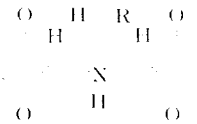
The conversion of A to B is specific to cellulose, being formed only on paper, cotton wool or cellulose powder. No reaction was obtained with starch, dextran, cellobiose or the monosaccharides. Moreover, the reaction requires an aqueous media (supplied by steam in the case of filter paper chromatograms). Thus no reaction will occur on acetylated paper and heating benzene solutions of pigment A with cellulose powder does not produce pigment B.

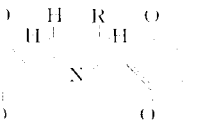
Spectroscopic analysis of the fluorescent light from pigment B indicated that it consisted of a broad red band including part of the yellow. The absorption peak at 570 mμ cannot be responsible for the fluorescence which only occurred in the presence of filtered u.v. light.

The chemical structure of pigment A was elucidated by GRASSMANN AND VON ARNIM[1] who proposed structures (1) and (2) but it is probable that in polar solvents structure (3) may make an important contribution.

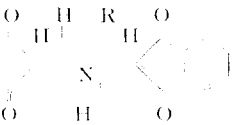
(1)



(2)



(3)



The above experimental data are consistent with the following interpretation, that pigment B is due to stabilization of structure (3) on the cellulose chains. This would account for the formation of B occurring only in aqueous media, its instability to alkali and stability to dilute acid and the immediate intensification of colour when A is converted to B on filter paper. Moreover, structure 3 when adsorbed on the linearly orientated cellulose chains could be fixed in a planar form which would be compatible with its fluorescent properties[6-8].

Due to the virtual non-extractibility of the pigment from the paper the possibility of its use for quantitative estimation of hydroxy-proline is limited to densitometric measurement on the paper. However the reaction will serve as a rapid and extremely sensitive test for hydroxy-proline (and to a less extent proline) on filter paper chromatograms.

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Evidence for a new precursor of methionine in *Neurospora crassa*

A "leaky" methionine-requiring mutant of *Neurospora crassa*, UT 27[1], accumulates a compound which promotes the growth of a series of *Neurospora* mutants blocked in different stages of methionine biosynthesis[2]. The chemical and biological properties of this compound indicate that it is a new intermediate in the conversion of homocysteine to methionine.

Mutant mycelia were obtained after growth for a period of two weeks in aerated carboys containing 2.5 liters of minimal medium. The harvested mycelia were extracted with water in a blendor and concentrated *in vacuo*. Occasional cultures exhibiting near wild type growth did not yield active extracts.

Bioautographs of the aqueous extracts were prepared by placing developed paper chromatograms on minimal medium containing 0.5% L-sorbose, 0.1% sucrose and 2.0% agar which had been previously inoculated with conidia of the cystathionineless *Neurospora* mutant 36104[2]. The major activity of the extract was due to a single substance which differed widely in R_F values from known intermediates in methionine biosynthesis (Table I). S-Adenosylhomocysteine was not available for testing, but published R_F values[3] for this compound differ greatly from those obtained for the accumulated intermediate.

TABLE I

Compound	*R_F values**	
	Solvent A	Solvent B
Methionine	0.62	0.76
Methionine sulfoxide	0.29	0.62
Homocysteine	0.20	0.54
Cystathionine	0.09	0.37
UT 27 Extract	0.49	0.60

* One dimensional (ascending). Solvent A: *n*-butanol, water, acetic acid (50:20:12); Solvent B: pyridine, water (13:7).

The compound gives a positive test for sulfur with the iodine-azide and iodoplatinate reagents. Hydrogen peroxide treatment results in the formation of a new compound which retains biological activity. Peroxide treatment in the presence of molybdate ion destroys biological activity. These data suggest that the compound contains a thioether linkage which is oxidized to a biologically active sulfoxide by peroxide and an inactive sulfone by peroxide in the presence of molybdate ion. Biological activity is retained when the compound is heated under conditions which destroy the sulfonium derivatives of methionine[1,3].

Other ninhydrin-active compounds in the extract migrate with the active principle on paper chromatograms, however, the presence of an amino group is indicated by the loss of activity on treatment with nitrous acid. Acetylation also destroys the biological activity of the compound for *Neurospora* mutants that fail to respond to *N*-acetylmethionine.

The biological activity of the compound for a series of mutants of *Neurospora* (Table II) suggests the following sequence for methionine biosynthesis:

$$\xrightarrow{H98} \text{Homocysteine} \xrightarrow{UT\ 112} \text{Intermediate} \xrightarrow[(UT\ 27)]{UT\ 122} \text{Methionine}$$

TABLE II

GROWTH OF *Neurospora* MUTANTS ON METHIONINE PRECURSORS

Compound	*UT 122*	*UT 112*	*H98*	*UT 70*	*UT 156*
Methionine	+	+	+	+	+
UT 27 Extract	—	+	+	+	+
Homocysteine	—	—	—	+	+
Cystathionine	—	—	—	+	+
Cysteine	—	—	—	—	+

A (+) sign indicates a positive growth response.

Further work on the isolation and identification of the active intermediate and a second compound which occurs in smaller quantities in the mycelial extracts is in progress.

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