Figure 1 illustrates the most common type of SDT change observed in 13 out of 15 subjects[9]. The threshold means of a representative subject at T_1 (previous to the drug) were 3.9△ and 3.6△, base up and base down respectively. As the drug effect reached its peak, T_2, these thresholds fell to 1.7△ and 1.5△. At T_3, thresholds were once again approaching normal values: 2.9△ and 2.7△. The range of decrease in threshold (base up and base down) for the 13 subjects from T_1 to T_2 was 1.4△ to 3.0△. Two subjects showed increases in mean threshold, one of only 0.58△, the other 9.6△, probably due to interference from vivid hallucinations.

In contrast to psilocybin, an excitatory drug, which generally decreases the SDT mean, tranquilizers such as chlorpromazine increase the SDT mean. This increase is illustrated in Figure 2 after the administration of 50 mg of chlorpromazine to the same subject whose response to psilocybin is given in Figure 1.

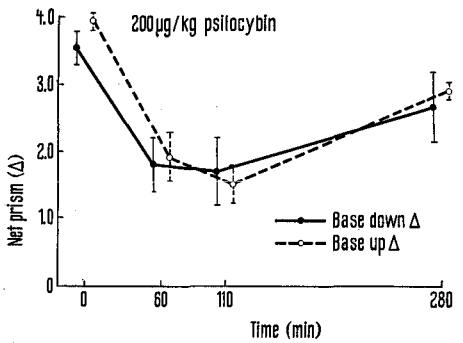

Fig. 1. Thresholds for spatial distortion induced by prism lenses prior to and during the time course of a drug experience elicited by 200 µg/kg psilocybin. Each point is the mean of 6 observations. The standard deviation about the mean is indicated by brackets.

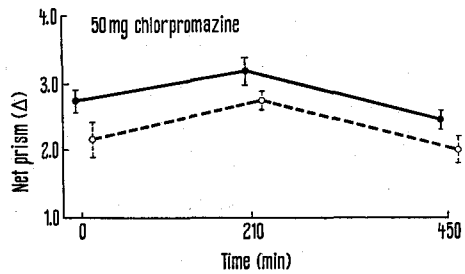

Fig. 2. Thresholds for spatial distortion induced by prism lenses prior to and during the time course of tranquilization produced by 50 mg of chlorpromazine. Each point is the mean of 6 observations. The standard deviation about the mean is indicated by brackets.

The significant effect of psilocybin on SDT is best represented through correlating the combined SDT means (base up and base down, N = 15) at T_1 with the comparable means obtained at T_2 ($r_s = +0.93$)[10,11]. This correlation is highly significant with the Wilcoxon Matched Pairs Signed-Ranks Test (N = 15) which indicates that the probability of such a distribution occurring by chance is 1 in 100[12]. Evidently a subject at psilocybin drug peak loses much of his compensation, i.e. the ability to 'correct' distorted visual space[13]. Our results imply a practical consequence; namely, that adaptation to corrective lenses should be diminished during the course of excitatory drugs of the psilocybin type and enhanced by tranquilizers. Preliminary observations show that this indeed seems to be the case[14].

Zusammenfassung. Die gerade noch wahrnehmbare, optisch induzierte Krümmung einer horizontalen Geraden wurde unter dem Einfluss von Psilocybin untersucht. Die Droge bewirkt einen deutlichen, jedoch vorübergehenden Kompensationsverlust gegenüber der optisch induzierten Krümmung.

R. M. Hill, R. Fischer and Diana Warshay

College of Optometry and Department of Psychiatry, College of Medicine, The Ohio State University, Columbus (Ohio 43210, USA), 30 August 1968.

[9] Each subject, $^1/_2$ h before T_1, was given sufficient base up and base down SDT training such that his answers fell within a one prism (△) diopter range of < 0.5 prism diopters of the mean for a given set. He was also familiarized with the appearance of color fringes and the slight displacement of the line (less than 2° on the average) accompanying the increase in prism power. The subjects, within a few trials, learned to ignore those ancillary phenomena.

[10] The r_s statistics given are Spearman Rank Correlation values.

[11] No correlation, however, was found between change in pupil size and change in SDT from T_1 to T_2 indicating that the concomitant vegetative and perceptual phenomena are unrelated.

[12] S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York 1956), p. 75 ff.

[13] I. Kohler, *The Formation and Transformation of the Perceptual World, Psychological Issues*, vol. 3 (4) Monograph 12 (International University Press, Inc., New York 1964).

