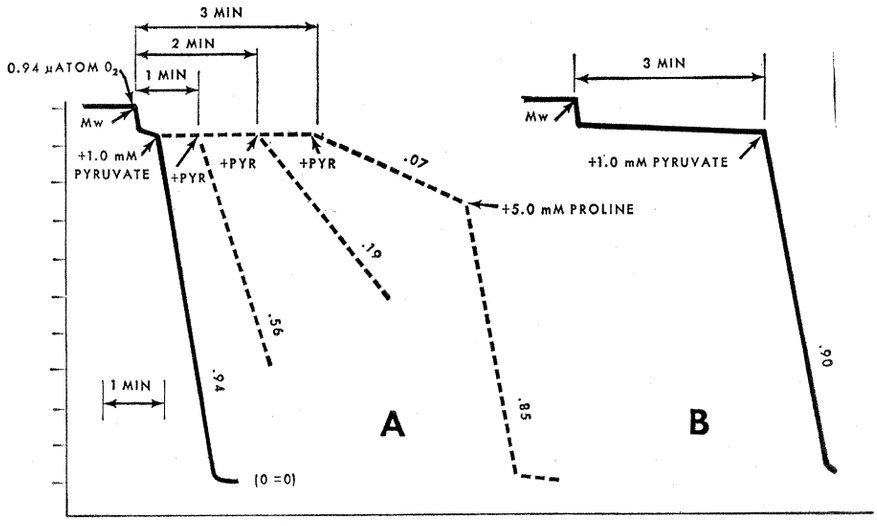

Fig. 4. Loss of pyruvate oxidation when added after the mitochondria. Mitochondrial isolation and suspension media are the same as described in Fig. 3. *A*, assayed at 30°C in the standard reaction medium containing 1.07 mg of mitochrondrial protein. *B*, assayed in standard reaction medium containing, in addition, 0.5 percent BSA, 5 m*M* ATP. Additions of mitochondria (*Mw*), pyruvate, and proline are shown by arrows. Ordinate is in units of microatoms of oxygen; the initial and final oxygen contents of the reaction medium, 0.94 and 0 μatom, respectively, are indicated. Abscissa is in units of time; 1 minute is indicated. The rates of oxygen consumption in microatoms of oxygen per minute are indicated along the curves.

ous α-ketoglutarate failed to prevent a decline in the rate of oxidation of pyruvate during long-term manometric reactions. Our finding that glutamate is also inadequate differs from his observation.

The correlation between the decrease in the rate of pyruvate oxidation and the increase in that of mitochondrial swelling suggests a functional relationship between the two. The protective effects of BSA, ATP, and Mg^{2+}, factors required for active mitochondrial contraction (*17*), support the view that inhibition of respiration and uptake of water may have resulted from a release of fatty acids from the mitochondria which was accelerated by the presence of Tris buffer. Swelling apparently leads to leakage of intramitochondrial precursors of oxaloacetate, thus accounting for the decline in the rate of pyruvate oxidation, as well as for the ability of proline to restore the original rate. Exogenous proline penetrates the mitochondria and is rapidly oxidized, via glutamate, to form intramitochondrial intermediates of the Krebs cycle (*14, 18*). Because they enter intact mitochondria very slowly, malate and glutamate were mostly ineffective in restoring pyruvate oxidation. The failure of added malate and glutamate to prime the oxidation of pyruvate also suggests that conditions which promote the leakage of endogenous substrates from the mitochondria do not necessarily increase the penetration of exogenous intermediates of the citric acid cycle into the mitochondria.

CHARLES C. CHILDRESS
BERTRAM SACKTOR
Medical Research Laboratory, Edgewood Arsenal, Maryland 21010

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Tetrachloroanisol: A Source of Musty Taste in Eggs and Broilers

Abstract. *Ingestion by hens and broilers of specific chloroanisols present in some wood shavings used in poultry cages can result in a musty taste in poultry products.*

A musty taste may occur in eggs and broilers from various commercial sources. This taste was noted in fresh eggs laid by hens at this institute. The hens had been fed a typical all-mash laying ration, ground oyster shell, and tap water. Changes of ration failed to eliminate the objectionable taste.

After wood shavings used in the bottoms of the cages to prevent egg damage were removed, the mustiness gradually disappeared. Within 2 to 3 weeks, the fresh eggs were normal in taste.

When 5 percent ground wood shavings from the batch previously used in the cages was added to the ration, hens produced musty eggs within 1 week. Japanese quail laid musty eggs within a few days, and the meat of broilers developed a similar taste.

To determine whether wood shavings, in general, contained a factor which caused the objectionable taste, samples of ten lots of wood shavings consisting of 130 bales were added to the diet (5 percent) as ground wood. None of the 130 samples contained the factor.

Twenty-five varieties of wood were similarly tested and two, limba (*Terminalia superba*) and obeche (*Triplochiton nigericum*), contained the factor. However, only 2 of 18 samples of limba caused the production of musty eggs. No further samples of obeche wood were tested.

The factor was found only in the superficial layer of the wood. This finding indicated that an outside influence, such as fungal growth on the wood or treatment of it with a wood preservative, might be responsible for the taste.

