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# VOLATILIZATION OF MUTAGENS FROM BEEF DURING COOKING

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## SUMMARY

The process of cooking beef substances which are mutagenic in the Ames *Salmonella*/microsome bioassay [1,2]. In this study, the formation and disposition of basic mutagens produced by cooking beef at different temperatures were examined. Mutagenic activity increased exponentially with cooking temperature between 137°C and 252°C. However, the amount of mutagenic activity remaining in the meat was only 1—7% of that which was volatilized into the air. The ingested dose of mutagens may therefore be significantly influenced by factors which restrict the dissipation of mutagens from the container, as well as by cooking temperature. Inhalation of airborne mutagens from cooking, as an alternative route of exposure, should be investigated when considered in light of some epidemiological data showing an excess of lung and bladder cancer among cooks and kitchen workers.

## INTRODUCTION

The search for a relationship between diet and human cancer has been approached by many different methods of inquiry [10]. One line of attack has been the identification and quantification of carcinogens and mutagens in cooked meat. Earlier studies [4] focussed on the measurement of polycyclic aromatic hydrocarbons, especially benzo[*a*]pyrene, in cooked and smoked foods. These investigations showed that polycyclic aromatic hydrocarbons were formed primarily from the pyrolysis of fats and that the levels of polycyclic aromatic hydrocarbons in meat were directly proportional to the temperature and duration of cooking. The recent development

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of a quantitative in vitro test for carcinogens and mutagens, the Ames *Salmonella*/microsome bioassay, has enabled the detection, isolation and identification of new classes of mutagens in cooked meats. Sugimura et al. [7,8,9,11,13] and Matsumoto et al. [5,6,14] have identified the chemical structures of several potent, basic mutagens produced by the pyrolysis of amino acids and proteins at temperatures exceeding 300°C. Recently, Commoner et al. [2,12] detected basic mutagens in ground beef cooked at 200°C and in beef extracts boiled at 105°C. Though the active compounds in Commoner et al.'s samples were not characterized, they appeared to be chromatographically distinct from the mutagenic products identified by Sugimura [7,8,9,11,13] and Matsumoto [5,6,14]. Taken together the above studies imply that virtually all cooking procedures leave mutagenic residues in meat, but the factors which affect the formation and disposition of mutagens are less clear. We wish to report here that when ground beef is cooked, over 90% of the basic mutagens formed are volatilized into the air. Cooking procedures which lead to the redeposition of airborne substances onto the surface of the meat will thus increase the levels of mutagens and result in a larger ingested dose. Inhalation of cooking emissions may also be a significant route of exposure to mutagens.

## MATERIALS AND METHODS

Beef was cooked in open and closed systems. Lean ground beef (< 30% fat) in 86 g patties was heated on a ceramic hot plate to surface temperatures ranging from 137°C to 252°C. The temperature of the cooking surface was measured with a Chromel-Alumel thermocouple beneath the meat and recorded with a strip chart recorder. The surface temperature increased continuously from the beginning to the end of the run. Those temperatures indicated were the means of all 1-min temperature readings during the run. All hamburgers were cooked for 10 min except those at 223°C and 252°C which were cooked for 15 min.

In the open system, the meat was cooked on a 14-cm Petri dish so that smoke and vapors were released into the air. The closed system consisted of a 1-l wide-mouth Erlenmeyer flask connected in series with a condensation trap in a dry ice-acetone bath followed by a fritted glass bubbler containing 150-ml of distilled $H_2O$. As meat was heated in the flask, dry compressed air flowing at 2.5 l/min continuously carried vapors and smoke into the condensation trap and the bubbler. The temperature inside the meat increased from ∿30°C at the beginning of each run to ∿80—90°C at the conclusion. All meat appeared edible, with the possible exception of that cooked at temperatures greater than 223°C, which was charred.

