# Experimental Carcinogenic Studies on Hydrogenated Coal Oils

## II. Fischer-Tropsch Oils

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PREVIOUS experiments on three products (reaction water, light oil, heavy oil) obtained by the gasification, hydrogenation and polymerization procedures to which coal is subjected in the Fischer-Tropsch process had shown that none of these materials manufactured in a pilot plant of the U.S. Bureau of mines at Bruceton, is capable of causing cancers of the skin of mice following prolonged cutaneous applications.[1] The Occurrence of a few hepatomas in mice after their cutaneous and intramusculär administration suggested a possible tumorigenic action on this organ as a sequela of primary cirrhotic changes.

In the Fischer-Tropsch synthesis a mixture of carbon monoxide and hydrogen is transformed with the help of a solid catalyst at elevated temperatures up to 275°C and pressures up to several hundred pounds per square inch into liquid and solid hydrocarbons somewhat resembling petroleum. The hydrocarbons generated by this process belong to more than 90% of the aliphatic series, and mainly of the straight-chain variety. Both paraffins and olefins are present. Ring compounds, both aromatic and naphthenic, are present, Particularly in the higher boiling fractions, as are also branched-chain aliphatics. The complex mixture contains compounds ranging from the pentanes, with five carbon atoms per molecule, to waxes melting at 100°C or above, corresponding to a molecular weight of 1,000 or more. Small amounts of oxygenated compounds, such as alcohols, aldehydes, ketons and acids, are also produced and appear in the process stream. The major Proportion of them will have no more than five carbon atoms per molecule. Some of the oxygenated compounds are unsaturated.

Through the courtesy of Mr. J. A. Markovits, Synthetic Fuels Demonstration Plant, Louisiana, Missouri, large-scale production samples of Fischer-Tropsch products were obtained for carcinogenic testing on mice, rats, and rabbits. This report presents the results of these studies.

### Experimental Procedures

THE following Fischer-Tropsch fractions were used :

Fraction No. 1. Synthesis Condensate is a thin, yellowish, aromatic liquid corresponding to a mixture of one part Diesel oil with four parts gasoline.

Fraction No. 2. Cracking Stock is an aromatic, brownish oil having a boiling point above 250°C.

Fraction Bo. 3. Diesel Oil is an aromatic brown-yellow oil having a boiling range of 204"-316°C.

Fraction No. 4. Raw Gasoline is a thin amber liquid having a boiling point up to 204°C.

Fraction No. 5. Used Coolant Oil is a thick brownish oil having the consistency of fudge. It was diluted with small amounts of xylene for cutaneous applications, so that it could be dispensed from a dropper.

All fractions were tested for carcinogenicity by repeated application to the skins of mice and rabbits and by intramuscular injection into the thighs of rats. For cutaneous administration, the fractions were applied by dropper twice a week to the nape of the neck of mice and to various parts of the skin of rabbits.

For the testing of the various fractions by the cutaneous route, 25 male and 25 female mice of the C57 black strain about six weeks old were used. Applications of the fractions were continued throughout life. For the cutaneous administration of the fractions to rabbits, three-month-old animals of the Dutch strain were employed. Each fraction was applied to the skin of five rabbits. However, several fractions were applied to the same rabbit at different sites (dorsal surfaces of the ears and three areas of the back). The sites used for each fraction in the various rabbits followed a predetermined scheme providing for

[1] Part I—"Bergius Oils" was published in *Industrial Medicine and Surgery*, February, 1956. pp. 51-55.

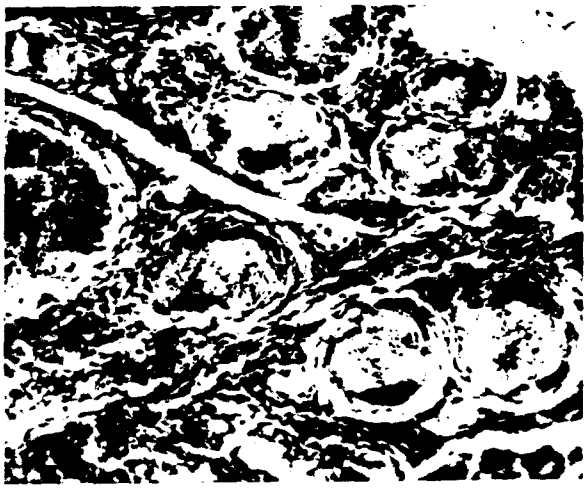

Fig. 1.
Giant-cell granuloma of the thigh showing huge giant cells containing powdery and smudgy blackish matter. 205x.

