Reports and Letters

Volatility of Metallo-Porphyrin Complexes

Small quantities of heavy metals are associated with crude oils. During refining these metals tend to pass overhead with the heavier distillates. The presence of vanadium, nickel, copper, and iron in distillate charge stocks greatly increases gas and coke yields and reduces gasoline production (*1*).

There are two hypotheses regarding the mechanism of occurrence of metals in distillates. One is that mechanical entrainment of residua is responsible (*1, 2*), and the other that the metals distill in the form of volatile metallo-organic compounds. (*3, 4*).

The demonstration of the presence of metallo-porphyrin complexes in crude oils (*5*) has been taken as evidence for the latter hypothesis (*4*). Reports of the sublimation of etioporphyrin (*6, 7*), monoimido- and diimido-etioporphyrin II (*8*), and octapropylporphine (*9*) indicate that these compounds possibly possess sufficient vapor pressure to distill with crude oils. No reference, however, could be found to the sublimation or other direct determination of the volatility of the heavy-metal complexes.

This paper describes the sublimation of the vanadium, nickel, copper, and iron (ferric chloride) complexes of etioporphyrin I under conditions that permitted the free porphyrin to sublime, thus demonstrating that these heavy-metal complexes may equally well distill with the heavier fractions of crude oils. The results of trial distillations of mixtures of metals-free petroleum fractions and pure synthetic porphyrin complexes will be reported in the near future.

Etioporphyrin I was selected as representative of the alkyl-substituted porphyrins that occur in crude oils. The procedure for the preparation of the etioporphyrin I was essentially that of Fischer (*6*), except that the product was purified chromatographically. The preparation of the nickel, copper, and iron complexes was performed in the usual manner. A description of the method for the preparation of the vanadium complex is in preparation.

The apparatus consisted of a sublimation cell, a vacuum system, a microscope equipped with a Leitz-Weygand hot stage, and equipment for cooling one surface of the cell. The sublimation cell was a hollow, glass cylinder of inner diameter 14 mm and inner height 10 mm; the end-pieces of the cylinder were optically flat. A mechanical forepump, an oil diffusion pump, a trap, and a Pirani gage for measuring pressure comprised the vacuum system. One flat base of the cell rested on the microscope hot stage, and the walls of the cell were surrounded with a washer of crumpled aluminum foil as insulation. A hypodermic needle connected with narrow copper tubing to a cylinder of carbon dioxide was arranged to direct a jet of expanding gas onto the exposed flat surface of the cell.

The porphyrin sublimand was placed in the cell and was distributed in an even layer over the entire surface of the base. Heating was begun only after a pressure of approximately 0.4 μ had been attained in the system. When the cell had cooled, the unsublimed residue was completely removed from the cell, and the weight of sublimate was determined. The results are summarized in Table 1.

The spectra of the sublimates were determined over the range 220 to 700 mμ, using a Beckman DU spectrophotometer. In all cases it was demonstrated that the metal complexes had sublimed and that the sublimate and sublimand were the same substance. There was no indication of significant thermal decomposition or dissociation of the metal complexes.

Table 1. Summary of sublimation data.

Substance	Wt. sample (mg)	Temperature range (°C)	Wt. sublimate (mg)	Duration of run (hr)
Etioporphyrin I	0.5	220–316	0.5	1.0
Etioporphyrin I—Copper complex	0.7	225–305	0.4	6.5
Etioporphyrin I—Nickel complex	0.6	210–305	0.6	3.3
Etioporphyrin I—Vanadium complex	6.6	220–316	5.6	3.5
Etioporphyrin I—Ferric chloride	0.6	225–298	0.6	3.5

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* Multiple fellowship of Gulf Research and Development Company.

19 September 1955

Transformation of Normal Human Fibroblasts into Histologically Malignant Tissue in vitro

On 18 Sept. 1953, fragments of a 4-day-old infant's foreskin were explanted into sponge matrix tissue culture. The most luxuriant cultures were used in studies on the invasion of HeLa cells into the connective tissue outgrowth (*1*). The least vigorous culture, now designated as strain D, was incubated at 37°C and fed at intervals as indicated by changes in the *p*H of the medium. The nutrient was composed of human serum, beef embryo extract, and Hanks' balanced salt solution in the ratio of 5/1/10 with penicillin and streptomycin at a concentration of 50 μg/ml.

Growth was very poor, and as an extreme measure before discarding the culture, the area of the explant was incised with a scalpel and patched. Subsequently spindle-shaped cells grew out into the new clot and soon extended into the old, granular clot, filling up the area of implantation as well as much of the surrounding cellulose sponge with new connective tissue.

Explants from this culture, cemented with a plasma clot on the surface of other slices of cellulose sponge, grew and were

subcultured every 6 to 10 weeks. Ten months after explantation, the technique of subcultivation was changed, since more rapid outgrowth of connective tissue was observed when explants were sandwiched between the wall of the roller tube and the slice of sponge. This has been our technique for growing strain D up to the present time.

