THE OCCURRENCE OF ERGOTHIONEINE IN PLANT MATERIAL*

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(Received for publication, July 12, 1955)

The question of the synthesis of ergothioneine by animals has been investigated in this Laboratory by the administration of isotope-labeled compounds considered to be likely precursors. No evidence for the formation of radioactive ergothioneine was obtained with C\textsuperscript{14} histidine in the rat, methyl-labeled C\textsuperscript{14}-methionine in the rat or chicken, S\textsuperscript{35}-methionine in the rat, guinea pig, or pig, and S\textsuperscript{35}-cystine in the human (1, 2). Studies with germ-free chickens have shown that the intestinal flora does not contribute significant amounts of ergothioneine (3). We have concluded that ergothioneine is not synthesized by any species of animal so far studied and is dietary in origin.

This evidence for the non-synthesis of ergothioneine by animals has been difficult to reconcile with the fact that ergothioneine apparently occurs universally in animal bloods, since attempts by various workers over a period of years have failed to demonstrate the presence of ergothioneine in plant material (3–6). We have therefore examined oats, which have been shown to give rise to blood ergothioneine when fed to animals (6–8), and have been able to demonstrate, by isolation of the crystalline compound, that this grain does in fact contain ergothioneine.

EXPERIMENTAL

A likely explanation for previous failures to detect ergothioneine in plant materials which are known to serve as dietary sources of ergothioneine could be the occurrence of the substance in a bound form. However, we have presented evidence to indicate that the precursor effect of corn is not associated with the protein fraction of this grain (2). It seemed possible that earlier difficulties might be due to a low concentration of the substance in plants (2), coupled with the fact that many naturally occurring substances interfere with the colorimetric methods which are used for the detection of ergothioneine. Accordingly, we directed our efforts toward the detection of free ergothioneine. Oats were chosen as the starting material because Baldridge and Lewis (6) and Baldridge (7) have shown them to be particularly effective as a dietary source of blood ergothioneine, while

* This work was aided by a grant from the National Science Foundation.
wheat and barley have been found to be less efficient in this respect (8), and corn has given variable results in the hands of different investigators (2, 5, 8, 9).

Rolled oats (The Quaker Oats Company, Chicago) of the kind sold for human consumption were fed to rats which had previously been depleted of blood ergothioneine by the feeding of a purified diet (10). Within 10 weeks the animals had blood ergothioneine levels of approximately 10 mg. per 100 ml. These values are considerably higher than those observed with corn (2) or other grains. However, attempts to identify ergothioneine in extracts of the oats, either directly or after alumina chromatography, were unsatisfactory because of the presence of substances which interfered with the color test with diazotized sulfanilic acid. More promising results were obtained when oat extracts were treated with basic lead acetate to remove interfering substances prior to chromatography. With this procedure we finally obtained effluent fractions which gave the characteristic magenta color of ergothioneine in the diazo test (11). This procedure was used in a quantitative fashion to follow the progress of purification during subsequent isolation experiments. The details of a fractionation procedure which led to the isolation of pure, crystalline ergothioneine are described below.

**Extraction of Oats**—2 kilos of rolled oats,1 which contained 34 mg. of ergothioneine on the basis of chromatographic analysis, were ground mechanically and added to 12 liters of hot water. The mixture was heated, with stirring, on a steam bath at 80–90° for 15 minutes and then 12 liters of 95 per cent ethanol were admixed. The liquid phase was removed as completely as possible by filtration through cheese-cloth in a press and evaporated to dryness under reduced pressure. The residue weighed 65 gm. and contained 23 mg. of ergothioneine.

**Treatment with Lead Acetate and Ion Exchange Resin**—The 65 gm. residue was mixed with 1.5 liters of water, and 190 ml. of a basic lead acetate solution (12) were added. The precipitate was separated by centrifugation and washed once with a small volume of water. The combined solutions were adjusted to pH 2 with concentrated sulfuric acid, and the precipitated lead sulfate was separated by centrifugation. The solution was passed through a column prepared from 1.5 kilos of the acetate form of Amberlite IRA-410 exchange resin (Rohm and Haas Company, Philadelphia) and the resin was washed with 1 liter of water. The total effluent was evaporated to dryness under reduced pressure. The residue weighed 34 gm. and contained 20 mg. of ergothioneine.

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1 These oats were part of a 100 pound lot purchased from The Quaker Oats Company. Crystalline ergothioneine was also isolated in comparable yield from “old fashioned” Quaker oats purchased at local food stores.
Alumina Chromatography—The residue from the ion exchange column was dissolved in 100 ml. of water and then 300 ml. of absolute ethanol were added. The precipitate which formed was separated by centrifugation and washed twice by dissolving it in water and reprecipitating with ethanol. The combined supernatant solutions were passed through a column prepared from 4 kilos of alumina (Alcoa, grade F-20) in a column 6 cm. in diameter. The column solvent was prepared by mixing 3 volumes of ethanol with 1 volume of water. 10 liters of solvent were used, and the effluent was collected in 1 liter fractions. The major portion of the ergothioneine appeared in Fractions 7, 8, and 9. These were combined and evaporated under reduced pressure. The dry residue weighed 857 mg. and contained 20 mg. of ergothioneine.

