formed there than in the high-temperature processes discussed here. In the cooling gas mass over the fires, freshly formed active carbon particles would readily scavenge the aromatic hydrocarbons and would protect them from photooxidation during extended air transport. Tropospheric mixing could account for the relative compositional uniformity of the sedimentary PAH fraction and for the fact that its composition is independent of that of the biota and the chemical conditions in the sedimentary environment.

Therefore, we suggest that natural fires form, and that air currents disperse, a complex PAH assemblage that eventually accumulates in soils and recent sediments. This hypothesis is open to tests, for instance, through the analysis of air samples or of sediment cores that predate the industrial revolution.

Our findings have analytical, geochemical, and environmental implications. The sedimentary PAH fraction is far more complex than was previously recognized. In fact, a complete resolution into individual components and their quantitative determination is difficult, if not often impossible (*8*). Regardless of the detailed mode of origin, the sedimentary PAH fraction may be accompanied by other classes of organic compounds, equally complex. This would imply that our present insight into the composition of nonbiological sedimentary organic compounds may be more limited, and our analytical capabilities more restricted, than generally accepted.

Our analyses demonstrate that the sedimentary PAH fraction contains, in addition to the already recognized carcinogens (benzo[*a*]pyrene and benz[*a*]anthracene), other, not previously recognized carcinogens (dibenzothiophene, methylchrysenes, and others) and a wealth of other polycyclic compounds that may comprise many biologically active compounds. Therefore, we need to reexamine the environmental toxicology of the sedimentary PAH fraction. Finally, our interpretation would imply that carcinogenic and mutagenic hydrocarbons occurred on the earth's surface during geologic time spans. This raises the question of whether these compounds might have contributed significantly to the processes of natural selection and mutation, and to the evolution of species.

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References and Notes

1. W. Kern, *Helv. Chim. Acta* **30**, 1595 (1947); M. Blumer, *Science* **134**, 474 (1961). A bibliography on the occurrence and analysis of environmental PAH is available from M.B.
2. W. Giger and M. Blumer, *Anal. Chem.* **46**, 1663 (1974).
3. R. A. Hites and W. G. Biemann, abstract, ANAL 40, 168th national meeting of the American Chemical Society, 1974.
4. Experimental details and additional data are presented elsewhere (M. Blumer and W. W. Youngblood, *Geochim. Cosmochim. Acta*, in press). Samples may be identified as follows. (i) South Louisiana "crude oil": standard sample, provided by J. W. Anderson, Texas A & M University; (ii) "marine": surface sediment at station A of Giger and Blumer (*2*); (iii) "marsh, Cape Cod": surface sample, Little Sippewisset marsh, tidal, Falmouth, Massachusetts; (iv) "soil, Cape Cod": surface soil from a hummock within Little Sippewisset marsh, oaks predominate: and (v) "soil, Maine": coniferous forest, North Anson, Maine.
5. J. Borneff, F. Selenka, H. Kunte, A. Maximos, *Environ. Res.* **2**, 22 (1968); G. Grimmer and D. Duevel, *Z. Naturforsch. B* **25**, 1171 (1970); G. Grimmer, J. Jacob, A. Hildebrandt, *Z. Krebsforsch.* **78**, 65 (1972); see also the bibliography mentioned in (*1*).
6. R. C. Lao, R. S. Thomas, H. Oja, L. Dubois, *Anal. Chem.* **45**, 908 (1973).
7. D. M. Smith, J. J. Griffin, E. D. Goldberg, *Nature (Lond.)* **241**, 268 (1973).
8. M. Blumer, *Angew. Chem.*, in press.
9. This work was supported by the Office of Naval Research (NOO-14-66 contract CO-241) and the National Science Foundation (grant DES 74-22781). Contribution No. 3467 of the Woods Hole Oceanographic Institution.

