STUDIES ON BROMINE-OXIDIZABLE SULFUR-CONTAINING COMPOUNDS IN MOLD METABOLISM

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While making a quantitative study of the metabolism of sulfur by Penicillium chrysogenum Q176, wherein the only source of this element was inorganic sulfate, it has been found that at least one-third of the organic sulfur is contained in metabolites that yield sulfuric acid when treated with bromine water.

The Penicillium strain was grown in submerged culture in a modified Czapek-Dox medium (chemically defined). The 110-ml cultures were grown on a rotary shaking machine for about 8 days. When penicillin production reached a maximum, the fermentation broth was separated from the mycelium by suction filtration. Bromine oxidations were restricted to the broth.

Approximately 500 ml of the raw fermentation broth were put in a porcelain dish and slowly evaporated to 40 ml. To this concentrated broth were added 180 ml of a mixture of equal volumes of acetone and distilled water. The aggregate was shaken well and allowed to stand at room temperature for 24 hours. The flourlike precipitate formed was separated from the solution by ordinary filtration. Tests for organic sulfur in the precipitate were negative. Solubility tests, employing the components in the original medium and the acetone-water solvent system, showed rather clearly that the precipitate did not contain sulfates. However, unmetabolized phosphates and lactose and, perhaps, related synthesized organic metabolites containing these compounds as a part of the molecule were present.

After the precipitate was removed, a small amount (2 ml) of concentrated hydrochloric acid was added to the dilute acetone, raw broth solution. Barium chloride (0.5 N) was added in slight excess. The solution was heated to boiling and allowed to stand at room temperature for 48 hours. The barium sulfate precipitate was collected on a piece of filter paper, employing slight suction, and then ignited and weighed. The sulfur contained in the barium sulfate represented that fraction of the sulfur originally present that was not metabolized by the organism.

After removal of the inorganic sulfur, a permanent excess of bromine water was added, with shaking, to the acetone-treated and acidified broth. The mixture was allowed to stand at room temperature for 24 hours. Since the solution was already acidic, additional hydrochloric acid was not added. Barium chloride (0.5 N) was then added in slight excess, after which the solution was heated to boiling and allowed to stand at room temperature for 48 hours. The barium sulfate was collected, ignited, and weighed in the manner previously described.

As a check on the specificity of the bromine oxidation, the broth from three cultures was combined in each of three different cases. Only one quantitative de-
termination was made on each aliquot. The amount of inorganic sulfur was determined on one, the amount of inorganic plus bromine-oxidizable sulfur was obtained on another, and the total sulfur was determined directly on the third. Under these conditions, the bromine-oxidizable sulfur was obtained without prior removal of the inorganic fraction, but after the flourlike precipitate induced by dilute acetone had been removed. The method employed in the bromine treatment was consistent with that already described. By having found the value of inorganic sulfur in the first aliquot, the amount of bromine-oxidizable sulfur was calculated. The values of the sulfur accounted for by the bromine oxidation were of similar magnitude, regardless of whether the fermentation broth had been altered by first removing the inorganic sulfate or treated directly with the bromine water.

Expressed in terms of the total sulfur originally present in the culture medium, an average of the values showed that 8.5 per cent of the sulfur was contained in compounds in the broth that gave rise to sulfate when treated with bromine. This represented more than one-third of the total organic sulfur obtained by the complete oxidation method of Evans and St. John (1944).

The sulfur-containing metabolites that were attacked by bromine cannot be specifically designated. According to Blumenthal and Clarke (1935), compounds such as cysteine, cystine, thiamine, biotin, and glutathione would not yield sulfate. However, compounds such as ROC (the xanthogenic acids) and

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\begin{align*}
\text{CH}_2\text{NH}_2\text{C} &= S \quad \text{thioacetamide) would be attacked; that is, (a) the compounds oxidized apparently contain sulfur in the form of sulphydryl linked to a carbon atom to which nitrogen or oxygen is attached, or (b) the compounds oxidized may contain sulfur linked to carbon by a double bond.}
\end{align*}
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In the present experiments, it is likely that compounds such as those mentioned above gave rise to sulfuric acid when treated with bromine water. That such compounds exist in mold metabolism has been indicated by Steinberg (1941) who, working with Aspergillus niger, showed that a wide variety of organic sulfur-containing compounds could be assimilated and utilized. Sulfur in certain compounds, such as thiourea, was not efficiently utilized; whereas that in others, such as thioacetamide, was very effective in promoting growth of the organism. Although the actual mechanism of assimilation was not clear, the experimental evidence shows that many different sulfur-containing compounds may exist during growth.

It appears, therefore, that there are unrecognized forms of sulfur in compounds produced by biological systems. The need for further study of the sulfur-containing intermediate compounds in mold and bacterial metabolism is suggested. It is possible that the identification of these bromine-oxidizable compounds would throw light on the many puzzling physiological activities of the sulphydryl and disulfide groups.
REFERENCES

