled carbon in the protein fraction of *Torula* cells.

EXPERIMENTAL

Torula utilis cells were grown in an aerated synthetic medium, harvested, and shaken in an O_2 atmosphere in a Dubnoff incubator in presence of carboxyl-labeled glycine[1]. At experimental intervals, the protein of the cell suspensions was precipitated with 5% trichloroacetic acid and washed by centrifuging four times with trichloroacetic acid containing 5% inert glycine. Following additional washings with hot 95% ethyl alcohol-ether (3:1) the protein fractions were collected on filter paper, weighed, and counted with the Geiger-Muller counter.

Uptake of radioactivity as a function of time

To determine the relationship of time to uptake of the label, washed cells amounting on the average to 60 milligrams of dried protein were placed in beakers containing 2 ml medium and a trace of labeled glycine having activity of 33,800 counts per 2 minutes. These were shaken in oxygen at 29 degrees centigrade, and at intervals a beaker was removed, protein of the cells precipitated, dried, weighed, and uptake of radioactivity counted. Results were plotted in terms of specific activity divided by administered dose of radioactivity, that is, counts per milligram of the protein fraction per unit time, divided by standard count of the label. This factor is a ratio of labeled carbon to inert carbon in the yeast protein material.

* Supported in part by a grant from the Damon Runyon Memorial Fund
** ^{14}C obtained through courtesy of United States Atomic Energy Commission, Oak Ridge, Tennessee.

References p. 571.

The time uptake curve is sigmoid (Fig. 1), and bears resemblance to the growth curve of BUCHANAN. A lag phase is present, and there is an area of logarithmic increase in uptake of the label between 60 and 95 minutes, and a phase of negative acceleration between 95 and 130 minutes. The maximum stationary phase, starting at about 130 minutes is obscurred by scattering of the points.

Uptake of the label as a function of temperature

In this experiment average weight of protein exposed to radioglycine was 60 mg. Standard count of the labeled amino acid was 41,000 per 2 minutes. Beakers were shaken for 1 hour at temperatures progressing from 10° to 50° C.

It can be seen from Fig. 2 that the mechanism of label uptake is extremely sensitive to temperature. This organism takes up glycine most efficiently somewhat above the temperature at which yeasts are commonly grown, with maximum activity occurring in the 35°–36° C range. At 25° C, uptake was less than half that of the optimum temperature. The precipitous decline in activity at temperatures above the optimum is in accord with the accepted concept of heat sensitivity of enzyme systems.

Uptake of the label as a function of p_H

The previous experiments were carried out at a p_H of 5.0 to 5.5, assuming that the traditional p_H range at which yeast are grown was the optimum for metabolic activity. For this experiment, buffers were prepared ranging from p_H 3.6 to p_H 5.6 (0.1 molar acetate), and p_H 6.05 to p_H 8.0 (0.1 molar phosphate). These were added to media, (1:1), and cells incubated 1 hour, with shaking in oxygen at 35° C. Average weight of protein exposed to radioactivity was 15 mg, and standard count of the labeled glycine was 33,800 per 2 minutes. Label uptake at p_H 5.0 was negligible (Fig. 3), and at p_H 5.6, activity was less than half that at the optimum p_H, 6.0. It would appear that *Torula utilis* turns over glycine more rapidly at a p_H approaching neutrality than in a more acid range. Further, label uptake in the area from p_H 6.0 to 6.8 is of a higher order than uptake from p_H 5.0 to 6.0.

Effect of phenol on uptake of the label

The action of a disinfectant in stopping the total activity of a cell system should be reflected in the component enzymic operations of the system. It would be expected therefore that phenol should depress uptake of the radioactive label. To test this, to 100 ml of a cell suspension, containing approximately 5 grams of protein was added 2.5 grams of phenol. As control, a 0.2 ml sample was placed in 1.8 ml medium and shaken one hour in oxygen at 29° C. The medium contained a trace of glycine having activity of 33,800 counts per two minutes. This first sample which served as base line had an uptake factor of 15.4. Twelve minutes after exposure to phenol another 0.2 ml sample was removed and 1.8 ml medium added, and the mixture shaken for an hour with labeled glycine. Yeast metabolism was already seriously deranged; in twelve minutes the uptake factor had fallen to 0.86. Subsequent samples were taken at 30, 60, 120, and 240 minutes. The resulting curve (Fig. 4) is that of a typical disinfection experiment, substituting ability of the survivors to incorporate the label, rather than counting numbers of surviving cells.

