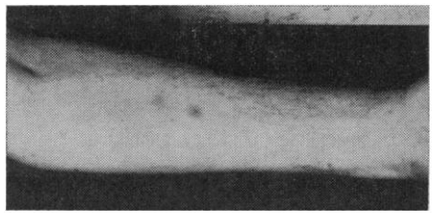

Fig. 1. Black-and-white reproduction of an original color transparency made of a typical passively transferred, delayed skin reaction to extract of timothy pollen as it appeared 24 hours after intradermal challenge. The darkening adjacent to the reaction is due to a previous test with an unrelated antigen.

fer would seem to be ruled out by the results of the specific transfer experiments (Expts. 8, 9, 10, and 12) and by the several failures encountered (Expts. 2, 3, 6, and 11). Evidently, successful transfer depends both on the number of cells injected and the degree of sensitivity of the donor. Thus the only failure with a 4+ donor occurred when 66 million cells were injected (Expt. 6), and the other three failures all occurred with donors who were only 2+ (Expts. 2, 3, and 11). Taken together, these results show that typical delayed hypersensitivity has been transferred by the injected cells and that the reaction is specific for the antigen to which the donor is sensitive.

Table 1 also shows the numbers and purity of the cell populations injected into the recipients. Microscopic examination of the daub preparations revealed that the cell populations consisted of from 94 percent to 99 percent of cells morphologically indistinguishable from the peripheral lymphocyte. The vast majority of these appeared to be small lymphocytes. A few polymorphonuclear neutrophils and rare basophils and eosinophils made up the balance of the cells seen, while occasional monocytes could not be definitely excluded. Very few platelets were seen on slide preparations from any of the injected cell suspensions. Quantitative data obtained on the lymphocytes in the blood of the column effluents showed that from 37 to 90 percent of the lymphocytes in the donor's blood were recovered in the effluents, whereas in the final suspensions used for injection only from 9 to 31 percent of the lymphocytes were recovered. It is evident that segregation of subpopulations of lymphocytes during the two fractionation steps cannot be excluded. The details of the washing procedure are of interest because the final suspensions showed no aggregates when they were observed on the white cell counting chamber, and the distribution of cells permitted an accurate and reproducible count as well as facilitated the morphological examinations. The fact that the lymphocytes were washed three times decreases the probability that a soluble factor from disrupted platelets, polymorphonuclear neutrophils, or other minor cellular components could remain adherent to the lymphocytes and thus confer upon them the ability to transfer.

In conclusion, while our experiments do not prove that the small lymphocytes of the peripheral blood are necessary for the transfer of delayed hypersensitivity to timothy grass and ragweed antigens in man, they do show that some preparations of circulating lymphocytes from sensitized subjects are both sufficient and specific for the transfer.

RAYMOND G. SLAVIN
JAMES E. GARVIN
Departments of Internal Medicine and Biochemistry, Northwestern University Medical School, Chicago 11, Illinois

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Benzo(a)pyrene and Other Polynuclear Hydrocarbons in Charcoal-Broiled Meat

Abstract. *The possible production of carcinogenic polynuclear hydrocarbons in the charcoal broiling of food has been investigated. Fifteen steaks were cooked and the polynuclear compounds were extracted, separated by chromatography, and identified spectrometrically. Many polynuclear hydrocarbons were identified, but no nitrogen heterocyclic compounds were detected. The carcinogen benzo(a)pyrene was present in the average amount of 8 micrograms per kilogram of steak.*

It has been suggested many times that high-temperature cooking of food might give rise to carcinogenic hydrocarbons of the polynuclear aromatic group. Indeed, there has been one report of the finding of dibenz(*a,h*)anthracene in charcoal-broiled meat (*1*). Five years ago an analysis of charcoal-broiled steak was carried out in this laboratory and several polynuclear hydrocarbons were separated and identified, including pyrene, fluoranthene, chrysene, benz(*a*)anthracene, and benzo(*g,h,i*)perylene. The analytical method used was cumbersome and lengthy, involving alkaline and acid hydrolyses occupying several weeks, and undoubtedly introduced serious losses, particularly of any unstable hydrocarbons present. The experiment has been repeated with a simpler and more sensitive method now available (*2*).