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Chemotherapeutic Drugs Increase Killing of Tumor Cells by Antibody and Complement

Abstract. *When the ascitic forms of two antigenically distinct guinea pig hepatomas induced by diethylnitrosamine are treated in vitro with chemotherapeutic drugs, their sensitivity to killing by xenogeneic antibody plus guinea pig complement increases. The effect is dependent on drug dose, is reversible, and does not appear to be due to increased antigen expression or fixation of the early acting components of guinea pig complement.*

The variation in sensitivity of different types of nucleated cells to the cytotoxic action of antibody and complement has generally been ascribed to differences in antigen concentration (*1*), immunoglobulin class (*2*), source of complement (*3, 4*), and phase growth of cells (*5*). Data from our laboratory showed that cells of two antigenically distinct guinea pig hepatomas induced by diethylnitrosamine (designated line 1 and line 10) were resistant to the cytotoxic action of specific rabbit antibodies to tumor and guinea pig complement. In addition, cells of line 10, but not of line 1, were resistant to 19*S* antibody to Forssman antigen and guinea pig complement. However, both tumor cell lines sensitized with either specific antibody to tumor or 19*S* antibody to Forssman antigen were susceptible to killing by human complement (*4*). Variable susceptibility to antibody and complement could not be ascribed to differences in antigen concentration, to antibody class, or to lack of fixation of C4 (the fourth component of complement) and C3 (*4*).

We have reported that cells of line 10 could be killed by antibody and guinea pig complement if the cells were first treated with actinomycin D, mitomycin C, puromycin, or hydroxyurea (*6*). Other investigators have shown that treatment of cultured human lymphoid cells with puromycin or cycloheximide increased their sensitivity to HL-A alloantibody and complement (*7*). Actinomycin D was not effective. We now report that line-1 and line-10 tumor cells can be killed by antibody and guinea pig complement if the cells are first treated with drugs used in the treatment of cancer.

The ascitic forms of line 1 and line 10 were used in our experiments (*8*). Ascitic fluids were collected 6 to 8 days after intraperitoneal injection of 3×10^6 cells. Ascites tumor cells (60×10^6) were freed of erythrocytes by lysing them with 5.0 ml of tris-NH_4Cl(*9*), washed twice with barbital-buffered saline containing 1 g of gelatin (*10*), and resuspended in RPMI 1640 containing 15 percent calf serum (*11*). All centrifugations were at 200*g* for 5 minutes at room temperature.

Line-1 or line-10 tumor cells (2.5×10^6) were added to 5.0 ml of tissue culture medium (RPMI 1640 containing 15 percent fetal calf serum) containing one of the following compounds: 5-fluorouracil (Roche, lot 0776-0843); cytosine arabinoside (NCI, Cancer Therapy Evaluation Branch, NSC-63878); 6-mercaptopurine (NCI, NSC-755); vincristine sulfate (NCI, NSC-67674); methotrexate (NCI, NSC740); cyclophosphamide (Meade Johnson); adriamycin (NCI, NSC123127); or azacytidine (NCI, NSC102816). Control suspensions were prepared concurrently with tissue culture medium alone. After incubation for 17 hours at 37°C

in an atmosphere of air and 5 percent CO_2, the cells, in 2.0-ml portions, were washed three times with 10 ml of warmed fresh RPMI 1640 containing 15 percent fetal calf serum without inhibitors, and resuspended to a density of 10^6 cells per milliliter of culture medium.

The method for determining cytotoxicity mediated by antibody and complement has been described (*4*). A drug was considered effective if the sensitivity of the drug-treated cells to killing by antibody plus complement was twofold or more higher than that of the untreated cells. The guinea pig complement used here had from 180 to 200 CH_{50} (hemolytic complement, 50 percent effective) units per milliliter as determined by the method described in (*10*).

Increased susceptibility of line-10 tumor cells to cytotoxicity mediated by antibody plus guinea pig complement as compared to control cells cultured in medium free of drugs was noted after prior incubation of the cells with adriamycin, vincristine sulfate, and azacytidine (Table 1). Cells treated with cyclophosphamide, 5-fluorouracil, and 6-mercaptopurine showed a lesser degree of sensitivity, while cytosine arabinoside–treated cells were essentially resistant. Different preparations of line-10 cells were not equally susceptible after treatment with the same drugs. This is illustrated by comparing the results of tumor cells treated with methotrexate or azacytidine. Line-10 cells that were resistant to killing by antibody plus guinea pig complement after treatment with the indicated doses of the drugs remained resistant even after treatment with higher concentrations of these drugs.