[14] Acknowledgments: These studies are part of a plan for the investigation of psilocybin by R.F. approved by the Food and Drug Administration IDN 3530 and were assisted by Grant No. 66-341 of the Foundations' Fund for Research in Psychiatry, U.S. Public Health Service, National Institute of Health, General Research Support Grants, administered by The Ohio State University, College of Medicine and Comly Coleman Fund. Our thanks are due to Sandoz Pharmaceuticals, Hanover, New Jersey, who through the FDA-PHS Psychotomimetic Agents Advisory Committee provided us with psilocybin.

The Fate of Walker 256 Carcinosarcoma Cells Labeled with Tritiated Cytidine (Cr-5-H^3)

There is considerable clinical evidence to demonstrate that neoplastic cells enter the circulating blood not only as a result of invasive growth, but also during certain commonly used clinical procedures[1,2]. It has been demonstrated that cancer cells are released into the circulating blood during surgery[3,4]. The fate of the circulating cells is still a matter of speculation. It is presumed that the vast majority perish in the blood or are filtered out in the tissues where they remain in a dormant stage[5,6]. Apparently, only a small number of them reproduce to form a metastasis.

The possible relationship between circulating cancer cells and the establishment of metastases has not been explained satisfactorily despite numerous investigations and deserves more intensive study.

Recent use of the tritium-labeled DNA and RNA precursor, tritiated cytidine (CR-5-H^3), has permitted in vivo radioactive labeling of individual tumor cells.

In contrast to the incorporation of solely DNA precursors, which occur primarily in dividing cells, the incorporation of RNA is a property of all viable nucleated cells. Such cells are easily detected by autoradiography.

The purpose of this investigation was to label Walker 256 carcinosarcoma ascites cells with tritiated cytidine and to determine their localization in the tissues of healthy recipient animals after i.v. injection.

Material and methods. Female Wistar rats about 200–250 g from Charles River Farms were used for this experiment.

Procedure: A rat which had been inoculated i.p. 8–10 days before with Walker 256 ascites tumor cells and with obviously demonstrated ascites was used as a donor. 0.5 ml containing 250 mc of tritiated cytidine (S.A. 6.0 c/MM, Schwartz Bioresearch, Inc., Orangeburg, New York) was injected i.p.

The animal was sacrificed 1 h later. The ascitic fluid (approximately 100 ml from each animal) was aspirated from the peritoneal cavity of the rat and diluted with saline (average 1 part ascites to 8 parts saline) to a concentration of 10^6 cancer cells/ml. The tumor cell count was performed in a hemocytometer. Smears were made for staining and autoradiograms. Viability was controlled by SCHREK's method[7] and found to be 100%.

Each recipient animal was anesthetized with i.p. sodium pentobarbital (approximately 15 mg), the femoral vein was exposed and 10^6 cancer cells in 1 ml of solution were injected i.v. Each ml contained about 3 mc of tritiated cytidine. The animals from each group were sacrificed at intervals of 1, 15, 30 and 60 min and 2, 3, 4, 12 and 24 h after injection. The lungs were ligated and removed in toto immediately after sacrifice.

Autoradiographic and histologic techniques: The ascitic fluid smears were fixed in methanol for 15 min. The lung tissues obtained at autopsy were fixed in buffered 10% formalin. After the tissues were embedded in paraffin, sections were cut at a thickness of 5 μ from several levels of the block and autoradiograms were prepared by dipping the slides in Kodak NTB_3 nuclear track emulsion. The autoradiograms were stored at 4 °C for 3 weeks, developed in Kodak Dektol at 15 °C for 2 min, fixed, washed, and stained with Ehrlich's acid hematoxylin and eosin. The smears were stained with Giemsa. Background was negligible in all autoradiograms.

Results. The results of this study have shown that when a single dose of tritiated cytidine was inoculated i.p. into a donor rat, a sufficient amount of the radioisotope was incorporated after 1 h to result in the heavy positive labeling of more than 99% of the ascitic tumor cells (Figure 1).