DISCUSSION

The four physical and chemical conditions which have been demonstrated to affect uptake of radioactive carbon into yeast protein are factors which affect living cells in their gross metabolism and in their specific enzyme reactions. In the *Torula* system, rate of exchange of a radioactive carbon atom derived from substrate glycine, with an inert atom bound in the protein fraction of the yeast cell is apparently governed by the same factors which govern enzyme activity. Although this is not considered as direct evidence that the radioactive protein fraction isolated represents a true incorporation of amino acid units into protein molecules, it is interesting that GREENBERG *et al.* studied uptake of labeled glycine into protein of liver homogenates and reported the incorporation was apparently true synthesis of protein[8]. These workers were unable to demonstrate evolution of $^{14}CO_2$ from the unhydrolysed protein upon heating with ninhydrin reagent. Also Rittenberg demonstrated in rats that incorporation of labeled amino acids into protein was a replacement reaction; the isotopic amino acid replacing identical acids situated in the tissue protein[4]. If it can be considered that this replace-

References p. 571.

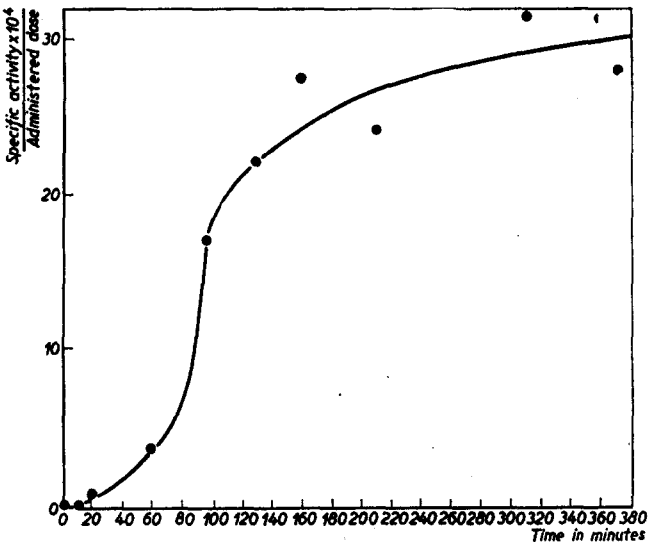

Fig. 1. Effect of time on uptake of radioactivity. Samples averaged 60 mg of protein and were exposed to labeled glycine having activity of 33,800 counts per two minutes

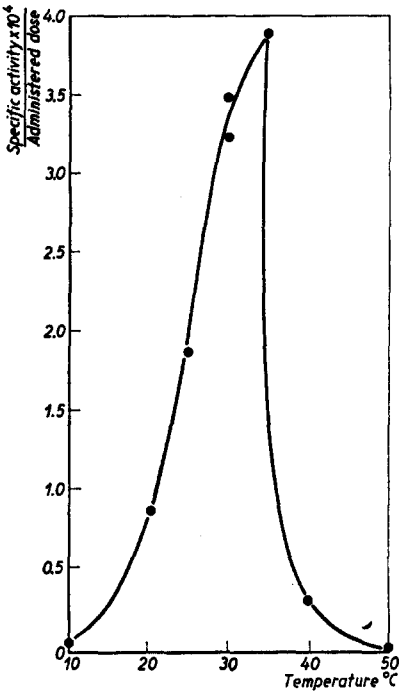

Fig. 2. Effect of temperature on uptake of radioactivity. Samples averaged 60 mg of protein and were exposed to labeled glycine having activity of 41,000 counts per two minutes