The cooked meat was homogenized for 5 min with 2 vol. of distilled water and filtered through glass wool into a 500-ml separatory funnel. Grease from the cooking surface and from the walls of the flask, material collected in the condensation trap, and the contents of the bubbler were

rinsed with distilled water into 3 separatory funnels. The samples were acidified to pH 2 with concentrated hydrochloric acid and extracted twice with 2 vol. of glass-distilled methylene dichloride to remove the acidic and neutral fractions. Sodium lauryl sulfate was added as required to eliminate emulsions. Basic fractions were obtained by adjusting the aqueous phases to pH 11 with concentrated ammonium hydroxide and re-extracting twice with 2 vols. of glass-distilled methylene dichloride. Sodium chloride was added as required to eliminate emulsions. Basic extracts were reduced to a few milliliters on a rotary evaporator and transferred to tared vials. Residues were dried under nitrogen and the vials reweighed to determine the mass.

Basic extracts were dissolved in dimethyl sulfoxide and 0.1-ml aliquots containing 0.125, 0.25, 0.50 and 1.0 mg were tested for mutagenic activity in histidine-dependent strains of *S. typhimurium* according to the method of Ames et al. [1]. In preliminary tests, mutagenic activity was detected principally in tester strain TA98 in the presence of Aroclor-induced rat-liver enzymes (S-9). Mutagens were also detected in TA1538 (with S-9), but at lower specific activities. Little or no activity was detectable in TA100 or TA1537. In subsequent bioassays only strain TA98 with S-9 was used. Duplicate plates were used for each measurement. Spontaneous reversion rates for TA98 in our laboratory ranged from 23 to 43 revertants/plate.

The specific activity of each fraction, expressed as net revertants/mg of extract tested, was calculated from the linear regression of the dose/plate vs. the observed number of revertant colonies corrected for spontaneous controls. Correlation coefficients of greater than 0.9 were obtained for each extract within the tested range of 0.125—1 mg/plate. 2-Aminofluorene (2-AF), a basic mutagen and carcinogen, was tested concurrently with every batch of samples in order to monitor variations that may occur in the Ames bioassay. 2-AF was tested at 5, 10 and 20 $\mu$g/plate and its activity ranged from 45 to 94 (mean = 73) net revertants/$\mu$g. The results of mutagenic activity were also presented as 2-AF equivalent units so that data from bioassays conducted at different times could be compared. The use of the 2-AF equivalence unit should not be interpreted as carcinogenic equivalence because the chemical identity and toxic properties of the mutagens in beef are, as yet, unknown.

## RESULTS AND DISCUSSION

The results, shown in Table 1, confirmed Commoner et al.'s observations that basic mutagens were present in cooked beef. The amount of mutagens in the meat increased with the temperature of the cooking surface. Furthermore, the amounts of mutagens in the closed system greatly exceeded those in the open system, indicating that mutagenic compounds were volatilized from the meat during cooking. These volatilized compounds not only collected in the condensation trap and the bubbler, but also condensed

TABLE 1

MUTAGENIC ACTIVITIES OF BASIC FRACTIONS (DETERMINED IN STRAIN TA98 IN THE PRESENCE OF S-9)

| Cooking system | Surface temperature (°C) | Residue weight (mg) | | | | Specific activity (net revertants/mg) | | | | 2-Aminofluorene equivalents (μg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | G | C | B | M | G | C | B | M | G | C | B |
| Open | 137 | 53.5 | NM | — | — | 6.0 | — | — | — | 3.4 | 7.1[a] | — | — |
| | 153 | 15.4 | 7.2 | — | — | 31.8 | 96.8 | — | — | 5.2 | 7.3 | — | — |
| | 173 | 10.6 | 4.3 | — | — | 95.7 | 474 | — | — | 10.8 | 21.6 | — | — |
| | 186 | 21.7 | 33.0 | — | — | 132 | 156 | — | — | 35.4 | 63.8 | — | — |
| | 252 | 22.8 | 11.4 | — | — | 113 | 115 | — | — | 57.6 | 29.1 | — | — |
| Closed | 137 | 55.0 | 19.4 | NM | 6.8 | 96 | 261 | — | 251 | 55.9 | 53.6 | 12.4[a] | 18.0 |
| | 152 | 19.9 | 20.2 | 15.5 | 19.6 | 121 | 192 | 269 | 188 | 53.8 | 86.3 | 93.1 | 82.3 |
| | 186 | 18.2 | 19.9 | 18.3 | 25.6 | 334 | 835 | 511 | 442 | 75.2 | 206 | 116 | 140 |
| | 223 | 15.2 | 43.7 | 584 | 280 | 156 | 2240 | 271 | 196 | 53.0 | 2183 | 3533 | 1226 |