The cells grow readily, as a latticework of spindle-shaped cells and collagenous fibers, forming a loose connective tissue throughout the sponge interstices. They also produce a dense layer of spindle-shaped cells on the outer surfaces of the sponge. Occasional bizarre, large cells have been seen from time to time, but the overwhelmingly predominant pattern has been one of a differentiated connective tissue in which collagenous fibers are readily produced. Four new subcultures may be made every 4 to 6 weeks from each culture tube. In practice, however, cultures are maintained for several months without subculture if new cultures are not needed for experimentation.

No known carcinogens have been added to the cultures, although on repeated replenishing of the medium, drops of nutrient adherent to the lips of the tube were charred on flaming, and occasionally fragments of this material were seen to drop into the tube as the rubber stopper was inserted. When the cell strain was 23 months old, one stock tube (D-189), a 15th subculture generation, which had been planted 4 months before, showed a remarkable pattern. Many large, bizarre cells were found extending from the edges of the sponge onto the adjacent glass wall. During the following 2 weeks the growth of pleomorphic cells became more extensive. The sponge was then removed, part of it was fixed in Zenker-formalin for histologic examination, and the remainder was subcultured.

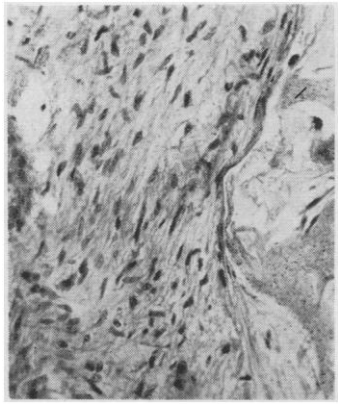

Fig. 1. Microscopic section of a cellulose sponge tissue culture of strain D, in which the pattern of growth is that of a normal connective tissue. The cells are of uniform size and shape and do not appear malignant. Hematoxylin and eosin stain (× 125).

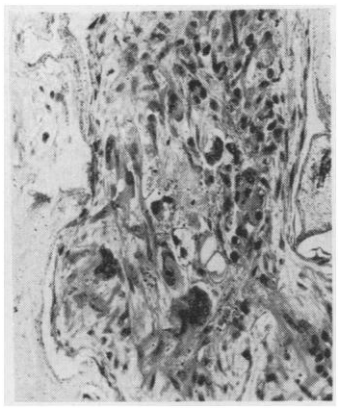

Fig. 2. Microscopic section of a culture of the spontaneously transformed connective tissue, strain D-189 (derived from strain D) in which the cytologic features of a malignant tumor are seen. Many cells are large and hyperchromatic. The nuclei in these are very large, and some are multilobate. Enormous nucleoli are seen in some cells. Hematoxylin and eosin stain (× 125).

The histologic pattern of the normal-appearing strain-D cell (Fig. 1) had been replaced in many areas on the outer surface of the sponge by a densely cellular tissue that appeared neoplastic. There were many large, hyperchromatic cells, some with single, large nuclei containing enormous nucleoli, others with four to six nuclei (Fig. 2). Dividing cells were numerous, and many of the mitotic figures were multipolar. The pattern seen in sections of this culture had the criteria on which the diagnosis of highly malignant anaplastic tumor is made. The altered tissue (D-189) in its fifth subculture generation since its recognition grows very rapidly.

The transformation of normal cells into malignant ones in tissue culture, with or without the addition of known carcinogens, has been observed in several laboratories in cultures of mouse and rat tissues (*2–4*). In each instance the malignant nature of the transformed cells was demonstrated by the production of sarcomas when the cultures were inoculated into animals of the same strain as the one from which the original cells had been obtained. It is not unexpected that human cells are capable of the same kind of transformation in tissue culture. In the instance reported here, the final proof of a malignant transformation, transplantation and growth in a genetically proper human host, is of course impossible. However, the biologic behavior of this new strain may be examined in animal transplant for a partial appraisal of its malignant properties.

Goldblatt and Cameron (*4*) have implicated anaerobiosis as a common factor in the production of malignancy *in vitro*. Since strain-D cells have been regularly covered by a blanket of cellulose sponge and plasma clot 0.5 to 1.0 mm thick on each subculturing for 13 months, anaerobiosis could have contributed to the formation of a morphologically malignant variant. Cellulose sponge itself may also be implicated as a causal factor, for Oppenheimer (*5*) has found that a variety of polymers including several cellophanes can induce sarcomas is the subcutaneous connective tissue of the rat.

Gey (*6*) has pioneered in the development of cell strains from malignant human tumors as well as of isogenic strains from normal areas of the same surgical specimen. Cell strains of such parallel origin may lend themselves to the discovery of distinctive metabolic properties of cancer cells.

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10 October 1955

Human Arterial Hypertension: a State of Mild Chronic Hyperaldosteronism?

Many efforts in the search for a direct relationship between the adrenocorticoid hormones and arterial hypertension in human beings have met so far with no success. Chromatographic separation of urinary steroid extracts using an aluminum oxide column did not show any significant differences between normal and hypertensive subjects (*1*). However, the identification of aldosterone, a new adrenal mineralocorticoid hormone, by Simpson and Tait (*2*) has made it possible to restudy the problem with a more specific method (*3*).

Details of the assay procedure have been described previously (*4*). Only the main procedures will be outlined. Twenty-four hour urine aliquots adjusted to *p*H 1 were immediately extracted with four successive 1/5 portions of chloroform. The remaining urine was