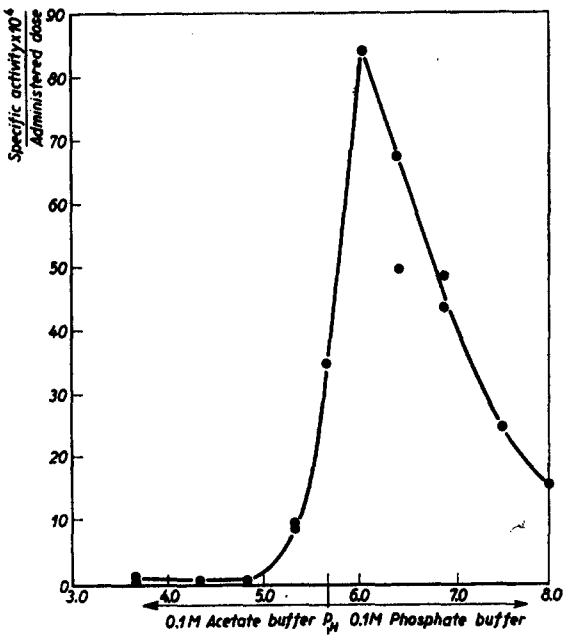

Fig. 3. Effect of p_H on uptake of radioactivity. Samples averaged 15 mg of protein and were exposed to labeled glycine having activity of 33,800 counts per two minutes

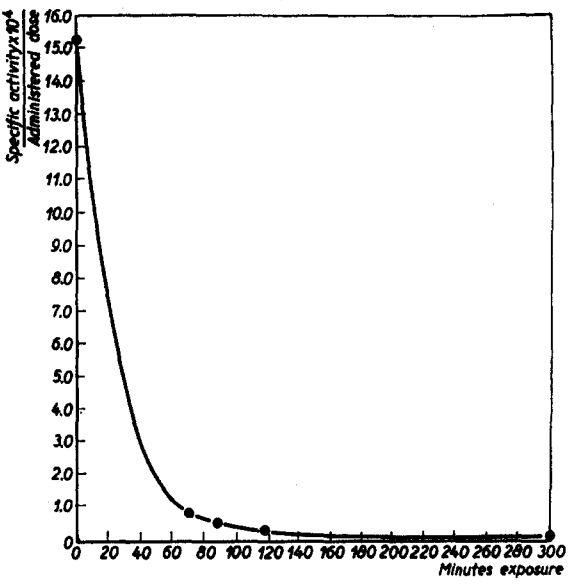

Fig. 4. Effect of phenol on uptake of radioactivity. Samples averaged 10 mg of protein and were exposed to labeled glycine having activity of 33,800 counts per two minutes

ment reaction is analogous to the anabolic phase of protein metabolism, and that the experiments reported here were concerned with replacement reactions, then it can be postulated that these factors which influence uptake of the radioactive label, and which govern enzyme activity, directly influence synthesis of protein.

The data presented here are further evidence that uptake of the radioactive label into the protein fraction of *Torula utilis* cells is an enzymatic process, and that the rate at which this process operates is influenced by time, temperature, p_H, and specific inhibitors.

SUMMARY

Torula utilis incorporates labeled carbon into its protein fractions by an enzymatic process. The ratio of this uptake is influenced by time, temperature, p_H and disinfectants. This enzymatic uptake might be an expression of protein synthesis operating as an exchange reaction.

RÉSUMÉ

La levure *Torula utilis* incorpore du carbone marqué dans la partie protéinique des cellules par un procédé enzymatique. La vitesse de ce phénomène est influencée par le temps, la température, le p_H et les désinfectants. Il pourrait être l'expression d'une synthèse protéinique opérant par réaction d'échange.

ZUSAMMENFASSUNG

Durch einen enzymatischen Vorgang wird markierter Kohlenstoff in die Proteinfraktion der Zellen von *Torula utilis* aufgenommen. Die Geschwindigkeit dieses Vorganges ist von der Zeit, der Temperatur, dem p_H und den Desinfiziermitteln abhängig. Diese enzymatische Kohlenstoffaufnahme könnte bedeuten, dass die Eiweiss-synthese als Austauschreaktion vor sich geht.

REFERENCES

[1] F. FRIEDBERG AND A. H. WEBB, *J. Bact.*, 58 (1949) 151.
[2] G. EHRENSVÄRD, *Cold Spring Harbor Symposia on Quant. Biol.*, 13 (1948) 81.
[3] D. M. GREENBERG, F. FRIEDBERG, M. P. SCHULMAN, AND T. WINNICK, *Cold Spring Harbor Symposia on Quant. Biol.*, 13 (1948) 113.
[4] D. RITTENBERG, *Cold Spring Harbor Symposia on Quant. Biol.*, 13 (1948) 173.

Received September 4th, 1950