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Bioassay of Complex Mixtures Derived from Fossil Fuels

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The conversion or processing of shale, coal, or petroleum involves elevated temperatures and altered pressures, and under these conditions polynuclear aromatic hydrocarbons are likely to form. Certain compounds of this type exhibit carcinogenic activity for a variety of organ sites in experimental animals and epidemiological evidence strongly implicates their role as carcinogens in man.

It is then not unexpected that many liquid fractions derived from shale and coal are carcinogenic when subjected to bioassay. Benzo(a)pyrene, [B(a)P], is frequently considered to be an indicator substance. It is clear that when a small quantity of B(a)P is present in a fraction, the fraction will exhibit carcinogenic activity in a bioassay (mouse skin). However, it does not follow that the lack of detectable B(a)P insures that the fraction will be noncarcinogenic.

Several fractions have been analyzed for their content of B(a)P and then subjected to bioassay. A method for testing complex mixtures for their carcinogenic potential is described. The carcinogenic potency of these fractions are compared to petroleum fractions.

In the United States, experience with shale oil and its derived products is limited. Through the early research of British workers it is apparent that a potential hazard exists (*1, 2*). The assessment of this hazard and the factors contributing to carcinogenic potency of shale oils comes mainly from the early work of Schoental and Berenblum and the extensive work over many years in the United Soviet Socialist Republic by Professors Bogovsky and Vosamae and their colleagues (*3–5*).

The work in our laboratory on the investigation of the carcinogenic potency of complex mixtures began about 1950 by Horton et al. (*6*). Their work demonstrated that when benzo(a)pyrene is present in mixtures (higher levels in catalytically cracked stocks) they are likely to be positive in a bioassay. In addition, other carcinogens may be present that will contribute significantly to the observed potency. The occurrence of additional compounds of the long chain paraffinic type, so-called cocarcinogens, may contribute to the potency (*7, 8*).

Continued investigations lead to the findings that benzo(c)phenanthrene may be an important contributor (*8*). A summary of the factors influencing the potency of complex mixtures (petroleum derivatives, coal tar, etc.) was published in 1966. One of the significant factors reported in this series of papers and later quantified by Bingham and Falk is the potentiation of polynuclear aromatic hydrocarbons by paraffinic compounds (*9*). Concentrations of 0.01 to 0.02% benzo(a)pyrene in such solvents as benzene and toluene are near the limit of detectable response using such a mouse bioassay. However, when n-dodecane is the solvent an effect is produced at 0.00002%.

The experiments reported present experience with several complex mixtures and compare their relative carcinogenic potency in this bioassay system (Table 1).

Methods

Mice (C3H males) are acclimatized for two weeks prior to beginning an experiment. They are toe numbered, assigned to one of the test groups using a randomization procedure, and housed in groups of five to a cage (stainless steel). Food (Purina Lab Chow) and water are provided *ad libitum*. The cages are changed weekly.

The solutions are applied with a microliter pipet, usually 50 μl two to three times per week to the interscapular region of the backs. Hair is removed

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Table 1. Bioassay of complex mixtures for carcinogenic potency.

Oil	Benzo(a)pyrene, %	Number of mice	Number of mice developing papillomas	Number of mice developing carcinomas	Average latent period, weeks
Shale oil sample #1 (heat transfer process)	<0.00001	20	1	17	43 ± 4
Shale oil sample #2 (heat transfer process)	—	30	1	18	36 ± 2
Shale oil sample #3 (retort combustion process)	<0.00001	30	3	22	43 ± 5
Crude oil (Texas)	0.002	20	0	0	—
Crude oil (asphaltic)	0.0005	20	0	0	—
Paraffinic distillate (uncracked crude)	<0.00001	30	4	2	64 ± 6
Industrial fuel oil	0.10	20	1	18	17 ± 2
Residuum (catalytically cracked)	0.4	30	0	30	8 ± 1
Benzo(a)pyrene in toluene	0.005	50	6	1	80 ± 8
Benzo(a)pyrene in toluene	0.2	30	3	27	31 ± 4