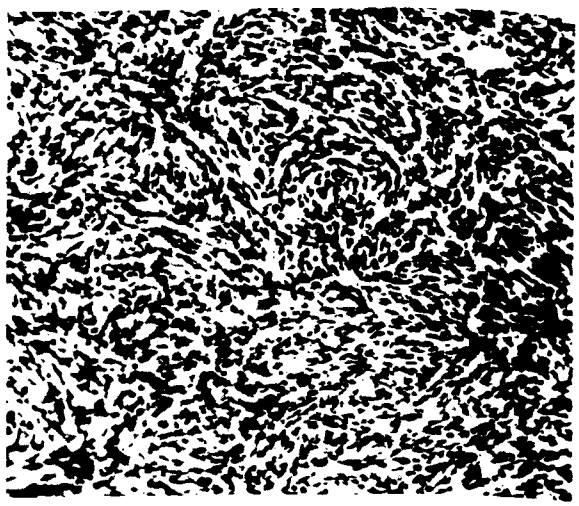

Fig. 2.
Spindle-cell sarcoma of the thigh at the site of injected Fircher-Tropsch product. 205x.

proper rotation so that each fraction was tested on each site selected. As in the mice, the hair was clipped with scissors during the early part of the experiment before more or less permanent loss of hair appeared at sites painted with the heavy fractions. The applications were continued for a maximal period of 25 months. The rabbits then were sacrificed.

For the purpose of intramuscular injection, a total of six doses were given divided in three series of two injections each with a one-week interval between the individual injections within a series and with a two-month interval between the first and second series and a four-month interval between the second and third series. The rats used were of the Wistar strain and about three months old. Each set consisted of 15 rats of mixed sex. The surviving rats were sacrificed at the end of a two-year observation period. Postmortem examinations were performed on all animals. Histologic examinations of the various organs and tissues were made whenever the necropsy findings revealed any significant pathology (five rabbits, 41 rats, 85 mice).

### Experimental Observations

CUTANEOUS APPLICATIONS TO RABBITS AND MICE:

Sone of the rabbits developed any neoplastic lesions at the sites where the five fractions were applied. The hair growth was not arrested at any time during the experiment at areas exposed to fractions Nos. 1, 2, and 5. Minor scaly changes were present in some but not all of the areas painted with fractions Nos. 3 and 4. The histologic examination of the skin from these areas did not as a rule reveal any abnormalities.

Similar essentially negative findings were made in the mice which were painted with the five fractions. There was some minor and transitory loss of hair and sometimes a scaly and slightly thickened skin where fractions Nos. 1, 2, and 3 were applied. Loss of hair, as well as dryness scaliness, and thickening of the skin were often but not always more marked and sometimes permanent in animals painted with fractions Nos. 4 and 5. Two mice painted with fraction No. 4 developed small papillomas. No other neoplastic reactions of the skin were observed. One mouse painted with fraction So. 1 showed at autopsy a reticulum-cell sarcoma of the liver. The majority of the mice dying during the latter part of the observation period revealed chronic interstitial nephritis and more or less extensive amyloidotic lesions of the liver, spleen, and sometimes of the renal glomeruli and adrenals. Since similar nephritic and amyloidotic changes are not uncommon in normal C57 black mice of the same age, they are most likely not causally related to the cutaneous applications of the various frac-

Fig. 3.
Fibrosarcoma of the thigh at the site of the injected Fischer-Tropsch product. 205x.

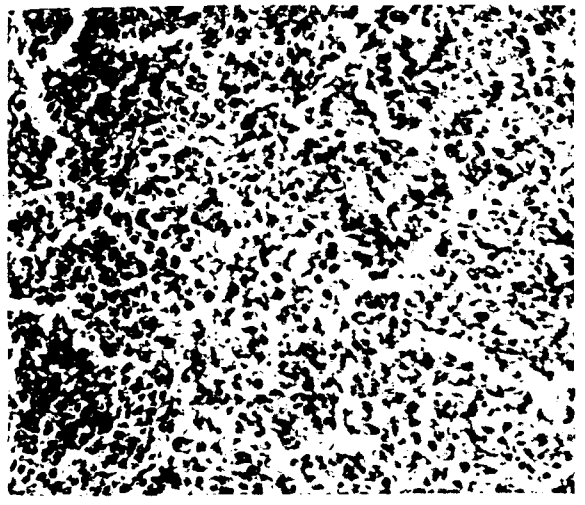

Fig. 4.
Round-cell sarcoma metastasis in the liver of a sarcoma originating in abdominal lymph nodes. 205x.