Fifteen steaks were charcoal broiled, the outer layers were removed and extracted, the polynuclear material was extracted from the lipids, and the polynuclear aromatic hydrocarbons were separated by column and paper chromatography. Identification and measurement were made by ultraviolet absorp-

Table 1. Polynuclear hydrocarbons in charcoal-broiled steaks.

Compound	Total (μg)	Concentration in μg		
		Per steak	Per kilogram	Per 100 cm^2
Anthanthrene	29	2	2	0.3
Anthracene	71	5	4.5	0.7
Benz(*a*)anthracene	76	5	4.5	0.8
Alkyl-benzanthracene	40	2.7	2.4	0.4
Benzo(*b*)chrysene	7.5	0.5	0.5	0.08
Benzo(*g,h,i*)perylene	76	5	4.5	0.8
Benzo(*a*)pyrene	133	9	8	1.3
Benzo(*e*)pyrene	97	6.5	6	1.0
Chrysene	21	1.5	1.4	0.2
Coronene	37	2.5	2.3	0.4
Dibenz(*a,h*)anthracene	3.5	0.2	0.2	0.04
Fluoranthene	321	21	20	3.2
Phenanthrene	180	12	11	1.8
Pyrene	286	19	18	2.9
Perylene	34	2	2	0.3

tion and by fluorescence spectrometry.

The results of the analysis (Table 1) show that the mixture of hydrocarbons extracted from the broiled meat is similar qualitatively to that obtained in the pyrolysis of other organic materials, such as cigarettes and coal. Most notable is the presence of benzo(*a*)pyrene to the extent of 9 μg steak (8 μg/kg). This is the quantity of benzo(*a*)pyrene in the smoke of approximately 600 cigarettes (*3*). Meat protein is apparently not pyrolyzed.

The most likely source of the polynuclear hydrocarbons is the melted fat which drips on the hot coals and is pyrolyzed at the prevailing high temperature. The polynuclear hydrocarbons in the smoke are then deposited on the meat as the smoke rises. The significant absence of pyrolysis of protein indicates that other methods of cooking meat such as oven broiling or roasting would be unlikely to produce carcinogenic hydrocarbons.

The analysis was performed by broiling 15 large steaks (1.1 kg each), with a total surface area of approximately 1 m^2, to "well-done" on a standard charcoal broiler. Wood charcoal was ignited with purified isooctane and allowed to reach a uniform glowing red heat before the meat was placed over the fire. The distance of the meat from the coals was about 6 inches (15 cm). Both sides of each steak were cooked. The outer half centimeter of each side was cut off and extracted with acetone in a Soxhlet apparatus for several hours. Acetone and water were removed by distillation in a vacuum. The bones and fat were washed with 1 liter of acetone and 2 liters of benzene, and the solution was evaporated under nitrogen until only fat remained. The two residues were combined; the total weight was approximately 500 g.

The polynuclear compounds were separated from the bulk of the aliphatic material by solvent partition. The lipid residue was dissolved in 500 ml of hexane [this and all other solvents were freed of polynuclear aromatic material by appropriate procedures (*4*)] and shaken with two 500-ml portions of nitromethane, the total extract being then distilled to dryness under nitrogen in a vacuum. The extraction of polynuclear aromatic material from the hexane solution was completed with three 200-ml portions of dimethylsulfoxide. To this extract were added 750 ml of hexane and 1.4 liters of water; the hexane layer was distilled to dryness and the oily residue was added to that from the nitromethane extract to give 8.8 g of oil. This was dissolved in 40 ml of isooctane and chromatographed on a 20- by 3.5-cm column of silica gel (100 to 200 mesh). Filtration of a further 200 ml of isooctane through the adsorbent eluted much dark brown material, after which the eluate was almost colorless. The adsorbed aromatic material was finally eluted with 250 ml of benzene, and the solvent was distilled under nitrogen. The residue (490 mg) was diluted to 2.5 ml with benzene, and 250 μl of solution was chromatographed on each of ten 15- by 50-cm strips of Whatman No. 1 paper that had been impregnated with *N,N*-dimethylformamide. The chromatograms were developed by the descending technique, with isooctane as mobile phase (*5*) and the analogous fluorescent zones on all of the papers were combined, ultraviolet absorption spectra taken and fractions were rechromatographed as necessary in the same system until spectra were obtained which could be identified with those of known polynuclear compounds. The identifications were confirmed, where possible, by the fluorescence emission spectra.