When these isotopically labeled tumor cells were subsequently injected into the blood of recipient animals, they were readily identified in autoradiographs of the lung tissue at the earliest time of sacrifice (1 min) (Figure 2). The pattern and degree of labeling did not appear to change significantly up to 12 h. At the early

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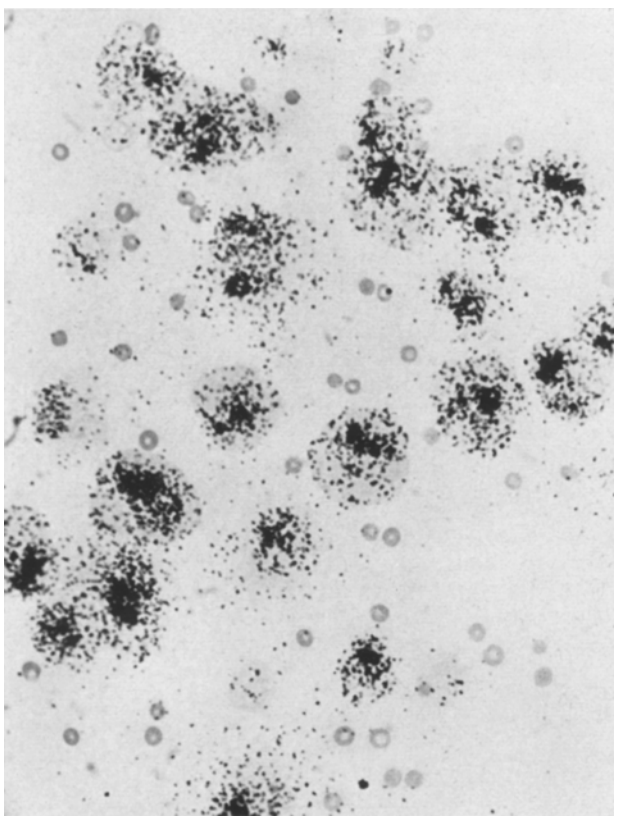

Fig. 1. Positively labeled ascites tumor cells before their inoculation into the blood. Autoradiograph. × 400.

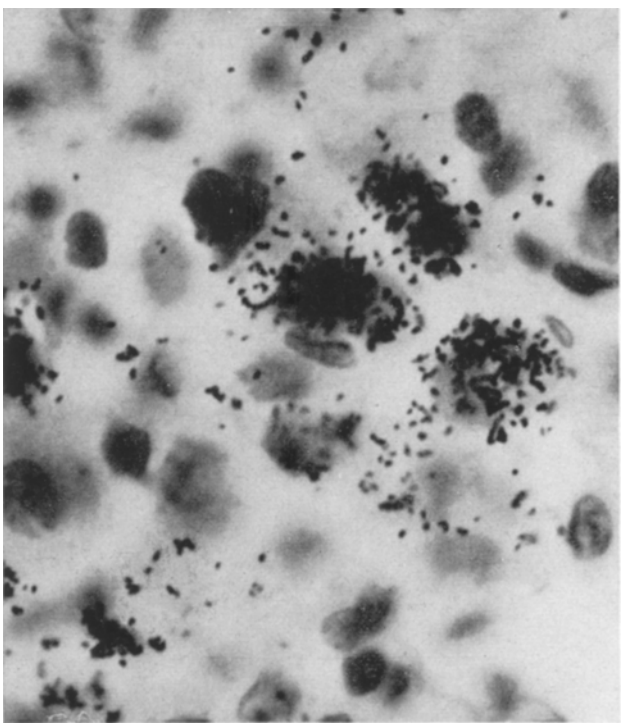

Fig. 2. Intensely labeled tumor cells in the lung tissue 1 min after their inoculation into the blood. Autoradiograph (oil immersion).

time intervals (up to 4 h) the label tended to appear highly concentrated. At 12 h there was a considerable lessening in the grain count of many individual cells but the label was still conspicuous. Autoradiographs of the lung tissue at 24 h still showed a significant presence of grains although they were more widely dispersed (Figure 3). Diffusion of the isotope into the surrounding tissue was apparent.

There were no detectable toxic effects following the administration of tritiated cytidine in any of the animals studied.

Tumor growth occurred in 100% of the rats inoculated s.c. with labeled ascitic cells.

Discussion and conclusions. The results reported in this study demonstrate the usefulness of the in vivo technic in labeling Walker 256 ascites carcinosarcoma cells with tritiated cytidine to study the localization of circulating cancer cells and the possible establishment of metastases. The method employed was basically simple. A single dose of CR-5-H^3 inoculated i.p. positively labeled the tumor cells in a short time (Figure 1).

The s.c. inoculation of ascitic fluid containing 2,000,000 labeled tumor cells into recipient young rats resulted in 100% takes proving their viability.

The injection of these cells into the circulating blood of healthy recipient animals resulted in their appearance in autoradiographs of the lung tissue as early as 1 min after their injection (Figure 2). The cells did not appear to change much up to 4 h but at 12 and 24 h there was a significant decrease in the counts per cell, as well as a more wide dispersion of the grains throughout the entire cell. This was due in part to obvious enlargement of the cells and to cellular breakdown, as many tumor cells were found in various stages of disintegration at this time. It is conceivable that there was some isotope dilution due to cellular proliferation, but this was not obvious.

