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UREA FORMATION FROM PYRIMIDINES IN FRUIT-BODIES OF HIGHER BASIDIOMYCETES

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THE reductive pathway of pyrimidine degradation is widespread in nature[1]. Uracil is degraded via dihydrouracil and ß-ureidopropionic acid to ß-alanine, carbon dioxide and ammonia. By analogy, thymine is catabolized via dihydrothymine and ß-ureidoisobutyric acid to ß-aminoisobutyric acid, carbon dioxide and ammonia. The reductive pathway has been described from bacteria, Neurospora, animals, and, more recently, from tissues of higher plants[2-6]. The findings have been demonstrated under a variety of experimental conditions, using isotopes *in vivo* and *in vitro*, and in studies with isolated enzymes. ß-Aminoisobutyric acid and ß-alanine have been found

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in some plant tissues in considerable amounts.[7-10]

In several microorganisms a pathway of uracil and thymine degradation has been demonstrated that markedly differed from the reaction path involving reduced pyrimidines. In strains of Corynebacterium and Mycobacterium uracil is oxidized to barbituric acid. By analogy, thymine gives rise to 5-methylbarbituric acid[11-13]. The initial step in this mode of pyrimidine catabolism is an oxidative attack in position 6 of the pyrimidine nucleus. Barbituric acid is further degraded to urea and malonic acid, whereas 5-methylbarbituric acid is hydrolyzed yielding urea and methylmalonic acid[14].

Studying urea biosynthesis in fruit-bodies of urea accumulating higher basidiomycetes, we observed an unexpected high incorporation of radioactivity from uracil-2-^{14}C and orotic acid-2-^{14}C in urea. The present publication deals with this phenomenon and will discuss some possibilities of urea formation from pyrimidines.

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Materials and Methods.

Fruiting bodies of Agaricus bisporus (Agaricales) and Lycoperdon pyriforme (Gastrales) were supplied with either uracil-2-^{14}C or orotic acid-2-^{14}C by injecting the radioactive solutions into the inner parts of the carpophores (i.e., the sterile parts of Agaricus caps, and the gleba of Lycoperdon puffballs). The plant materials were killed by boiling 70% ethanol, and three-times extracted by grinding the fruit bodies with 70% ethanol. The alcohol extract was concentrated, and chromatographed two-dimensionally (ascending) on Schleicher & Schüll 2043 b papers, using propanol-water (az.) in the first direction, and phenol-water (3:1, g/v) in the second. Radioactivities in components of the 70% ethanol extract were determined by counting directly on the paper, using a Geiger-Müller tube. Urea and ureides were detected with Ehrlich's reagent; dihydropyrimidines are readily detectable with Ehrlich's reagent after an alcaline hydrolysis. Amino acids were localized with a ninhydrin spray. Urea was identified by elution from chromatograms and rechromatography in 6 different solvents by comparison with an authentic sample of urea. Incubation of eluted urea-^{14}C with commercial urease resulted in the liberation of ammonia and carbon dioxide-^{14}C. For estimation of specific activities (cpm/mmoles), urea was quantitatively determined after elution from papers, using the microdiffusion procedure of Conway[15]. Radioactivity of ureatically liberated $^{14}CO_2$ was determined on planchetts either as $Na_2^{14}CO_3$ in infinite thinness or as $Ba^{14}CO_3$ in infinite sample thickness.

Experiments and Results.

Caps of Agaricus bisporus were supplied with uracil-2-^{14}C (3,5 mc/mM; 4 µc/100 µl feeding solution). After increasing feeding periods, the percent distribution of radioactivity in components of an alcohol extract was determined (Table 1).

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Table 1. Catabolism of uracil-2-^{14}C in caps of Agaricus bisporus. Percent distribution of radioactivity in components of the 7o% ethanol extract.

Feeding time (hr)	uracil	urea	unknowns
2	92	3	5
8	71	1o	19
24	62	16	22

In feeding experiments using orotic acid-2-^{14}C, urea was the predominantly labelled compound, too. Analogous results were obtained when puffballs of Lycoperdon pyriforme were supplied with uracil-2-^{14}C or orotic acid-2-^{14}C (Table 2).

Table 2. Catabolism of ^{14}C-pyrimidines in puffballs of Lycoperdon pyriforme. Percent distribution of radioactivity in components of the 7o% ethanol extract.

	orotic acid-2-^{14}C		uracil-2-^{14}C	
	2 hr	8 hr	2 hr	8 hr
orotic acid	85	63	o	o
uracil	o	o	98	86
urea	3	21	1	8
unknowns	12	1[illegible]	1	6

The results of determination of specific activity are shown in table 3.