Two different preparations of line-1 tumor cells treated with adriamycin, azacytidine, 5-fluorouracil, methotrexate, or 6-mercaptopurine were rendered more susceptible than untreated cells to killing by antibody to Forssman antigen plus guinea pig complement, while cells treated with vincristine, cytosine arabinoside, or cyclophosphamide showed little or no increase in susceptibility. Cells treated with adriamycin were susceptible to killing by antibody to line 1 plus guinea pig complement in both experiments, while azacytidine and methotrexate were effective with cells used in experi-

Table 1. Effect of chemotherapeutic drugs on the killing of line-1 and line-10 tumor cells by antibody and guinea pig complement. Cells were cultured for 17 hours at 37°C in the presence or absence of drugs, washed twice with tissue culture medium, and suspended in tissue culture medium to a density of 10^6 per milliliter. Cytotoxicity was determined as follows: A mixture of 0.1 ml of cells plus 0.1 ml of antibody was incubated for 30 minutes at 30°C, washed once with 1 ml of warm tissue culture medium; 0.1 ml of guinea pig complement (C) diluted 1/8 was added and the mixture was incubated for 60 minutes at 37°C. At this time 0.1 ml of a 0.4 percent solution of trypan blue was added, and the mixture was incubated for 60 minutes at 37°C. At this time 0.1 ml of a 0.4 percent solution of trypan blue was added, and the number of cells stained with trypan blue was determined. In all experiments, more than 90 percent of the initial cell population was recovered. Each experiment was performed with a different fresh preparation of line-10 or line-1 cells; the number of dead cells varied from preparation to preparation (see column headed, Medium alone). Abbreviations: Anti-F, antibody to Forssman antigen; anti–line-1, antibody to line-1; anti–line-10, antibody to line 10; C, complement.

Treatment		Percentage of cells stained with trypan blue					
Agent	Amount (μg/ml)*	Anti-F (1/2) + C	Anti-F (1/2)	Anti–line-10 (1/20) + C	Anti–line-10 (1/20)	C alone	Medium alone
			Line-10 cells, experiment 1				
Tissue culture medium		16		13		17	18
Adriamycin	50	46		75		18	15
Vincristine sulfate	20	50		62		26	23
Cytosine arabinoside	100	22		30		20	23
Azacytidine	20	69		62		25	25
			Line-10 cells, experiment 2				
Methotrexate	500	11		11		0	0
Cyclophosphamide	100	25		29		3	4
5-Fluorouracil	500	18		14		2	3
6-Mercaptopurine	500	11		23		4	4
			Line-10 cells, experiment 3				
Tissue culture medium		10	9	16	4	6	6
Adriamycin	50	49	16	86	10	19	15
Vincristine sulfate	20	64	14	84	13	15	11
Azacytidine	20	24	8	39	9	8	5
Methotrexate	500	44	11	57	10	10	9
		Anti-F (1/100) + C	Anti-F (1/100)	Anti–line-1 (1/30) + C	Anti–line-1 (1/30)	C alone	Medium alone
			Line-1 cells, experiment 1				
Tissue culture medium		10	1	1	1	0	0
Adriamycin	50	62	0	71	2	1	0
Vincristine sulfate	20	22	0	5	2	1	0
Azacytidine	20	56	1	59	2	2	0
Methotrexate	500	48	1	44	1	0	1
			Line-1 cells, experiment 2				
Tissue culture medium		26		1		0	0
Adriamycin	50	52		50		0	2
Vincristine sulfate	20	35		0		2	1
Cytosine arabinoside	100	33		1		2	0
Azacytidine	20	61		7		6	8
Methotrexate	500	54		0		2	0
Cyclophosphamide	100	31		0		0	1
5-Fluorouracil	500	69		1		2	2
6-Mercaptopurine	500	53		2		0	1