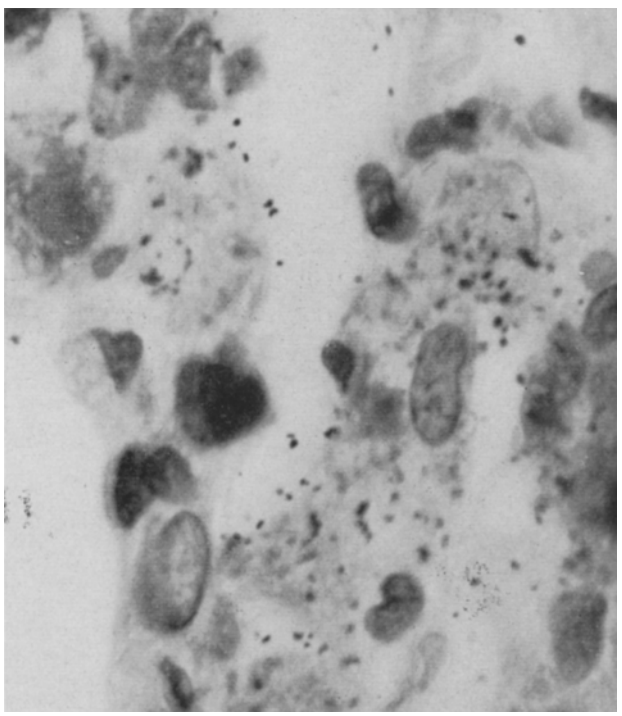

Fig. 3. Sparsely labeled tumor cells in the lung tissue 24 h after their inoculation in the blood. Autoradiograph (oil immersion).

The pattern of localization of the tumor cells in the lung parenchyma is of interest. Many isolated tumor cells could be found scattered throughout the tissue at all times although more aggregates of cells seemed to be present at the later time intervals. At 12 h there was evidence of passage outside the capillaries and penetration of the alveolar wall.

There was no evidence that a significant amount of free cytidine was present in the ascites fluid. In 3 early experiments we washed the ascites cells with saline several times after withdrawal, with no significant difference in the cell labeling or the appearance of the cells in the recipient animals. We could find no evidence of labeling of normal cells in the recipient animals by free cytidine H_3 in the injected fluid. Only the isolated tumor cells showed any evidence of label. No isotope could be seen in any other organ, where tumor cells did not lodge, nor was there any evidence of isotope in the lungs of animals which did not contain tumor cells after inoculation.

It is impossible to say that there is no turnover of isotope with the destruction of the tumor cells after 8–12 h. However, at these times though there is local spread of the isotope granules, there was no evidence of localization within cells of the recipient tissue. With the very marked dilution of the isotope in the recipient animals, the amount present for pick-up by the animal cells would be so small as to be negligible. It seems reasonable to suppose that the labeled cells seen are all tumor cells, labeled at the time of injection.

There was no apparent toxicity caused by the administration of tritiated cytidine into the peritoneal cavity or by the injection of labeled cells into the blood. All the animals survived these procedures.

The absence of toxic effects from the tritiated cytidine and its ability to label 100% of viable tumor cells in a short time make this method a valuable one in the study of the localization of individual tumor cells after their injection into the blood[8].

Riassunto. Si vollero studiare in vivo le cellule neoplastiche del Walker 256 marcate con Tritium Citidine (CR-5-H^3). L'iniezione i.p. di una sola dose di CR-5-H^3 risultò dopo un'ora di incubazione in un'adeguata marcatura degli elementi neoplastici. L'immissione nel torrente sanguigno delle cellule marcate, risultò mediante studi autoradiografici, nell'identificazione di quest'ultime nel parenchima polmonare. Il trattamento con CR-5-H^3 è stato uniformemente ben tollerato in tutti gli animali studiati. L'innesto di ascite marcata riproduceva in animali integri tumori della struttura di quello del datore.

E. E. Cliffton and D. Agostino
with the technical assistance of
Miss Jean Nolan

The Clotting Mechanisms Section of the Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research and Cornell University Medical College, New York (N.Y. 10021, USA), 13 May 1968.

[8] This investigation is supported in part by the Public Health Service Research Grant No. CA-08748, from the National Cancer Institute and by Institutional Funds.

[9] Associate Member, Sloan-Kettering Institute for Cancer Research; Associate Professor Clinical Surgery, Cornell University Medical College.

[10] Research Associate, Sloan-Kettering Institute for Cancer Research