The 857 mg. fraction was chromatographed again on alumina. In this step, 80 gm. of alumina were used and 20 ml. fractions were collected. Fractions 6, 7, and 8 contained 283 mg. of solids and 15.4 mg. of ergothioneine. These fractions were combined, evaporated to dryness, and used in the next step.

Precipitation with Cuprous Oxide—The 283 mg. of solids were dissolved in 3 ml. of 0.5 N sulfuric acid and treated with cuprous oxide according to the procedure of Hunter, Molnar, and Wight (13). The resulting precipitate was decomposed with hydrogen sulfide, the solution was adjusted to pH 7 with barium hydroxide, and the hydrogen sulfide treatment was repeated. The solution which was obtained was evaporated to dryness. The residue weighed 81.6 mg. and contained 9.2 mg. of ergothioneine.

Alumina Chromatography—The residue was chromatographed on 20 gm. of alumina with 80 per cent ethanol as the solvent. 5 ml. fractions were collected. Fractions 8 through 13 contained most of the ergothioneine. They were combined and evaporated to dryness. The residue weighed 8.6 mg. and contained 7.2 mg. of ergothioneine.

Crystallization—The dried residue was dissolved in 0.03 ml. of water, 0.25 ml. of ethanol was added, and crystallization was induced by rubbing the vessel walls with a fine glass rod. The mixture was kept at 5° for 24 hours, the mother liquor was removed, and the crystals were washed with ethanol. 6.3 mg. of needles were obtained. As a final purification step, the material was dissolved in water and passed through a small column prepared from 1 gm. of IRA-410 acetate. The solid material from the column effluent was recrystallized from 0.02 ml. of water and 0.25 ml. of ethanol. Needles weighing 5.6 mg. were obtained.

The material was indistinguishable from ergothioneine in crystalline form and solubility. Like ergothioneine, it decomposed above 250° on the micro melting point stage. The color intensity in the diazo test was the same as that given by ergothioneine. The substance showed the same
behavior as ergothioneine in ascending paper chromatography with
n-butanol-acetic acid ($R_F$ 0.23) and with n-butanol-methanol-benzene
($R_F$ 0.45). The ultraviolet absorption spectrum in 95 per cent ethanol
was similar to that described for ergothioneine (2). Infra-red spectra of
the isolated material and of pure ergothioneine were obtained by incor-
porating finely ground 0.5 mg. samples into potassium bromide disks under
high pressure. The spectra were identical (Fig. 1).

![Infra-red absorption spectra](image)

**Fig. 1.** Infra-red absorption spectra of KBr blank (bottom curve), ergothioneine
(top curve), and the crystalline substance isolated from oats (middle curve).

**DISCUSSION**

The discovery of ergothioneine in a plant material clarifies considerably
the question of the origin of animal ergothioneine. It makes understand-
able the occurrence of ergothioneine in herbivorous animals and further
strengthens our belief that ergothioneine is not synthesized by animals in
general. These considerations are based on the assumption that oats are
not unique among edible plants in containing ergothioneine. This latter
point has not been thoroughly investigated; however, other cereal grains
have been shown to act as dietary precursors of blood ergothioneine, and
it seems reasonable to believe that they too contain the compound. In
this respect, it is of interest that the ability of corn to serve as a precursor
for blood ergothioneine is also shown by aqueous acetone extracts of
corn (2).

The oats used in these studies contained appreciably larger amounts of
ergothioneine than the corn which was used in our earlier work (2). The
value of 1.7 mg. per 100 gm. found by chemical analysis of the oats must
be taken as a minimal figure for the ergothioneine content. Other samples
of rolled oats of the same brand have given comparable values. It does
appear, however, from the results of Potter and Franke (8) that the ergo-
thioneine content of oats may vary from crop to crop. In view of the fact that we have found that some common microorganisms synthesize ergothioneine (14) and that included in this group are fungi which are commonly present in oats, it seems quite likely that at least part of the ergothioneine of this grain is of fungal origin. This might help to explain the variations in ergothioneine content of grain from different crops.

Appreciation is expressed to Dr. Julian R. Rachele for aid in carrying out the infra-red analyses.

SUMMARY

The heretofore unexplained ability of cereal grains to serve as dietary sources of blood ergothioneine has been investigated by an examination of oats for the presence of the compound. A chemical fractionation procedure has been devised which resulted in the isolation from oats of a crystalline compound identical in all respects with ergothioneine. This discovery of ergothioneine in a plant material makes understandable its wide-spread occurrence in animal species.

BIBLIOGRAPHY

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