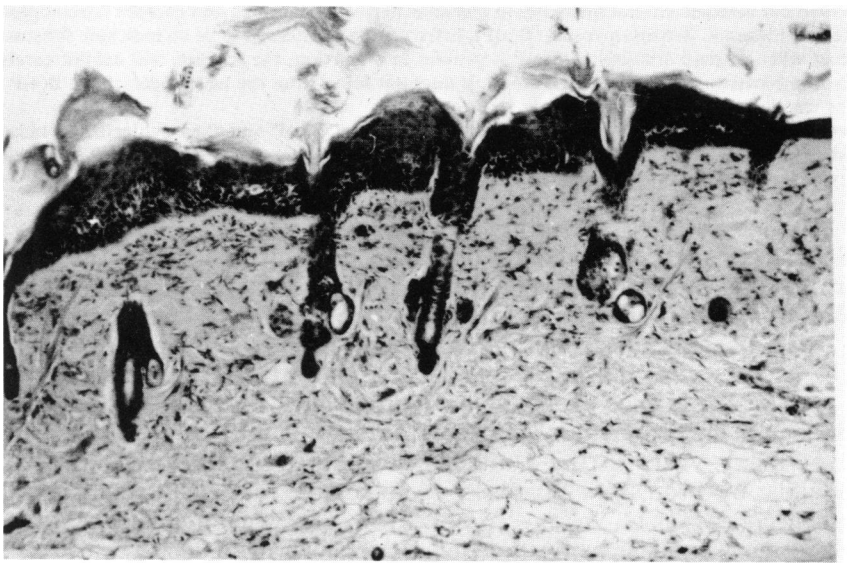

FIGURE 1. Early papilloma with hyperkeratosis and acanthosis. Most of the keratotic material broke off in the process of slide preparation. The dermis shows slight reactive fibrosis.

from the backs of the mice by using electric clippers prior to application of the solution and periodically as regrowth occurs. The mice are observed for the appearance of tumors and other skin lesions which are recorded three times each week. When a papillomatous lesion 1 mm^3 is seen the mouse is kept under observation. When the papilloma progresses and it is diagnosed grossly as a carcinoma, the mouse is killed and autopsied. A gross diagnosis of a carcinoma is made when a lesion upon palpation is attached to underlying tissues and apparently has invaded the connective tissue or muscular layers. All tumors and skin lesions are fixed in formalin for histological examination.

The average rates of induction of tumors by the different solutions involved in any given experiment are estimated by various calculations involving the time of appearance of the first papilloma in each mouse (in many animals, more than one tumor developed). In addition, the total number of papillomas per mouse and the number of mice developed papillomas and carcinomas are listed. The results obtained with three shale oil samples and several petroleum crude oils and petroleum fractions are presented for comparative purposes. In the instances where benzo(a)pyrene analyses were available they are presented.

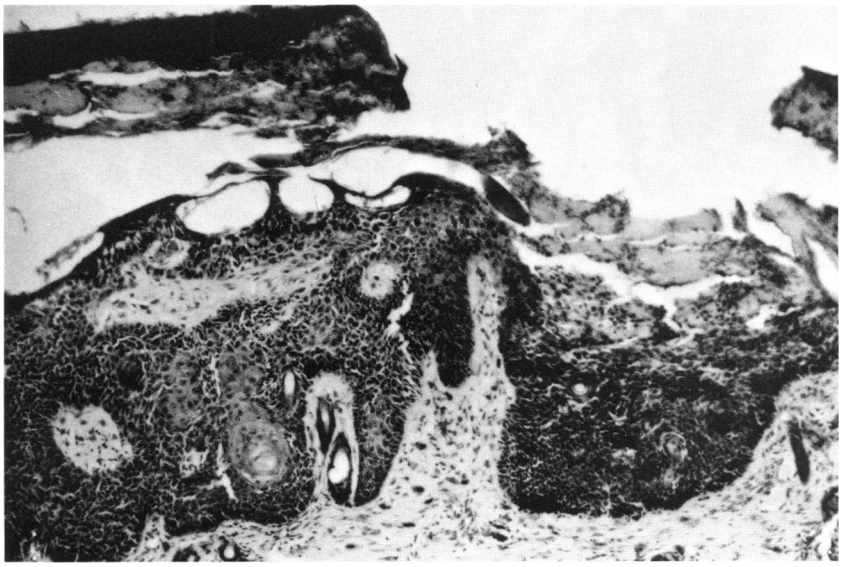

FIGURE 2. Advanced papilloma. Both acanthosis and keratosis are more pronounced. Also reactive fibrosis is present and atrophy of hair follicles.