Three wood preservatives containing chlorinated hydrocarbons (dichlorobenzene, trichlorobenzene, and hexachlorocyclohexane), when used in feeding tests, produced an abnormal flavor distinctly different from that originally found.

The active factor was isolated from wood shavings by extraction with methanol, reextraction from methanol with ethyl ether, and fractionation of the ether extract by thin-layer chromatography on silica gel G (Merck; thickness of layer 1 mm) with ethyl acetate (15 percent) in hexane as the solvent. Feeding experiments with extracts from fractions of the plate material, followed by testing of the eggs, were used to locate fractions containing the active material. Portions of these active fractions were concentrated on cotton wool for distillation directly into the ionization chamber of a mass spectrometer. The resulting spectrum was identified as that of a tetrachloroanisol.

One milligram of pure 2,3,4,6-tetrachloroanisol (prepared by methylation of the parent phenol) per kilogram of weight was fed to hens and broilers. A musty taste, similar to that found when 5 percent active wood shavings was fed to them, developed in their eggs and in broiler meat. The musty taste of eggs was also produced by mixing 20 μg of the active factor into a normal egg.

Related chlorinated anisols and phenols were tested for activity: 2,4,6-trichloroanisol was more active than 2,3,4,6-tetrachloroanisol; but 2,4,5-trichloroanisol, pentachloroanisol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol were inactive.

The presence of tetrachloroanisol has since been demonstrated in eggs from hens fed on active wood shavings, in musty eggs from two commercial houses, in seven lots of active wood shavings, and in built-up litter taken from two commercial houses where musty eggs were produced. The compound has not been detected in normal eggs or in inactive wood shavings. Pentachloroanisol was found in all instances where the presence of 2,3,4,6-tetrachloroanisol had been established. Whether these compounds are present in wood shavings as a result of biotransformation or as a contaminant of wood preservatives is unknown.

C. ENGEL
A. P. DE GROOT
C. WEURMAN

Central Institute for Nutrition and Food Research TNO, Zeist, Netherlands

Note

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Purification and Reconstitution of the Periodic Fibril and Unit Structure of Human Amyloid

Abstract. *Extraction with alkaline sodium glycinate of the periodic fibril and unit structure of human amyloid and subsequent acidification of the extract provided a method for the purification as well as for the reconstitution of the periodic rod and of the unit structure. Guanidine completely solubilized and irreversibly dissociated both the rod and the unit structure of the human amyloid fibril.*

Recent electron-microscopic studies on negatively stained extracts of human amyloidotic tissue (*1*) revealed the presence of two distinct types of particles. One was a rod 100 Å wide; the other was a small pentagonal structure (unit structure) 90 Å in diameter (Fig. 1). Both morphologic units were present in all preparations of various tissues obtained from patients with either primary or secondary amyloidosis. Narrow electron-opaque bands divided the rods at intervals into smaller segments, the average distance from the center of one segment to that of the next being approximately 40 Å. A similar periodicity of amyloid fibrils, or segments thereof, has been described by several authors (*2*). Frequently, however, the width of the electron-opaque bands between segments varied irregularly, suggesting that the segments were easily disunited. When preparations containing predominantly long rods were exposed to high-frequency sound, a large increase in the number of unit structures (apparently single or double segments of the rods) was evident. Because the unit structures lay on their flat sides, the ends of the amyloid rod could be seen. The unit structures appeared to be composed of five globular sections surrounding a hollow core. A nonperiodic, 75-Å-wide fibril composed of two laterally aggregated parallel filaments with an apparent 100-Å beading has been described by Shirahama and Cohen (*3*) and solubilized, purified, and precipitated by Newcombe and Cohen (*4*). We attempted to reconstitute the periodic fibril of human amyloid, to purify it, and to characterize it by physical means.

The periodic structural components of amyloid in impure form were isolated, by a previously described method, from the spleen of a patient with secondary amyloidosis (*1*). The supernatant fluids of seven saline washings containing the periodic amyloid fibrils and unit structures were pooled, and the impure fibrils and unit structures were sedimented by centrifugation at 105,000*g* for 20 minutes at 4°C.

We used Newcombe and Cohen's method (*4*) for solubilizing and purifying the periodic fibrils and unit structures. The sediment of the washings centrifuged at 105,000*g* was shaken with Sörenson's glycine buffer (0.1*M*, *p*H 9.5). The glycinate extract was then centrifuged at 105,000*g* for 1 hour at 4°C, and the crystal-clear supernatant fluid was removed. A portion of it was examined with the electron microscope; another portion was used for electrophoresis on polyacrylamide gel. The remaining supernatant of fluid was dialyzed against 2 liters of 0.1*M* sodium acetate buffer (*p*H 4.5), the dialyzing solution being changed every 2 hours for 8 hours. A white, fluffy precipitate was formed, which was further dialyzed overnight against distilled water. The precipitated material was resuspended and centrifuged at 20,000*g* for 1 hour at 4°C. The white pellet obtained was again solubilized and precipitated, and this entire procedure was repeated twice.

Electron-microscopic examination of a portion of the original glycinate extract revealed only the unit structure,