Relationship of Leukemia to Lung and Stomach Tumors in Mice Fed Benzo(a)Pyrene* (33508)

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Tumors occur in the stomach and in the lungs of mice given carcinogenic hydrocarbons (1–18). Heredity is thought to be a factor in their pathogenesis (3, 4, 13). Lynch (13) observed that the yield of chemically induced adenomas of the lung was proportional to the incidence of lung adenomas occurring spontaneously in different strains of mice and concluded that the degree of susceptibility to induce lung tumors was inherited and that three or more genes must be involved. In a previous study we demonstrated the occurrence of gastric and pulmonary neoplasms in our inbred colony of CFW white Swiss mice fed benzo(*a*)pyrene (19). The gastric tumors were squamous cell papillomas and carcinomas occurring in the squamous portion of the stomach and adenomas occurring in the lungs. Leukemia likewise has occurred frequently in these mice fed benzo(*a*)pyrene (20, 21). Mice that developed leukemia also had a marked increase in the number of pulmonary adenomas. Our observations are reported.

Method and Material. The mice were white Swiss (CFW strain) and the basic ration was Purina laboratory pellets. Benzo(*a*)pyrene was obtained from the Nutritional Biochemical Laboratory, Cleveland, Ohio. The technique for preparing the benzo(*a*)pyrene-containing food has been described (20). This ration contained 0.25 mg of benzo(*a*)pyrene/g of food. Food and water were available *ad libitum*. The mice usually were 18–30 days old when placed on the benzo(*a*)pyrene-containing ration. The experiment continued for 80–140 days, at which time the mice were autopsied. A few mice were sick and were killed; occasionally a mouse died during the time of the experiment. The stomach was removed, opened, washed and examined macroscopically, after which some of the specimens were fixed in a 4.0% solution of formaldehyde. The lungs, heart, and thymus were removed and examined under a dissecting microscope. The adenomas on the pleural surface of each lung were counted. The spleen and sections from the liver were placed in formaldehyde. Any enlarged lymph nodes were removed and placed in the fixative. The lungs, thymus, heart, and mediastinal lymph nodes also were fixed in formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin.

Results. All the tumors were located in the squamous portion of the stomach, however the number varied. The majority were papillomas with a thick layer of keratin covering their surface. Some of these macroscopic papillomas were found microscopically to be malignant. Some of the gastric tumors had an ulcerated surface and histologically were typical squamous cell carcinomas. These gastric lesions have been described and illustrated in previous publications (19, 22). No neoplasms were observed in other portions of the gastrointestinal tract other than the stomach. The frequency of gastric tumors in 108 mice fed the benzo(*a*)pyrene ration is given in Table I. In a previous study, in a group of 175 control mice fed Purina laboratory pellets only 2 had a papilloma (19). Multiple gastric tumors occurred in the mice fed the benzo(*a*)pyrene-containing food.

The tumors in the lungs were adenomas. The size varied and an occasional tumor was consistent with a maliganant adenocarcinoma. No metastases were observed. In the study of 175 mice fed laboratory pellets, 37 had pulmonary adenomas (19). The maximum number of pulmonary adenomas observed in any mouse was 10; usually, the

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TABLE I. Relationship of Leukemia to Lung and Stomach Tumors in 108 Mice Fed Benzo(a)pyrene.

Total no. of mice	No. of mice with			
	Leukemia	Lung tumors	15 or more lung tumors	Stomach tumors
9	9	0	0	0
23	23	23	12	0
5	5	5	4	5
3	3	0	0	3
7	0	7	3	0
17	0	17	1	17
44	0	0	0	44
108	40	52	20	69

study of 39 mice fed 0.25 mg of benzo(a)pyrene/g of food, only 2 did not have a pulmonary adenoma; 15 had 6 or less, 1 had 10,

The diagnosis of leukemia is made by a number was less than 5/mouse (19). In a and 1 had 11 adenomas. Ten of the mice had 13 or more adenomas; 3 of these mice had more than 50 (19). The number of benzo(a)pyrene-fed mice with pulmonary adenomas in this experiment is shown in Table I.
histologic study of the liver and is based upon the presence of leukemic cells. Usually the thymus, spleen, and lymph nodes are enlarged and infiltrated with similar cells. In a previous study, 10.7% of 73 mice fed 0.25 mg of benzo(a)pyrene/g of food for 70–165 days developed leukemia (20). No leukemia occurred in the 175 mice of corresponding age fed the control ration (19). Only rarely have we observed leukemia in any of the CFW mice. In this experiment we have 40 mice with leukemia, 28 of which had pulmonary adenomas and 8 had stomach tumors (Table I).

The data in Table I were tested by the χ^2 test of independence and showed the following to be highly significant. Mice that had more than 15 pulmonary adenomas had a higher rate of leukemia than those that had less than 15 adenomas. Of the mice with leukemia there is a much higher proportion of lung adenomas. Of the mice with leukemia there is no relationship between stomach tumors and lung adenomas. In the nonleukmic group of mice there is a negative relationship between stomach tumors and lung adenomas.