FIGURE 3. Keratoacanthoma. On one edge epidermis is seen covered by keratinous material. Also at the surface large amounts of keratinous material. In the acanthotic parts retention of keratinous material. On one side the expansive growth is seen "pushing" the dermis and hair follicles. This type of keratoacanthoma can evacuate and then leaves a shallow cup-like hole. One can see the excavation more to the center of the lesion. At the edge the tumor seems to develop into carcinoma.

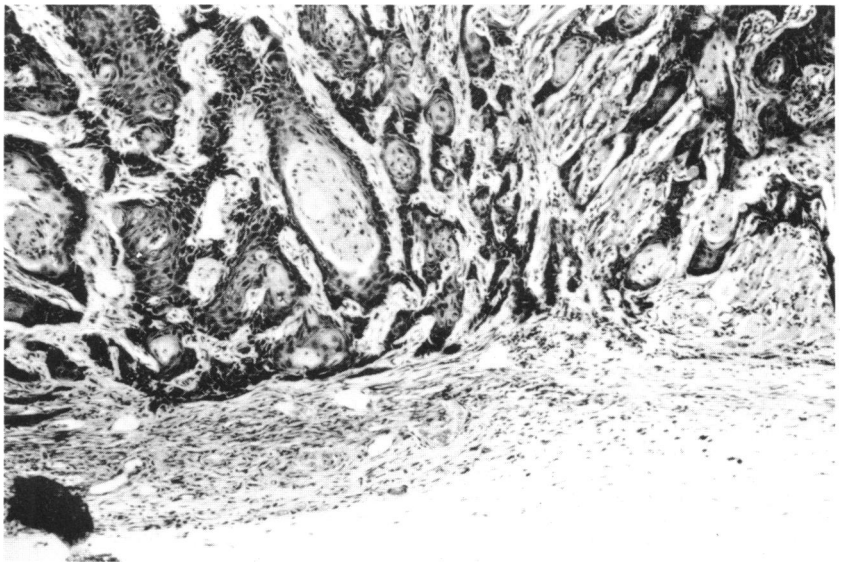

FIGURE 4. Basal cell carcinoma. Neoplasm is invasive. It consists of spindle cell-like cells arranged in clusters or band-like formation. In the center of these clusters the cells are more polygonal.

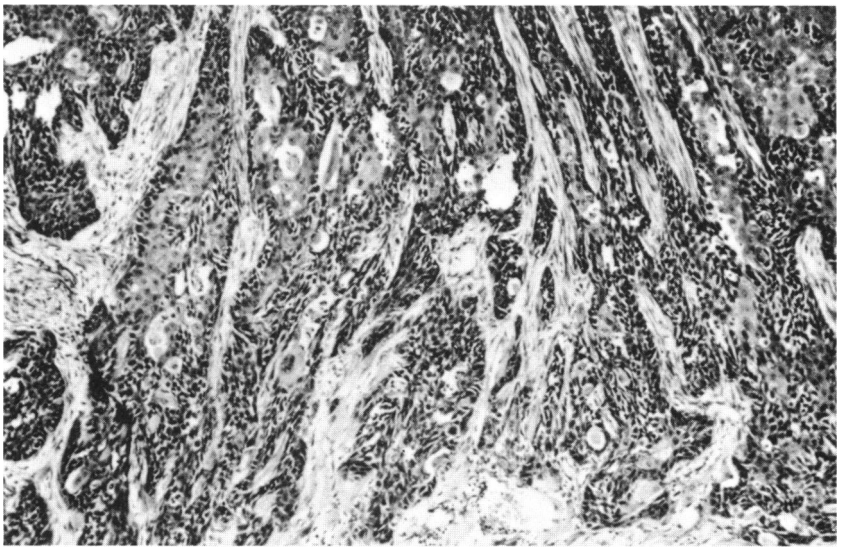

FIGURE 5. Squamous cell carcinoma. Invasive through the musculature as can be seen in one corner. Also invasive into subcutaneous fat tissue with reactive fibrosis.