[a] Mass of residue not measured. Total activity determined by washing vial with DMSO and applying to bioassay.
M, meat; G, grease; C, condensation trap; B, bubbler; NM, not measured.

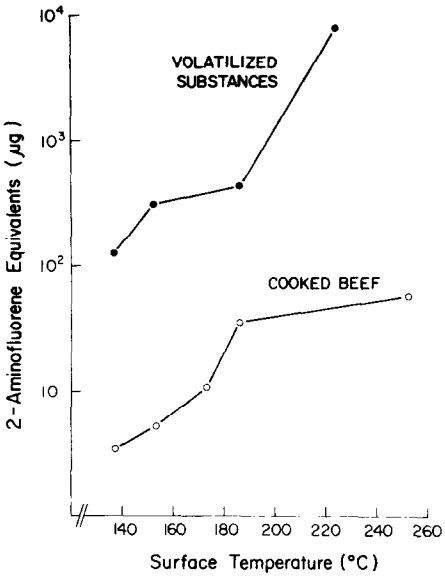


Fig. 1. Amounts of basic mutagens produced at various cooking-surface temperatures in terms of equivalent amounts of 2-aminofluorene. Open circles represent mutagens remaining in meat cooked in the open system. Closed circles represent mutagens volatilized from meat cooked in the closed system (mutagens found in: condensation trap + bubbler + (meat in closed system — open system ) + (grease in closed system — open system).

within the cooking flask itself to be returned to the meat and grease of the closed system. Figure 1 shows that the volatilized mutagens, which included smoke particles and liquid droplets, exceeded those remaining in the meat cooked in the open system by 14—100 times and comprised 93 ± 8% ($x \pm s$) of all the activity.

This study of the volatilized mutagens formed during the cooking process thus revealed 2 factors which may critically affect the dose of ingested mutagens: the temperature at which the meat is cooked and the disposition of the airborne mutagens after they are formed. More mutagens are formed at higher temperatures, but this effect appears to be self-limiting because the mutagens volitalize more readily from the meat at higher temperatures (Table 1). The effect of temperature may be quantitatively less important than the cooking conditions which affect the dissipation of mutagens from the meat. If the egress of the airborne mutagens from the container is restricted so that redeposition occurs, the levels of mutagens in the meat and grease increase dramatically (Table 1). This situation is analogous to those encountered with some commercial cooking devices which enclose the meat.

Since most of the mutagens produced during the cooking of beef are apparently released into the air, exposures may arise from inhalation as well as ingestion. Given the strong correlation between the mutagenic

and carcinogenic properties of chemicals, the investigation of an association between long-term exposures to airborne mutagens from cooking and the risk of cancer may be worthwhile. A recent retrospective study [3] found that female cooks (males were not investigated) have a higher relative risk for cancer of the bladder and lung than an appropriate control population. The relative risk ratio for cancer of the bladder was 4.7 ($P = 0.01$) and that of the lung, 2.93 ($P = 0.02$). Female kitchen workers were also found to have a higher risk of bladder cancer; the relative risk ratio was 2.73 ($P = 0.04$). These figures were adjusted for smoking habits and represent one of the few statistically significant observations made in this survey of many occupational groups.

These observations of the formation and volatilization of mutagens indicate that the temperature and method of cooking meat are important determinants of exposure. It is apparent that cooking procedures which reduce the redeposition of volatilized materials on food and ventilation systems which efficiently remove airborne emissions from cooked foods will serve to minimize the ingested and inhaled dose of mutagens.

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