gallbladder, pituitary gland, and large intestine had the highest levels of $^{35}S$ radioactivity equivalent to 0.82, 0.81, 0.81, and 0.76 ppm, respectively. This tissue sequence was not maintained by the $^{3}H$ residues; the liver had 3.38 ppm, gallbladder 1.10, kidney 0.81, and thymus 0.56 ppm equivalents. Of the tissues analyzed, the fats contained the lowest levels of radioactivity. Of the nine tissues from sheep B that were quantitated, the thyroid, liver, kidney, and adrenals had the highest levels of 1.26, 0.99, 0.77, and 0.49 ppm equivalents, respectively (Table II).

The two major nonpolar $^{35}S$ metabolites in urine (35–40% of the radioactivity in the chloroform extracts) were at $R_f$ 0.64 and 0.72. Most of the remainder of the radioactivity was $^{3}H$ metabolites with $R_f$ values of 0.48, 0.64, and 0.72. Less than 5% of the radioactivity was in the ferbam band area.

The two major nonpolar $^{35}S$ metabolites in feces (25 to 35% of the radioactivity in the chloroform extracts) were at $R_f$ 0.64 and 0.72. Most of the rest of the radioactivity was $^{3}H$ metabolites at $R_f$ values of 0.48 and 0.64, with no product at the 0.72 value.

Only four metabolites were found in the polar extracts of feces. The polar metabolites in the urine (60–70% of the radioactivity) resolved into five components, but no further attempts were made to chromatograph these products.

None of the above metabolites co-chromatographed with the available metabolite standards, dimethylamine and TMTD.

If the hydrogens are most stable and labeling in the dimethyl group represents a bonding area not readily exchanged with $H^+$ of water, the 82% elimination (62% in urine and 20% in feces) of the $^{3}H$ moiety indicates a molecular degradation of ferbam beyond the finding of Owens (1960), who speculated that ferbam operated through free radical rather than ionic mechanisms and proposed the following theoretical reaction:

$$((CH_3)_2NCS_2)_3Fe \rightarrow ((CH_3)_2NCS_2)_2Fe + (CH_3)_2NCS_2$$

The elimination of the $^{35}S$ moiety probably was not as $CS_2$ because the authors found no dimethylamine. Owens (1960) further concluded that ferbam retards citrate synthesis and indicated that the inhibition of enzymes resulted from complex formation with metals of the metal-containing enzymes or by interference in electron shifts between sulfhydryl or amino groups of the enzyme and substrate molecules. Owens (1969) and Weed et al. (1953) also found that the activity of the sulfhydryl-dependent enzymes was inhibited by ferbam. Our observations on the cellular distribution or "flooding" of the $^{35}S$ moiety through all the tissues of sheep support the conclusions of Owens (1969) and Weed et al. (1953) on the metabolic pathways of ferbam and its dithiocarbamic acid derivatives.

ACKNOWLEDGMENT

The authors wish to thank J. S. Palmer for collecting tissues and blood samples.

LITERATURE CITED

Budd, A. L., Newman, F. M., Thompson, B., Beckman Instrument Analytical Data Sheet No. 100, 1968.
Hunt, L. M., Gilbert, B. N., *Int. J. Appl. Radiat. Isot.* **23**, 246 (1972).
Lindsay, P. A., Kurneck, N. B., *Int. J. Appl. Radiat. Isot.* **20**, 97 (1969).
Owens, R. G., "Developments in Industrial Microbiology", Vol. I, Plenum Press, New York, N.Y., 1960, pp 187–205.
Owens, R. G., *Ann. N.Y. Acad. Sci.* **160**, 114 (1969).
Turpin, R. A., Bethune, J. E., *Anal. Chem.* **39**, 362 (1967).
Weed, R. M., McCullan, S. E. A., Miller, L. P., *Contrib. Boyce Thompson Inst.* **17**, 299 (1953).

**LaWanda M. Hunt***
**Bennye N. Gilbert**

U.S. Livestock Insects Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Kerrville, Texas 78028

Received for review October 6, 1975. Accepted January 12, 1976. This paper reflects the results of research only. Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

## Anti-Tumorigenic Effect of Maleic Hydrazide on Mouse Skin

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione), a tobacco sucker-control agent which has been detected in relatively high concentrations in cigarette smoke, was tested for tumorigenic activity on mouse skin. MH significantly inhibited the "initiation" phase of two-stage tumorigenesis caused by 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The inhibitory activity of MH resembled that of its parent compound maleic anhydride. At 5000 times its residue level in cigarette smoke, MH did not initiate tumor development on its own.