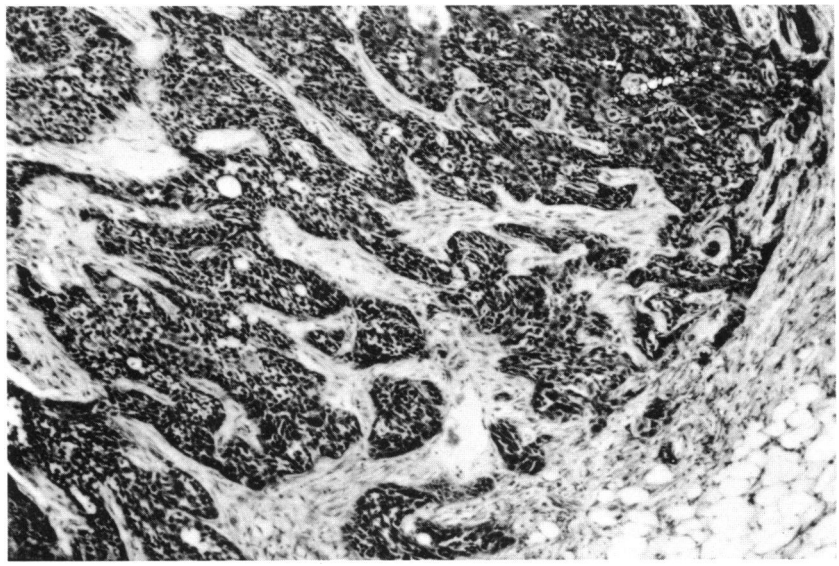

FIGURE 6. Base of squamous cell carcinoma. Muscle tissue almost completely destroyed by invasion.

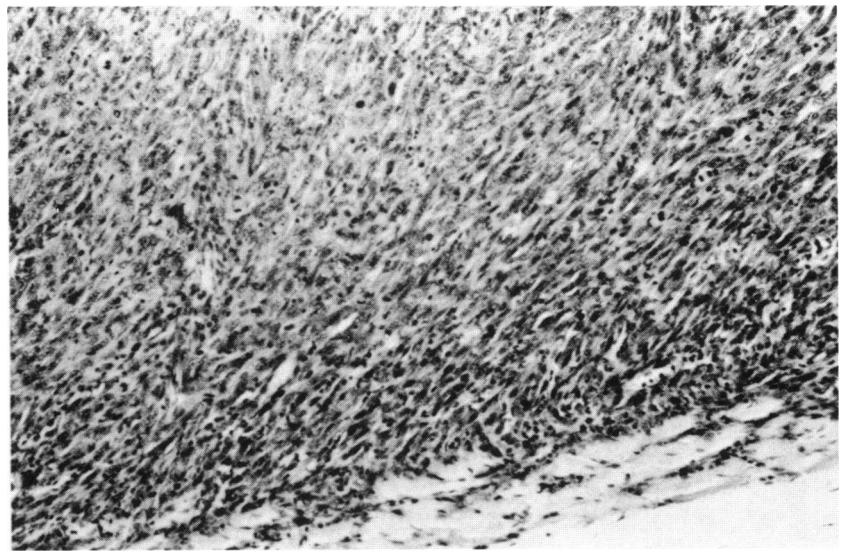

FIGURE 7. Acanthosis of epidermis with atrophy of hair follicles. Reactive fibrosis. In one half of the picture quite cellular.