'ions tested. The two papillomas of the skin, on he other hand, definitely have such a relationship, vhile that of the single reticulum-cell sarcoma of he liver is quite doubtful.

INTRAMUSCULAR INJECTION INTO RATS:

The character of the local reactions elicited ›y the various fractions at the site of infection in he tissues of the thighs exhibited distinct differences depending upon the particular fraction ntroduced. In the interstitial tissue of the thigh nuscle injected with fraction No. 1, the lesions onsisted of a multicystic inflammatory fat tissue mbedded in a hyaline necrotic muscle tissue. 'raction No. 2 produced multicystic foam cell ranulomas; fraction No. 3 elicited abscesses and broses; fraction No. 4 did not cause any emonstrable local lesions; while fraction No. 5 roduced the development of large foreign-body, 'ant-cell and foam-cell granulomas with numer- is small cysts filled with a powdery black material which also was present within the foam and giant cells (Fig. 1).

In addition to two benign tumors (adenofibroma of the breast, hemangioma of the adrenal), the 75 rats showed a total of 17 malignant neoplasma, seven of which involved the right thigh and were spindle-cell or fibrosarcomas. Four additional cancers were spindle-cell sarcomas located in abdominal lymph nodes. Other tumors were large round-cell sarcomas originating also from abdominal and, particularly, ileocecal lymph nodes. One cancer was an adenocarcinoma of the kidney, and another was a squamous-cell carcinoma of the lung (Figs. 2 to 6). Both the spindle-cell and round-cell sarcomas produced, in part, widespread metastasis to other organs (spleen, liver, kidney, lung). Table I presents the distribution of the various cancers observed in the different groups in relation to the time of death of the rats.

The location of the spindle-cell sarcomas involving the thighs in relation to the site of injection of the fractions as well as the close relation of some of these neoplastic reactions to the nonneoplastic reactive lesions provide adequate proof

TABLE I.
DISTRIBUTION OF CANCERS AND OF DEATHS AMONG RATS INTRAMUSCULARLY INJECTED WITH FISCHER-TROPSCH OILS

| | Months of Survival | | | | | | | Total Number | |
|---|---|---|---|---|---|---|---|---|---|
| | 0-6 | 7-9 | 10-12 | 13-15 | 16-18 | 19-21 | 22-24 | Rats | Cancers |
| I. | 1 | 3 | 1 | 1 | 2 | | 3 | 15 | 1 |
| II. | 3 | 0 | 2† | 1 | 2 | 4†* | 5#* | 15 | 5 |
| III. | 2 | 0 | 3 | 2†‡ | 3‡ | 3† | 2 | 15 | 4 |
| IV. | 1 | 0 | 1 | 0 | 3‡ | 4& | 6* | 15 | 3 |
| V | 2 | 1 | 1 | 3 | 3* | 3*** | 2 | 15 | 4 |
| Total | 9 | 4 | 8 | 7 | 13 | 16 | 18 | 75 | 17 |

Code
* Spindle-cell sarcoma of thigh.
† Spindle-cell sarcoma of abdomen.
‡ Round-cell sarcoma of abdomen.
# carcinoma of lung.
& Carcinoma of kidney.

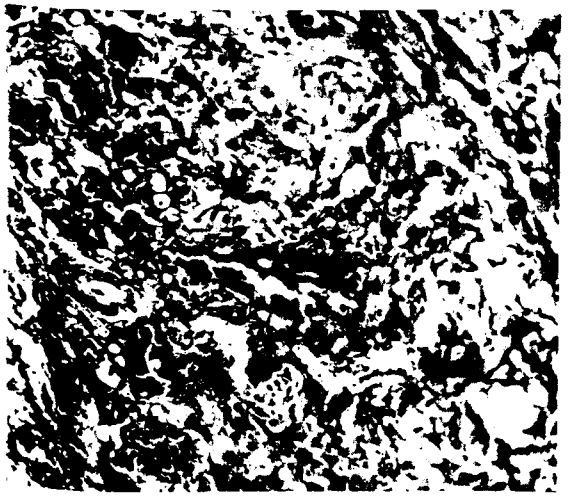

Fig. 5.
Adenocarcinoma of the renal cortex. 205x.