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione) is used extensively in agriculture as a herbicide and as a growth retardant to prevent suckering in tobacco plants. It is estimated that fully 80% of American grown tobacco is routinely treated with this chemical (Tso, 1972). The oral and parenteral toxicities of MH have been studied (Barnes et al., 1957; Nasrat, 1965) but the question of its carcinogenic properties is widely disputed among investigators (Dickens and Jones, 1965; Epstein and Mantel, 1968; Hunter et al., 1973).

Relatively high MH residues have been detected in tobacco and other crops (Ihnat et al., 1973), a factor which increased in importance when Liu and Hoffmann (1973), using sensitive assay techniques, found that over 1 μg, or about 4%, of the 30 μg of MH in an average American cigarette was transferred unchanged to the mainstream smoke, and thus was available to the smoker. Haeberer and Chortyk (1974) confirmed the presence of MH in cigarette smoke and also detected concentrations of up to 20 ppm in cigarette smoke condensate (CSC), commonly called "tar". These findings focus attention on the possible contribution of MH to the tumorigenicity of cigarettes.

Much of the experimental data on the tumorigenic activity of cigarettes are results from application of smoke condensate or its constituents to mouse skin. Salaman and Roe (1956) tested MH by the mouse skin bioassay and

Table I. Tumor-Initiating Activity of MH and DMBA[a]

| Treatment | No. of appl. | Promoter[b] | No. of mice | Mice with papillomas | Total No. of papillomas |
|---|---|---|---|---|---|
| MH, 500 μg | 10 | TPA, 1.5 μg | 30 | 0 | 0 |
| DMBA, 90 μg | 1 | TPA | 30 | 8 | 25 |
| DMBA | 1 | Acetone | 25 | 0 | 0 |
| Acetone | 10 | TPA | 10 | 0 | 0 |

[a] Initiators were applied to shaved backs of 55-day old ICR Swiss Mice. [b] Promoter applied 5 times weekly beginning 3 weeks after first initiator application.

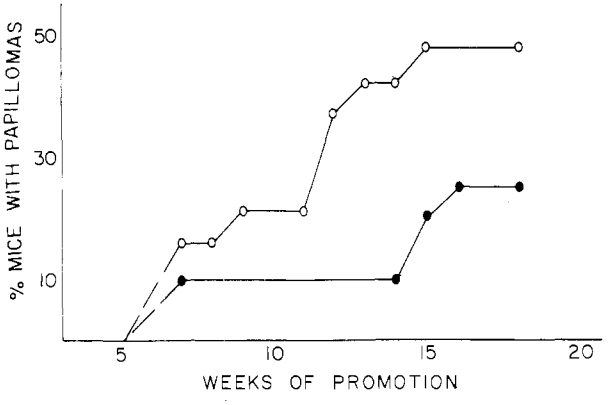


Figure 1. Effect of MH on the tumor-initiating activity of DMBA. Acetone (○) or 30 μg of MH (●) was applied 4 times in the 48-h interval prior to a single application of DMBA (90 μg) and 3 times during the 36 h after DMBA. Thrice weekly applications of TPA (1.5 μg) were begun 3 weeks later. The number of tumor bearers was significantly less ($P < 0.05$) in the MH-treated mice after 14 weeks of promotion.

concluded that it was inactive as a tumor initiator. It was not tested for other possible biologic effects. Tobacco smoke contains not only tumor inducers but also tumor inhibitors or "anticarcinogens", only a few of which have been identifed (Van Duuren and Melchionne, 1971; Akin and Chamberlain, 1974). MH retains structural similarities to maleic anhydride and maleic acid, the two precursors used in its commercial preparation. Maleic anhydride inhibits the initiating phase of two-stage tumorigenesis on mouse skin caused by the carcinogenic hydrocarbon 7,-12-dimethylbenz[*a*]anthracene (DMBA) (Klein, 1965). Maleic acid has anticarcinogenic activity as well (Crabtree, 1947).

The present report of experimental studies on mice describes an inhibitory effect of MH on DMBA-induced tumorigenesis, and provides evidence confirming the failure of MH to "initiate" tumor development on its own.

## EXPERIMENTAL SECTION

Female ICR mice in groups of 25–30 were 55 days old when experiments were begun. Hair was clipped from the treatment region, a 9-cm$^2$ area on the mouse's back, prior to and periodically during the experimental trials. Practical grade MH, recrystallized twice from water, was dissolved in hot water (1 part) and diluted to volume with acetone (5 parts). DMBA and the tumor-promoting agent, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), were dissolved in acetone.