Although many polynuclear hydrocarbons were identified in the steaks (Table 1), some hydrocarbons were present which could not be adequately identified or estimated because of their low concentration; among these are possibly picene, benzo(*j*)fluoranthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, dibenzo(*a,l*)pyrene, and dibenzo(*a,i*)pyrene. The concentration of any of these compounds, if present, is certainly below 0.5 μg per steak. At least two compounds were present in concentrations comparable with those of the identified compounds, but they could not be identified spectroscopically with any known polycyclic aromatic compound. One of these was compound "Y," which has been found previously in solvents, waxes, and so forth (*4*); the other is a compound of low R_F in the dimethylformamide/isooctane system and having absorption maxima at 405, 400, 383, 362, 340, and 325 mμ.

The concentrations given for the various hydrocarbons are, of course, minimum values. The losses that occur during repeated chromatography set an effective limit to the obtainable purification of some of the rarer components of the mixture. The identification of dibenz(*a,h*)anthracene and benzo(*b*)-chrysene is consequently not quite definite. That of benzo(*a*)pyrene, on the other hand, is unequivocal. In addition to benz(*a*)anthracene, a compound with a very similar spectrum but quite sharply separated from the former (and with higher R_F) was present. This is probably an alkylbenz(*a*)anthracene, which might or might not be a carcinogen. More of this material is needed for positive identification.

Several conclusions may be drawn tentatively from this preliminary experiment. The profile of polynuclear hydrocarbons present on the meat as a result of charcoal broiling is very similar to that present in other pyrolysis products, with one notable difference. Since no nitrogen-containing polynuclear compounds were detected, it can be inferred that pyrolysis of only carbon-, hydrogen-, and oxygen-containing compounds is involved. Carbazoles and acridines are very evident in the pyrolysates of nitrogen-containing materials, such as coal, vegetable matter (tobacco), and liquid smoke (produced

by dry distillation of wood) (*6*). Reports of the analysis of the pyrolysates of cigarette paper (C, H, and O only) have given no evidence of the presence of nitrogen heterocycles (*7*).

W. LIJINSKY
P. SHUBIK

Division of Oncology, Chicago Medical School, Chicago, Illinois

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Mitomycins and Porfiromycin: Chemical Mechanism of Activation and Cross-linking of DNA

Abstract. *Mitomycins and porfiromycin, generally nonreactive in the natural oxidized state, behave as bifunctional "alkylating" agents upon chemical or enzymatic reduction, followed by spontaneous loss of the tertiary methoxy (hydroxyl) group and formation of an aromatic indole system. Thus activated, mitomycins and porfiromycin react in vitro with purified DNA, linking its complementary strands. A high content of guanine and cytosine favors this cross-linking reaction, which is the basis of the lethal effect in vivo of these antibiotics. The activation and cross-linking reactions are discussed in terms of reactive sites on the mitomycin and DNA molecules.*

In a paper (*1*) on the mechanism of action of the antibiotic mitomycin C (*2*) evidence was presented that mitomycin in lethal concentrations cross-links the complementary strands of intracellular DNA and that it does not react in vitro with purified DNA unless this reaction mixture is supplemented with a homologous or heterologous cell lysate. The results presented here provide information concerning the chemical nature of mitomycin activation, both in vivo and in vitro. The degree of the resultant cross-linking of DNA was determined from the proportion of spontaneously renaturable DNA molecules under conditions of irreversible denaturation, by the method of equilibrium density-gradient centrifugation in CsCl or Cs_2SO_4 or by biological assay of residual transforming activity. The methods have already been described (*1*).