* Concentrations used for treating the tumor cells before the addition of antibody plus complement and are the lowest concentrations giving maximum sensitivity to killing by mediated antibody plus complement.

ment 1 but not experiment 2. Repeat studies with the cells of experiment 2 treated with higher concentrations of methotrexate and azacytidine showed that the cells were still resistant to killing. In experiments not shown here, prior treatment of line-1 cells with actinomycin D, puromycin, or mitomycin C showed increased susceptibility to tumor-specific antibody plus guinea pig complement. The data also show that antibody alone is not sufficient to kill drug-treated line-1 and line-10 cells. In experiments not reported here, we found no killing of drug-treated line-1 cells with antibody to line 10 plus guinea pig complement and of drug-treated line-10 cells with antibody to line 1 plus guinea pig complement (specificity controls).

Some experiments indicate enhanced sensitivity of cells to killing mediated by antibody plus complement after preliminary drug treatment is dependent on drug dose and is reversed within several hours by culturing in medium free of the drugs. Line-10 cells held at 4°C in the presence of the drugs show little or no increase in sensitivity to killing by antibody plus guinea pig complement. Preliminary data also indicate that the effect is not due to an increase in the number of Forssman or tumor-specific antigen sites or to increased fixation of guinea pig C4 and C3 onto the cell surface. Other investigators have shown that prior treatment of tumor cells with various drugs may increase or decrease their absorbing capacity for transplantation or antiviral antibody (*12*).

Others have shown that the presence of chlorambucil during exposure of polyoma-transformed hamster cells to antibody alone decreased the colony-forming efficiency of these cells (*13*). The relation of this observation to ours is not clear since it is difficult to explain mechanisms whereby antibodies become cytotoxic without complement.

The biochemical mechanisms of action of the drugs in our experiments have been studied extensively, but little is known of how tumor cell destruction is effected in vivo. These inhibitors may affect the synthesis of surface areas susceptible to the attack of terminal components of complement or reduce the effectiveness of cell surface repair mechanisms. Regardless of mechanisms, the beneficial effect of various drugs and metabolic inhibitors in the treatment of cancer might be due, in part, to their ability to increase the susceptibility of tumor cells to killing by antibody plus complement.

Some success has been reported in treating mouse leukemias with antibodies to which chlorambucil was coupled (*14*). O'Neill and Davies also reported that injection of melphalan into mice with EL4 leukemia before administration of antibody to EL4 resulted in 100 percent of the mice being free of disease at 150 days (*15*). Our results may furnish a rational explanation for their observations.