To test its effect on the tumor-initiating action of DMBA, MH was applied 4 times before and 3 times after a single application of DMBA. Control mice were treated with acetone before and after DMBA. Tumor promotion with TPA was begun 3 weeks later.

To test MH for tumor-initiating activity of its own, mice were treated with 10 applications of 500 μg each over a period of 2 weeks. The control group received a single application of 90 μg of DMBA. Three weeks later TPA treatment was begun in both groups. All mice were examined weekly for the development of papillomas measuring 1 mm in diameter or larger. Numbers of tumor-bearing mice in treatment and control groups were compared by the chi-square test.

## RESULTS AND DISCUSSION

The effect of MH on DMBA-initiated tumorigenesis is shown in Figure 1. Papillomas first began to appear in both groups after 5 weeks and were measurable at 7 weeks of promotion. From the 9th week onward about half as many MH-treated mice developed papillomas as did acetone controls. Also, the average number of papillomas per positive mouse was lower in the MH group, 2.0 as compared to 2.6 in the acetone group. The action of MH as an inhibitor of DMBA-induced tumorigenesis closely resembled that of its parent compound maleic anhydride.

The inhibition of a potent carcinogen by a weaker one has been well documented (Steiner and Falk, 1951) and certain carcinogens actually diminish the growth of established tumors (Haddow, 1935). MH, however, gave no indication of possessing tumor-initiating activity of its own. After 23 weeks no tumors were observed in the group receiving 5 mg of MH as initiator and TPA as promoter, while 27% of the mice initiated with 90 μg of DMBA developed at least 1 papilloma (Table I). These results agree with those of Salaman and Roe (1956) who reported negative initiator activity of MH with croton oil as the promoting stimulus.

CSC possesses tumor-initiating activity which is due solely to a group of polynuclear aromatic hydrocarbons (PAH) located in the neutral portion (Hoffmann and Wynder, 1968). The quantity of known carcinogenic PAH isolated from the condensate is less than 0.5 μg per cigarette (Wynder and Hoffmann, 1967). Recently, we reported that a PAH-containing neutral fraction of CSC had more than twice the initiating potency of the crude preparation (Akin et al., 1975), further supporting the concept that some of the agents in cigarette "tar" are anticarcinogenic. In smoke from tobacco treated with the sucker control agent, MH can be present in much higher concentrations than the PAH. The present findings indicate, that on mouse skin, the biological action of MH is that of an inhibitor to carcinogenesis and not as an initiator of carcinogenesis. Whether this effect of MH is important to the human smoking situation awaits further study.

## LITERATURE CITED

Akin, F. J., Chamberlain, W. J., *J. Natl. Cancer Inst.* **52**, 613 (1974).
Akin, F. J., Chamberlain, W. J., Chortyk, O. T., *J. Natl. Cancer Inst.* **54**, 907 (1975).
Barnes, J. M., Magee, P. N., Boyland, E., Haddow, A., Passey, R. D., Bullough, W. S., Cruickshank, C. N. D., Salaman, M. H., Williams, R. T., *Nature (London)* **180**, 62 (1957).
Crabtree, H. G., *Br. Med. Bull.* **4**, 345 (1947).
Dickens, F., Jones, H. E. H., *Br. J. Cancer* **19**, 392 (1965).
Epstein, S. S., Mantel, N., *Int. J. Cancer* **3**, 325 (1968).
Haddow, A., *Nature (London)* **136**, 868 (1935).
Haeberer, A. F., Chortyk, O. T., *J. Agric. Food Chem.* **22**, 1135 (1974).

Hoffmann, D., Wynder, E. L., *Natl. Cancer Inst. Monogr.*, **28**, 151 (1968).
Hunter, B., Mawdesley-Thomas, L. E., Worden, A. N., *Toxicology* 1, 301 (1973).
Ihnat, M., Westerby, R. J., Hoffman, I., *J. Assoc. Anal. Chem.*, **56**, 1164 (1973).
Klein, M., *J. Natl. Cancer Inst.* **34**, 175 (1965).
Liu, Y., Hoffmann, D., *Anal. Chem.* **45**, 2270 (1973).
Nasrat, G. E., *Nature (London)* **207**, 439 (1965).
Salaman, M. H., Roe, F. J. C., *Br. J. Cancer* **10**, 363 (1956).
Steiner, P. E., Falk, H. L., *Cancer Res.* **11**, 56 (1951).
Tso, T. C., "Physiology and Biochemistry of Tobacco Plants", Dowden, Hutchinson and Ross, Stroudsburg, Pa., 1972.
Van Duuren, B. L., Melchionne, S., *Prog. Exp. Tumor Res.* **12**, 55 (1969).
Wynder, E. L., Hoffmann, D., in "Tobacco and Tobacco Smoke", Academic Press, New York, N.Y., 1967, Chapter 7.

