Table 2. Skin tumor induction following repeated exposures to approximately 1 μg of 3,4-benzopyrene that was applied percutaneously three times per week.

Mice		Weeks preceding tumor appearance												Mice with no tumors*
Strain	No.	30	35	40	45	50	55	60	65	70	75	80	85	
C57BL	13	1	2				1	1		2	1		4	1
DBA/2	13					1	1	1	1	1	1	1		6
CAF_1	12							1	1	1	1			6

* Nontumor deaths after the 30th week.

exposure time are essentials that have been omitted previously in proposed methods for assaying comparative carcinogenic potency (7). In the absence of experimental verification, the exposure level at which a carcinogen ceases to have "initiating potency" and retains only "promoting action" cannot be predicted at this time.

From the early studies in experimental carcinogenesis, there has been general acceptance of 0.3 percent as providing a standard low concentration of carcinogenic hydrocarbon dissolved in benzene for skin painting experiments (8). An indication that 0.3 percent is in fact a high concentration of carcinogen may be found as early as 1940, when, despite reference to the concentrations used as minimal doses of carcinogen, tumors were reported in 18 of 20 mice that had been painted with a 0.05-percent solution and in 14 of 20 mice that had been painted with a 0.02-percent solution of benzopyrene in benzene (9). In our laboratory, the tumor-inducing potency of even lower concentrations was demonstrated with the application of 1 μg or less of benzopyrene as a 0.01-percent solution in benzene three times weekly for the life span of the animal or until a tumor was induced (Table 2). Evidently, when solutions of 0.01-percent benzopyrene applied in microgram or lesser quantities induce tumors in 50 to 100 percent of exposed mice during their life span, such terms as *low standard concentration, minimal dose* of carcinogen, *initiator, promotor,* and *cocarcinogen* need to be redefined in quantitative terms.

It cannot be denied that cocarcinogens may exist, with roles in carcinogenesis comparable to that of pharmacologic adjuvants and physiologic or chemical catalysts. But their existence is yet to be conclusively demonstrated by the experimental oncologist.

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26 September 1955

Preliminary Report on Biological Applications of Color Television

Electronic image processing (1) is the term we have used to describe a variety of electronic means for viewing objects or processes and picturing these in their original or in altered but meaningful forms. These techniques have been of particular interest to us when they permit visualization of biological phenomena that cannot otherwise be seen, or when the electronic systems employed bring out information that is concealed when the phenomena are viewed directly. Such systems are analogous to indicator systems used in morphological studies, such as stains.

Color television has been explored as one of these electronic image-processing techniques that can be applied to biological problems. Sixteen millimeter color kinescope (motion-picture) film records of a pilot experiment indicate that some theoretical advantages of this technique can be realized. An amphibian preparation (frog) was set up in the C.B.S. Studios in New York on 10 Apr. 1955, by Louise Warner of Georgetown University Medical School and Edward H. Bloch of the Western Reserve University School of Medicine; the quartz-rod illumination technique was employed. This preparation was selected because it is simple, the results are reproducible, and direct motion-picture records by this technique are familiar to investigators in the field of microscopic circulation as well as readily available for comparison by others. The C.B.S. color system was employed because it is a completely engineered system with inherent adaptability for these experimental purposes, and a fully developed kinescope recorder is available.

Microscopic circulation in frog mesentery and liver can be observed in normal color relationships at satisfactory magnifications and with good resolution. When lighting is reduced below levels for microcinematography, satisfactory films are obtained. Reductions in the Kelvin temperature of the light source can be compensated without sacrifice in the final picture. Thermal energy delivered to the tissue can be reduced to low levels, thus simplifying the problem of maintaining physiological conditions.

At will, the investigators could remove one or two colors from the picture and record the remaining color or colors. Gamma (the degree of contrast) could be varied independently for each color. "Crispening" circuits were employed to increase apparent contrast. Phase shifts between the camera color wheel and the monitor and recorder color wheels made it possible to record red subject matter as green or blue. The investigators could observe the exact picture that was being recorded on a monitor that was conveniently located near the subject material.

Examination of kinescope film made during this pilot experiment with unmodified equipment indicates that combinations of the afore-mentioned variables produce color motion-picture film that contains usable information that is not available by direct photography.

The extreme sensitivity to light that is an inherent property of such systems makes it conceivable that undisturbed human circulation can be observed and recorded at usefully high magnifications in the retina of man. With the substitution of appropriate filters for the color disks in the camera, chemical data could be correlated with living morphology in those instances in which dissociated light-transmission curves have chemical significance, as in the case of reduced and oxyhemoglobin.

The experimental film has been organized into a short motion-picture entitled, *Color Image Processing, Experiment 26, Joint Study of Electronic Image Processing.* It is one of 26 experiments conducted by the Special Devices Center, Office of Naval Research, U.S. Navy, and the National Institutes of Health, Department of Health, Education, and Welfare, in the course of the first part of a study of the usefulness of television and related techniques in the medical research environment.

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Note

1. The term *image processing* was introduced in the electronic literature by L. S. G. Kovasznay and H. M. Joseph (*Proc. I.R.E.* 43, No. 5, 1955) to describe electronic techniques for sharpening photographic images. We have borrowed and expanded the term to describe the larger field defined in this communication.