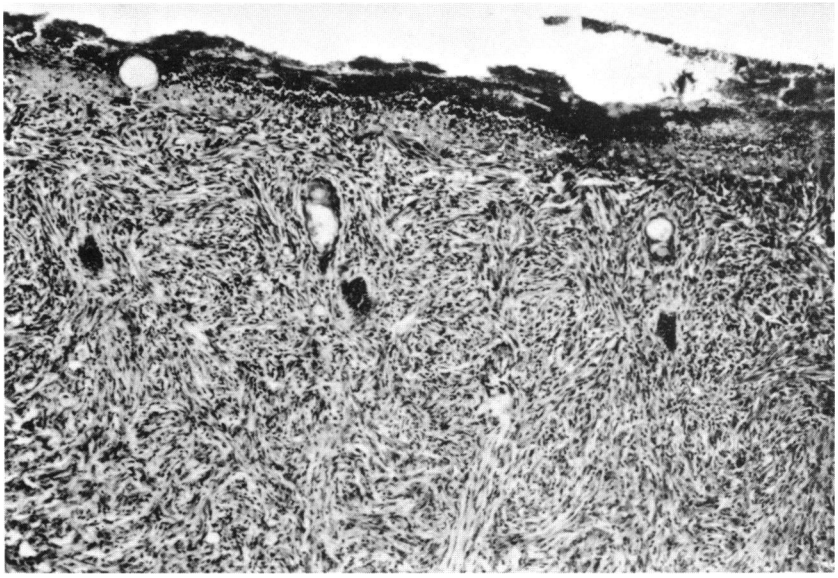

FIGURE 8. Fibroma, cellular type. A fasciculated structure with ulcerated surface is seen. Remnants of hair follicles are present.

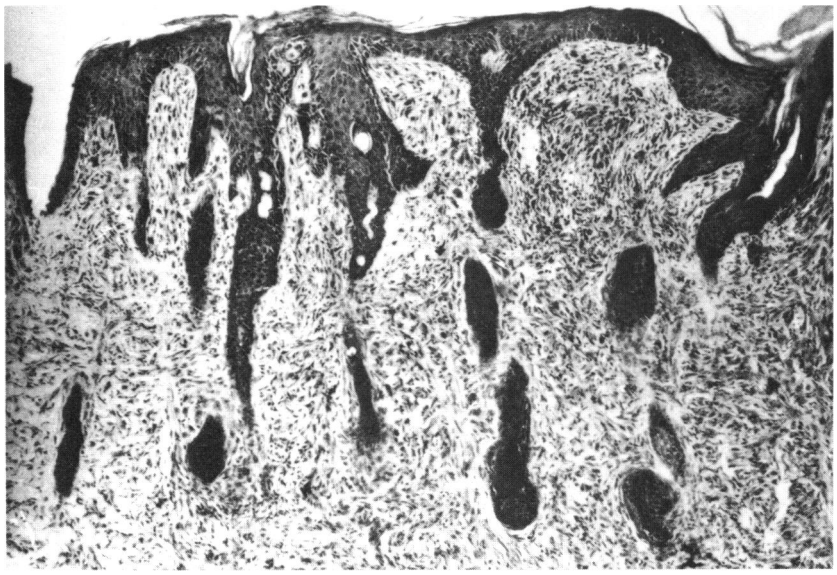

FIGURE 9. Fibrosarcoma showing ulcerated surface. Marked pleomorphism of cells and giant nuclei and mitotic figures are evident. Note the variation in size of cells and nuclei with irregular structure of the neoplasm. Note the large number of mitotic figures at the invasive edge of fibrosarcoma.

Results and Discussion

As may be seen from Table 1, results of the samples of shale oil are similar in potency to the crude petroleum fractions and uncracked distillates. Cracking of petroleum fractions produces carcinogenic substances including benzo(a)pyrene.

Lesions produced during bioassay of these mixtures are similar and the development of lesions may be seen in Figures 1-9.

Currently we are conducting experiments to investigate the carcinogenic potential of several samples of shale oil, raw shale and spent shale. The oils are applied topically in the manner described previously. The samples of raw shale and spent shale are applied as finely divided particles suspended in a white mineral oil. Mice are also exposed to topical application of white mineral oil, known concentrations of the carcinogen benzo(a)pyrene, and there are some that are untreated.

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