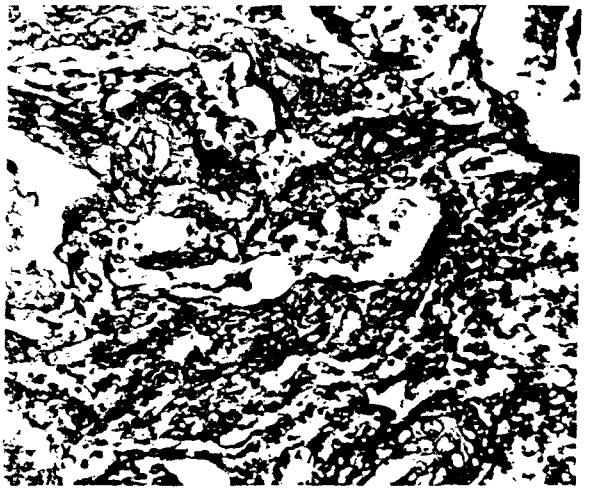

Fig. 6.
Squamous-cell carcinoma of the lung. 205x.

that the particular materials injected elicited these cancerous responses.

Less definite is the relation of the various abdominal spindle-cell sarcomas, the renal carcinomas, and the pulmonary carcinoma to the injected oils.

A lymphatic translocation of these materials from the thigh into abdominal lymph nodes is a distinct possibility, since similar shifts in the location of paraffin parenterally injected for cosmetic or surgical purposes from the primary sites of deposition (breast, nose, penis) into regional lymph nodes has frequently been reported.[2] The excretion of carcinogenic aromatic amines through the kidney, likewise, is a well-established fact and may provide a mechanism which may account for the occurrence of renal carcinoma in one of the rats given fraction No. 4. Since experimental evidence indicates that subcutaneously introduced carcinogenic polycyclic hydrocarbons as well as aromatic amines may elicit cancers of the lung in rats and mice, the presence of a squamous-cell carcinoma in the lung of one rat may well reflect a systemic carainogenic action of fraction No. 2, especially since only papillary adenomas of the lung have so far been observed among the various "spontaneous" neoplasms found in rats serving as starting points of pulmonary carcinomas.[3]

The four round-cell carcinomas involving abdominal organs and metastasizing into the lung, on the other hand, are most probably not neoplastic responses to the oils injected, but have to be considered as "spontaneous" cancers, because such tumors are not infrequent in normal untreated rats of the strain used.' The observations made in the five series of rats indicate that of the various fractions tested, fraction No. 5 seems to be most carcinogenic ; next in potency are fractions Nos. 2 and 3 : while the carcinogenic potency for rats of fractions Nos. 1 and 3 is uncertain.

### Conclusions

1. THE BIOASSAY of five products of the Fischer-Tropsch synthesis of hydrocarbons showed that several of these oils possess definite carcinogenic properties for rats, while doubtful ones for mice, and none for rabbits.

2. The main carcinogenic potency resided with two higher-boiling fractions, although a weak one was found also for raw gasoline.

3. The apparent species specificity of the carcinogenic action of the Fischer-Tropsch products parallels that previously demonstrated for Bergius oils and petroleum derivatives.

4. The observations made suggest that the carcinogenic effect of Fischer-Tropsch oils, like those obtained by the Bergius process, may not be restricted to the tissues in which these materials are deposited, but may extend also to remote organs.

5. Fischer-Tropsch synthesis products seem to be not only carcinogenically less potent than Bergius products but also appear to have also a narrower species and tissue susceptibility spectrum than the latter.

### References

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## To Keep Artery Walls Elastic

AFTER 10 YEARS of research on hardening of the arteries (which is rated as the No. 1 killer among diseases in the U.S.), Dr. Herman T. Blumenthal, Ph.D. 38, M.D. 43, has found evidence to support his thesis that emotional stress is the main cause of artrriosclerosis. The 43-year-old director of the Pathology Laboratory at St. Louis' Jewish Hospital pointed out his findings in a report delivered last month. Diverging from the generally accepted theory among most specialists and researchers in the field, Dr. Biumenthsl states that "cholesterol (fat stubstance which inhibits normal flow in blood vesseis) is the result, riot the cause of the disease." His theory is that emotional stress with accompanying changing blood pressure, works against the arterial walls to cause hardening and lesions. Through ictensive study of the mechanics of blood flow and pressure within the walls of the arteries, Dr. Blumenthal states that under normal pressures, the healthy arterial wall is elastic. However, under changing and higher-than-normal pressures, arterial walls at the vulnerable junctions lose their elasticity. He and his staff are now working on the isolation of the chemical substance which the body produces to keep artery walls elastic. In a few irregular cases, observed by Dr. Blumenthal, the body has offset abnormal wear and tear by cresting new elastic material. It is the researcher's next task to isolate the elastic substance and then find ways of developing a man-made material to use in repairing the strains of stress on the arterial life lines of aging humanity.

—*Washington University Alumni* Bulletin. St. Louis, May, 1956.