The mitomycins and porfiromycins (all five forms listed in Fig. 1*A*) are metabolically activated, as demonstrated by cross-linking in vitro of purified native DNA, when 1 volume of a freshly prepared cell lysate (10^9 cells of *Sarcina lutea* per milliliter exposed for 10 minutes to 100 μg of lysozyme per milliliter of SSC) is added for each 1 to 4 volumes of DNA (100 to 200 μg of DNA from *Bacillus subtilis* per milliliter) containing 10 to 200 μg of mitomycin per milliliter; the DNA of *S. lutea* is cross-linked at the same time. Heating (10 minutes at 70°C) or dialysis against SSC abolishes the mitomycin-activating capacity of the *S. lutea* lysate. The activity of the dialyzed lysate can be almost completely restored by the addition of TPNH (100 to 200 μg/ml) or of a TPNH-generating system, but not by DPNH, TPN or DPN (*2*); however, TPNH in the absence of lysate is ineffective as a mitomycin-activating agent. Approximately 90 percent of the activity is associated with the cell-free lysate fraction sedimenting at 198,000*g* for 90 minutes. Besides bacterial lysates, a commercial preparation of the *Clostridium kluyveri* diaphorase (*3*) was found to activate mitomycin in the presence of TPNH, whereas several reducing mitochondrial systems or smooth membrane liver fractions (*4*) were ineffective. Dicumarol, a reported inhibitor of some quinone reductases (*5*), did not interfere with the enzymatic activation of mitomycin. Several properties of the mitomycin-activating system of bacterial origin are analogous to the similar mitomycin-reducing system present in liver homogenates (*6*). One could conclude that TPNH-dependent enzymatic reduction of mitomycin appears a necessary prerequisite to DNA cross-linking.

Cross-linking of DNA in vitro can also be accomplished with chemically reduced mitomycins. Native, high molecular weight DNA of viral, bacterial, or mammalian origin (100 to 200 μg/ml of SSC) was mixed with various concentrations of mitomycin (2 to 200 μg/ml), and the reaction was started by

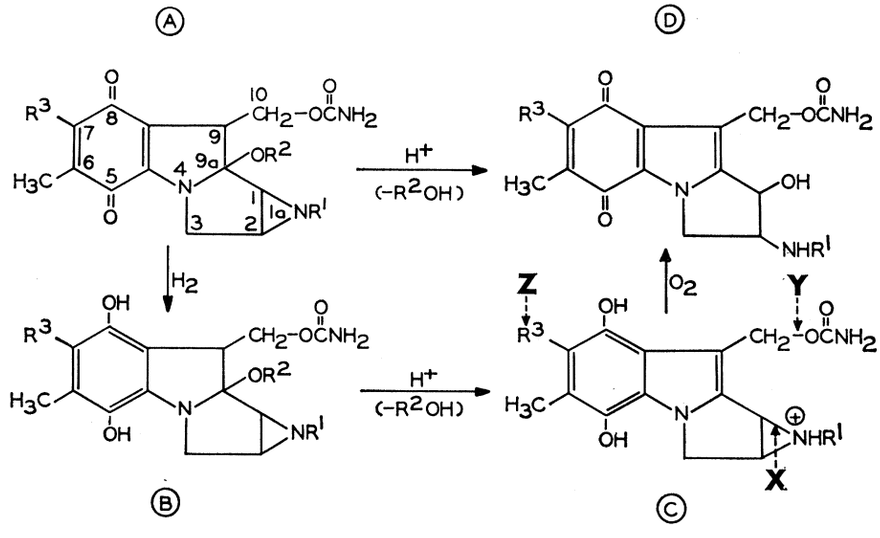

Fig. 1. Structure of mitomycins and porfiromycins, and of their reduction, reoxidation, and protonation products (*7*). *A*, The groups R^1, R^2, and R^3, respectively, are for mitomycin A: H, CH_3, CH_3O; for mitomycin B: CH_3, H, CH_3O; for mitomycin C: H, CH_3, NH_2; for porfiromycin: CH_3, CH_3, NH_2; and for 7-hydroxyporfiromycin: CH_3, CH_3, OH. *B*, Primary reduction product. *C*, Postulated structure after secondary rearrangements (*10*) following the reductive step and protonation of the aziridine nitrogen. Arrows point to the reactive sites and to the points of possible cleavage. *D*, Product of acid degradation (*7*).