**Frank J. Akin**
United States Department of Agriculture
Agricultural Research Service
Richard B. Russell Agricultural Research Center
Athens, Georgia 30604

Received for review October 8, 1975. Accepted December 29, 1975.

# Amino Acid Composition of the Endosperm Free Amino Acids and Proteins from a Maturing Common Wheat and Its Extracted Tetraploid

The compositions of the total amino acids and the free amino acids within the endosperm of the common wheat (*Triticum aestivum* L. em. Thell) cultivar Thatcher were compared with those of its AABB extracted tetraploid Tetrathatcher at intervals during kernel development. Rapid changes occurred in the compositions of the total amino acids within the endosperms of both wheats during development. The levels of free amino acids within the endosperm decreased with increasing maturity. At comparable stages of kernel development the total amino acids and free amino acids were of similar composition in both wheats.

The baking quality of synthetic and natural AABBDD hexaploid wheats is generally superior to the baking quality of extracted and natural AABB tetraploid wheats (Kerber and Tipples, 1969). This has been attributed to quantitative (Dronzek et al., 1970) and qualitative (Boyd and Lee, 1967; Boyd et al., 1969; Bietz and Wall, 1972; Orth and Bushuk, 1973a,b, 1974) differences in their gluten proteins. Dronzek et al. (1970) found that removal of the D-genome did not significantly alter the amino acid composition of wheat flour or the amino acid composition of the soluble Osborne protein fractions. The present investigation was undertaken to ascertain whether the D-genome has a significant effect on the amino acid composition of the free amino acids and proteins in wheat endosperm during kernel maturation.

## EXPERIMENTAL SECTION

**Plant Material.** The common wheat (*Triticum aestivum* L. em Thell) variety Thatcher and the AABB tetraploid (Tetrathatcher) derived by Kaltsikes et al. (1968) were grown in a controlled environment chamber (21 °C, 16 h light). Prior to planting, chromosome counts were performed on the germinated Tetrathatcher seeds to ensure that only 28-chromosome plants were grown. Grain development was determined by noting the date of anthesis for each head. Each sample consisted of 12 heads from 12 different plants selected at the same stage of development. Both wheats were fully mature 49 days after anthesis.

Endosperm tissue was excised from immature seeds by hand dissection, freeze-dried, and ground in a Wiley mill to pass through a no. 60 sieve. Flour from the mature samples was obtained by grinding in a Brabender Quadrumat Junior Mill.

**Amino Acid Analyses.** Endosperm samples were hydrolyzed as described by Orth et al. (1974) and analyzed according to the method of Spackman et al. (1958) on a Beckman 121 automatic amino acid analyzer. Precision was better than ±3% for all amino acids listed except methionine which was partially converted to the sulfoxide during hydrolysis. Cysteine, cystine, and tryptophan were destroyed during hydrolysis.

Kjeldahl nitrogen accounted for by the amino acid analyses ranged from 80 to 92%. Recoveries were lowest for the immature samples, probably because they contained substantially more nonprotein nitrogen (Hoseney et al., 1966). For the purpose of calculating amino acid distribution, the nitrogen recovered from the chromatographic columns was used to estimate the amino acid nitrogen content of the samples. The results were adjusted to a common recovery (90%) in order to eliminate the effect of varying nonprotein nitrogen levels.

**Free Amino Acid Analyses.** Free amino acid extractions were performed and the free amino acids separated on the Beckman 121 automatic amino acid analyzer as described previously (Dexter and Dronzek, 1975).

Several samples of varying maturity from the two wheats were analyzed in duplicate. The standard deviations were less than 3 and 10% for major and minor components, respectively.

The concentrations of the free amino acids were expressed as grams of nitrogen per 100 g of amino acid nitrogen computed from the total amino acid analyses of the endosperms.

## RESULTS AND DISCUSSION

**Nitrogen Content.** The total nitrogen content of the endosperms from Thatcher and Tetrathatcher decreased on a dry matter basis during the early stages of kernel development and increased as the kernels neared full maturity (Figure 1). Similar results were reported by Jennings and Morton (1963) for maturing Australian