10 October 1955

Scopoletin in Differentiating and Nondifferentiating Cultured Tobacco Tissue

Two morphologically distinct strains of cultured tobacco tissue, when extracted with ether, yield markedly different amounts of a fluorescent material. These two strains have been cultured for 5 years and are of a similar (but not identical) origin, being derived from root tumors on two separate seedlings of *Nicotiana affinis* (*1*). Both strains first grew as undifferentiated callus for 3 years. Strain 20-B then started to produce buds and has continued to do so for 2 years, while strain 3-S, which was cultured on the same medium and under the same conditions, has continued to grow as undifferentiated callus with occasional wound tracheids (Fig. 1). More recently, a substrain of 20-B, called 20-B-O, which has reverted to the original nondifferentiating condition, has been isolated (*2*). The tissues are all maintained in diffuse light at room temperature on a modified White's medium containing 3 g/lit of yeast extract.

The tissues were extracted with 2.5 ml of freshly distilled ethyl ether per gram of fresh weight for 16 hours in the cold room. Dry weights were determined on samples of tissue similar to those extracted. The materials present in the ether extracts were chromatographed on Whatman No. 1 paper with water as the ascending solvent. The fluorescent material, visible under ultraviolet light, moved as a distinct band and was eluted with ethyl alcohol. Optical densities of this eluted material were measured at 345 mμ in a Beckman spectrophotometer. For reasons given in a subsequent paragraph, the substance was presumed to be scopoletin, and concentrations were calculated using a molar extinction coefficient of 14,000 determined by Goodwin (*3*) for solutions of this compound.

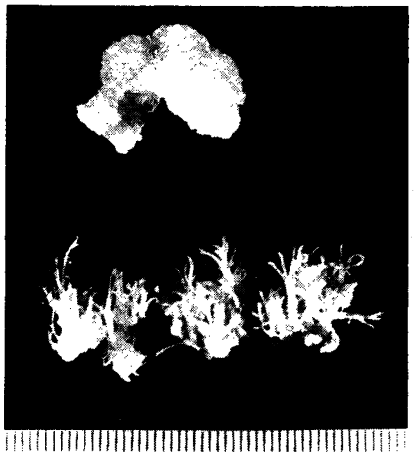

Fig. 1. Two strains of cultured tobacco tissue. (Top) Undifferentiated callus of strain 3-S; (bottom) differentiated shoots on strain 20-B. Scale in millimeters.

The results of the analyses are shown in Fig. 2. The figures are undoubtedly low estimates of the amounts of the compound present in the tissues, since the substance is light-labile, and other losses occur in the elution process. Each bar represents a mean of 12 extractions, with the standard deviation indicated. By the methods used, approximately 18 times as much of the fluorescent compound is extracted from tissue that produces organized structures (strain 20-B) as from tissue that has never so differentiated (strain 3-S). About 6 times as much is extracted from the differentiating strain as from its nondifferentiating substrain (20-B-O). Hot and cold acidic and basic aqueous extractions of macerated and unmacerated tissue yield similar relative differences.

On filter-paper chromatograms, the extracted fluorescent material moves with spots of known scopoletin (6-methoxy-7-hydroxy coumarin) giving R_f values of approximately 0.9 in butanol-acetic acid-water, and approximately 0.34 in butanol-ammonia-water (*4*). The fluorescent material, when eluted from the chromatograms with 95-percent ethanol, has an absorption spectrum that coincides with that reported by Goodwin (*3*) for scopoletin, and with the spectrum of known scopoletin determined simultaneously in alcoholic solution. Identical spectra were obtained for five separate preparations at various concentrations. Figure 3 shows one of these. The same absorption spectrum is observed in material prepared according to Best (*5*), followed by column chromatography on alumina (*3*). Four characteristic maxima occur at 230, 254, 300 and 345 mμ. Both known scopoletin and the extracted material give a green-fluorescent spot on paper chromatograms in basic solvents in daylight, but no daylight-visible spot in acidic solvents. These properties of the fluorescent material are accepted as good indirect evidence that it is indeed scopoletin.

Thus it appears that in these tissues, under the stated conditions of culture, scopoletin is associated with the presence of structures in which much differentiation exists. Similarly, Goodwin and Pollock (*6*) have observed that scopoletin occurs in *Avena* roots in relative abundance only in older parts where tissue differentiation is occurring. Whether the presence of scopoletin is a prerequisite for the formation of the organized structure or the organized structure produces the scopoletin is a question under investigation.

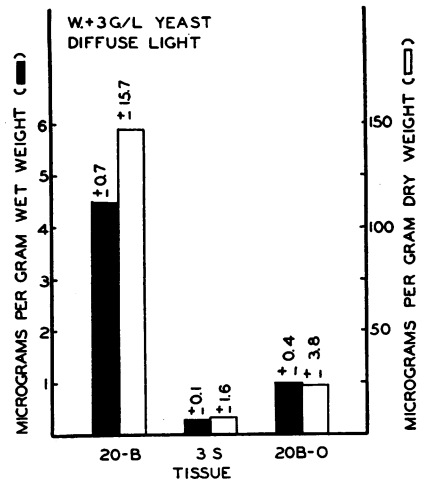

Fig. 2. Amounts of "scopoletin" extracted from three strains of tissue. The figures at the ends of the bars are standard deviations.

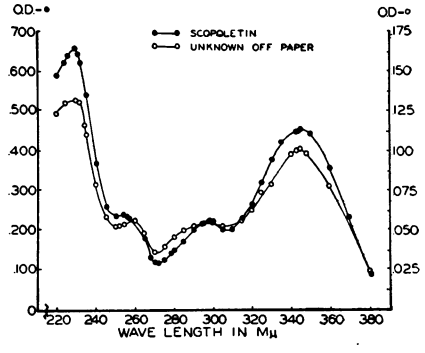

Fig. 3. Absorption spectra of known scopoletin (black dots, left-hand scale) and the fluorescent compound obtained from tobacco tissue (white dots, right-hand scale).

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26 September 1955