Table 3. Urea formation in puffballs of Lycoperdon pyriforme. Orotic acid-2-^{14}C or uracil-2-^{14}C were fed over 8 hours.

Compound fed	cpm/mmol urea-^{14}C	specific incorporation rate (%)
orotic acid-2-^{14}C (o,624 x $1o^9$ cpm/mmol)	o,552 x $1o^7$	8,83
uracil-2-^{14}C (o,2285 x $1o^9$ cpm/mmol)	o,3732 x $1o^7$	1,63

The data presented demonstrate a high incorporation of radioactivity from orotic acid-2-^{14}C and uracil-2-^{14}C in the urea of fruiting bodies of Agaricus and Lycoperdon. As shown by radioautography, there was no radioactivity in well-known members of reductive pyrimidine breakdown in animals and plants. In experiments in which both uracil-2-^{14}C and dihydrouracil were fed to fruit bodies of Agaricus, no radioactive dihydrouracil or ß-ureidopropionic acid were formed. Dihydrouracil was not degraded to urea. Thus it appears to be unprobable that uracil is reductively catabolized yielding carbon dioxide-^{14}C which is subsequently reutilized in urea biosynthesis via the Krebs-Henseleit ornithine cycle being operative in fruit bodies of the studied fungi[16, 17]. For, quite different labelling patterns were obtained when either $^{14}CO_2$ or uracil-2-^{14}C were fed to fruit-bodies of Agaricus and Lycoperdon. In experiments with $NaH^{14}CO_3$, radiocarbon was very readily introduced in members of the Krebs-Henseleit cycle and in a number of other compounds, including amino acids and amino acid amides. In experiments in which orotic acid-2-^{14}C and uracil-2-^{14}C were fed, however, there was no radioactivity in such compounds. Preliminary tests suggest that the unknown compounds formed after feeding ^{14}C-pyrimidines, are products of anabolic reactions of uracil and orotic acid (ribotides and related compounds?). In an investigation in which thin layers of hymenium-free Agaricus caps were fed with uracil-2-^{14}C, only a minor part of the radioactivity supplied (i.e., 0,32%) could be captured as $^{14}CO_2$. There was no evidence that barbituric acid was formed.

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Discussion.

Some ten years ago, it was shown that the dog and other animals which were maintained in nitrogen equilibrium, metabolized uracil and thymine, which resulted in an increased urea output. Thus, it was postulated that pyrimidines may be initially oxidized at carbon 5 which, in the case of uracil, yielded isobarbituric acid. Finally, this compound is metabolized to urea and oxalic acid.[18] The hypothetical scheme of pyrimidine degradation presented by Cerecedo, is not in accord with the bacterial mode of oxidative pyrimidine breakdown. However, the formation of urea as a final product of pyrimidine metabolism agreed with the finding that ^{15}N-labelled pyrimidines give rise to labelled urea by the intact rat[19,20]. Later investigations, however, on pyrimidine catabolism *in vivo* and *in vitro* demonstrated that the release of the ureido carbon as respiratory CO_2 represents the major pathway of uracil and thymine breakdown. Urea formation from pyrimidines in animals appears to be negligible at endogenous metabolic levels and of secondary importance at higher dose levels[21,22].

Our investigations demonstrated that in fruit bodies of some higher fungi which were supplied with pyrimidines labelled in position 2, urea was the main radioactive product. There was

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no evidence that the well-known reductive pathway of pyrimidine catabolism may be operative yielding $^{14}CO_2$ and ammonia which are subsequently utilized in urea synthesis via the reactions of the Krebs-Henseleit ornithine cycle. Thus, we are now discussing some mode of oxidative pyrimidine breakdown not including barbituric acid, or a reductive pattern that may imply ribotide derivatives of pyrimidines and their catabolites and a trans-carbamylation reaction, by-passing free carbon dioxide.

Abstract.

In fruit bodies of Agaricus bisporus and Lycoperdon pyriforme, urea-^{14}C is very readily formed from either uracil-2-^{14}C or orotic acid-2-^{14}C. There is no evidence that the over-all reductive pathway of pyrimidine breakdown followed by the re-assimilation of evolved $^{14}CO_2$ in urea synthesis via the Krebs-Henseleit ornithine cycle may be operative. Evidently, barbituric acid is not involved in uracil degradation. Possible reaction paths of urea formation from pyrimidines are discussed.

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