Discussion. This group of CFW Swiss mice, when fed 0.25 mg of benzo(a)pyrene for 100 days or longer, developed leukemia, pulmonary adenomas, and gastric tumors. The frequency of leukemia during the lifespan of this strain of mice, when fed control ration, has not been determined; however, our experience shows that it is very infrequent. In a previous experiment, no mouse developed leukemia in a group of 175 fed Purina laboratory pellets and examined between the ages of 70 and 300+ days (20). Only 2 mice in a group of 175 examined between the ages of 38 and 210+ days had a gastric papilloma (19). Thirty-three mice in a group of 151 between the ages of 62 and 210+ days had pulmonary adenomas. The maximum number of adenomas present in the lungs of any one mouse was 12 and the majority had 3 or less. Mice fed benzo(a)pyrene developed pulmonary adenomas frequently. In a group of 78 mice fed 0.25 mg of benzo(a)pyrene/g of food for 31–153 days 10 did not have a pulmonary adenoma, 29 had 6 or less, 1 had 9, and 4 had either 10, 11, 12 or 13. Eight mice had 15 or more pulmonary adenomas, 3 of these having more than 50 tumors (19).

In mice with leukemia there is a negative statistical relationship between the occurrence of pulmonary adenomas and stomach tumors, but there is a much higher proportion of lung tumors. Mice with more than 15 pulmonary adenomas had a higher rate of leukemia than those with less.

The age of mice fed benzo(a)pyrene is significant referable to the occurrence of stomach and lung tumors and leukemia. A group of 32 CFW mice, 313 days old, were fed 0.25 mg of benzo(a)pyrene/g of food for 108 days. At autopsy none of the mice had leukemia, 1 mouse had 50+ pulmonary adenomas, 15 had from 1–9 adenomas, and 16 had no pulmonary tumors. Only 15 of these 32 mice had gastric tumors; the number per mouse was less and usually the tu-

mors were much smaller than the tumors occurring in young mice fed the same benzo(a)pyrene-containing ration.

Pulmonary adenomas in mice are considered by some to be genetically influenced (13). It would appear from this study that benzo(a)pyrene or a metabolite may stimulate the production of pulmonary adenomas and leukemia in this strain of mice that normally has few pulmonary adenomas and rarely leukemia. No leukemia or lung adenomas were observed in a group of 22 BALB/C mice fed the same hydrocarbon for 100 days; 14 had gastric tumors.

Sugiyama *et al.* (23), in 1967, reported a chromosome abnormaltiy in the cells of rats with leukemia induced by 7, 12-dimethylbenz-(a)anthracene (7, 12-DMBA). This abnormality consisted in the trisomy of the longest telocentric chromosome. It is reasonable to suggest that a chromosomal abnormality occurs in mice fed benzo(a)pyrene to account for both the leukemia and the increased frequency of pulmonary adenomas. It would appear, however, from this study that it is necessary for the benzo(a)pyrene to come into direct contact with the squamous epithelium in the stomach for gastric tumors to develop. No tumors were observed in the esophagus which is lined by squamous epithelium similar to that in the stomach. No tumors occurred in the glandular portion of the stomach or in the intestines of these mice.

Summary. CFW mice fed 0.25 mg of benzo(a)pyrene/g of food develop gastric papillomas and squamous cell carcinomas, pulmonary adenomas, and leukemia. Mice that develop leukemia have a significant increase in the number of pulmonary adenomas. There is no relationship, however, between the occurrence of pulmonary adenomas and gastric tumors in this nonleukemic group of mice.

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1. Stewart, H. and Lorenz, E., J. Natl. Cancer Inst. **10**, 147 (1949).
2. White, J. and Stewart, H. L., J. Natl. Cancer Inst. **3**, 331 (1943).
3. Strong, L. C., J. Natl. Cancer Inst. **5**, 339 (1945).
4. Strong, L. C., J. Natl. Cancer Inst. **7**, 305 (1947).
5. Mulay, A. S., Nature **200**, 1114 (1963).
6. Borneff, J., Arch. Hyg. Bakteriol. **147**, 28 (1963); Abstr. no. 61, Excerpta Med., Sect. XVI **12**, 17 (1964).
7. Stewart, H. and Andervont, H. B., Arch. Pathol. **26**, 1009 (1938).
8. Slye, M., Holmes, H. F., and Wells, H. G., J. Cancer Res. **2**, 401 (1917).
9. Campbell, J. A., Brit. J. Exptl. Pathol. **15**, 287 (1934).
10. Murphy, J. B. and Sturn, E., J. Exptl. Med. **42**, 693 (1925).
11. Shimkin, M. B., Am. J. Cancer **35**, 538 (1939).
12. Campbell, J. S., Yang, Y. H., and Bolton, J. D., Arch Pathol. **79**, 588 (1965).
13. Lynch, C. J., Proc. Soc. Exptl. Biol. Med. **52**, 368 (1943).
14. Pietra, G., Rappaport, H., and Shubik, P., Cancer **14**, 308 (1961).
15. Walters, M. A. and Roe, F. J., Brit. J. Cancer **18**, 312 (1964).
16. Kelly, M. G. and O'Gara, R. W., J. Natl. Cancer Inst. **26**, 651 (1961).
17. Heston, W. E., J. Natl. Cancer Inst. **1**, 105 (1940).
18. Pollard, M. and Salomon, J., Proc. Soc. Exptl. Biol. Med. **112**, 256 (1963).
19. Rigdon, R. H. and Neal, J., Texas Rept. Biol. Med. **24**, 195 (1966).
20. Rigdon, R. H., Neal, J., and Mack, J., Texas Rept. Biol. Med. **25**, 422 (1967).
21. Rigdon, R. H., Neal, J., Anigstein, D., and Anigstein, L., Cancer Res. **27**, 2318 (1967).
22. Neal, J. and Rigdon, R. H., Texas Rept. Biol. Med. **25**, 553 (1967).
23. Sugiyama, T., Kurita, Y., and Nichizua, Y., Science **158**, 1058 (1967).

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