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References and Notes

1. E. Moller and G. Moller, *J. Exp. Med.* **115**, 527 (1962); H. J. Winn, *Ciba Foundation Symposium on Complement* (Little, Brown, Boston, 1965); S. Friberg, *J. Natl. Cancer Inst.* **48**, 1477 (1972); C. C. Ting, D. H. Lovrin, K. K. Takemoto, *Cancer Res.* **32**, 74 (1972); E. M. Fenyo, P. T. Peebles, A. Wahlstrom, E. Klein, A. J. Cochran, *Isr. J. Med. Sci.* **9**, 259 (1973).
2. T. Borsos, in *Progress in Immunology*, B. Amos, Ed. (Academic Press, New York, 1971).
3. P. I. Terasaki, J. D. McClelland, J. A. Cannon, W. P. Longmire, *J. Immunol.* **87**, 39 (1961); G. Haughton and M. P. McGehee, *Immunology* **16**, 447 (1969); A. R. Baker, T. Borsos, H. R. Colten, *ibid.* **21**, 33 (1971).
4. S. H. Ohanian, T. Borsos, H. J. Rapp, *J. Natl. Cancer Inst.* **50**, 1313 (1973); S. H. Ohanian and T. Borsos, *J. Immunol.* **112**, 979 (1974).
5. M. Cikes, *J. Natl. Cancer Inst.* **45**, 979 (1970); *Nature (Lond.)* **225**, 645 (1970); ——— and S. Friberg, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 566 (1971); R. A. Lerner, M. B. Oldstone, N. R. Cooper, *ibid.*, p. 2584; W. U. Shipley, *Cancer Res.* **31**, 925 (1971); N. A. Cooper, M. Polley, M. A. Oldstone, *J. Immunol.* **112**, 866 (1974).
6. M. Segerling, S. H. Ohanian, T. Borsos, *J. Natl. Cancer Inst.* **53**, 1411 (1974).
7. S. Ferrone, M. A. Pellegrino, M. P. Dierich, R. A. Reisfeld, *Tissue Antigens* **4**, 275 (1974).
8. B. Zbar, H. T. Wepsic, H. J. Rapp, *J. Natl. Cancer Inst.* **43**, 833 (1969); B. Zbar, I. D. Bernstein, H. J. Rapp, *ibid.* **46**, 831 (1971).
9. W. Boyle, *Transplantation* **6**, 761 (1968).
10. H. J. Rapp and T. Borsos, *Molecular Basis of Complement Action* (Appleton-Century-Crofts, New York, 1970).
11. RPMI 1640 containing 15 percent calf serum was made as follows: Medium 1640 (Gibco 187G), plus 15 percent calf serum (Gibco 694), plus 1 percent antibiotic-antimycotic mixture (Gibco 524).
12. S. Ferrone, B. C. Del Villano, M. A. Pellegrino, R. A. Lerner, R. A. Reisfeld, *Tissue Antigens* **2**, 447 (1972); M. Cikes and G. Klein, *J. Natl. Cancer Inst.* **48**, 509 (1973); M. A. Pellegrino, S. Ferrone, B. C. Del Villano, R. A. Reisfeld, *Transplant. Proc.* **5**, 91 (1973).
13. R. D. Rubens and R. Dulbecco, *Nature (Lond.)* **248**, 81 (1974).
14. T. Ghose, S. T. Norvell, A. Guclu, D. Cameron, A. Bodurtha, A. S. MacDonald, *Br. Med. J.* **3**, 495 (1972); D. A. L. Davies and G. J. O'Neill, *Br. J. Cancer* **28**, 285 (1973).
15. G. J. O'Neill and D. A. L. Davies, Abtracts, 5th International Congress of the Transplantation Society, Jerusalem, 1974, p. 167.

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Prolactin Receptors in Rat Liver: Possible Induction by Prolactin

Abstract. *A prolactin receptor, present in adult female rat liver, can be induced in males by estrogen. Hypophysectomy diminished receptor levels in the female and rendered males unresponsive to estrogen. A renal pituitary implant blunted the decrease in hypophysectomized females and induced the receptor in hypophysectomized males. The increased receptor level in hypophysectomized males with a renal pituitary implant was preceded by a sustained elevation of circulating prolactin. Our observations suggest that prolactin induces its own receptor.*

Adult female rat liver membranes possess a receptor specific for lactogenic hormones (that is, prolactin, placental lactogens, and primate growth hormones). Receptor levels are much lower in male than in female liver membranes. Estrogen administration to male rats induced the receptor. A role for the pituitary was suggested by the observations that hypophysectomy greatly decreased the hepatic receptor in female rats, and rendered males unresponsive to estrogen (*1*). In this study we have examined the role of the pituitary. The data suggest that prolactin can induce its own receptor in rat liver.

Adult male and female rats weighing 180 to 220 g were obtained from Fauna Breeding Company, Quebec. Animals were hypophysectomized by the parapharyngeal technique. Pituitaries from animals of comparable size were implanted beneath the renal capsule according to the schedules noted in the figure legends.

At the time of killing, animals were ether-anesthetized and decapitated. Blood, obtained from the cervical wound, was allowed to clot at 4°C. Serum was collected by centrifugation at 4°C and stored at −20°C. The methods employed for tissue fractionation, membrane preparation, the iodination of human growth hormone