

CLINICAL AND EXPERIMENTAL STUDIES
OF FLUORESCEIN DYES
WITH SPECIAL REFERENCE TO THEIR USE FOR THE DIAG-
NOSIS OF CENTRAL NERVOUS SYSTEM TUMORS*

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IN THE PAST, many investigators have recorded the use of dyestuffs to delineate malignant tissues with varying success. In most instances, it was found that the dye particles accumulated either in macrophage type cells of the tumor struma or in necrotic areas where the blood supply to the tumor had broken down. In no instance has an appreciable concentration of dye been demonstrated in intact viable tumor cells. This subject has been reviewed elsewhere.⁵

Sodium fluorescein has been injected into patients subjected to laparotomy with the hope that it might accentuate differences in the appearance of normal and malignant tissues. Approximately one gram of the dye (20 per cent aqueous solution) was injected intravenously four hours before the scheduled exploration of patients suspected of harboring an abdominal malignancy. If the injection rate was slow, no untold reaction occurred; rapid injection of the dye resulted in several cases of transient nausea and vomiting. It is well to warn the patient that his skin may be yellow for several days. Although staining of the skin usually disappeared in 24 hours, patients with liver insufficiency remained colored for longer periods of time.

Examination at time of operation was carried out under the ultraviolet light emitted by a CH-4 mercury vapor lamp equipped with a Wood's filter. In addition to the inspection of the tumors in situ, further study was made of sections of the removed specimens. A summary of these cases appears in Table I.

It was early noted that there was no specificity of the dye for tumor tissue but that inflammatory and cystic areas also fluoresced. The most consistent results were obtained with mucinous type carcinomas. In several instances lymph nodes involved by the carcinoma as well as the primary tumor exhibited a marked fluorescence. As noted elsewhere,⁷ carcinomatous peritoneal implants usually fluoresced in contrast to the surrounding peritoneum. Although many interesting phenomena were encountered, the technic per se was not of any practical value.

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The fluorescein technic was extended to include tumors of the central nervous system. In contrast to the lack of affinity of general body malignancies for fluorescein, brain tumors almost invariably showed definite fluorescence when viewed in ultraviolet light.⁸

This technic has been of definite clinical value in the localization and diagnosis of subcortical lesions. Briefly, biopsy material is obtained by aspiration through brain needles inserted into the suspected area. When examined under ultraviolet light, neoplastic tissue fluoresces a bright yellow. Normal cortical material retains a faint yellow-green fluorescence while normal white matter appears blue-white. Edematous tissue which often surrounds brain tumors fluoresces more than normal brain but much less than the tumor itself. In those instances in which the tumor tissue is not aspirated but the biopsy material is fluorescent (edematous) further probing of the same area will usually reveal the tumor. On the other hand, if the brain needle is introduced a second time, to one side, and the material aspirated is less fluorescent, the surgeon will know that he is probing further away from the tumor site.

TABLE I.—*Summary of the Degree of Fluorescence Noted in Tumors Other Than Those of the Central Nervous System.*

	No. Cases	Excellent	Good	Poor	Failure
Carcinoma of stomach	36	3	14	14	5
Lymphosarcoma of stomach	1	1	..
Squamous cell carcinoma of the esophagus	2	..	1	1	..
Carcinoma of the colon	15	..	2	13	..
Total	54	3	17	29	5

This method of examination is simple and can be carried out in the operating room itself. The biopsy specimen is collected on a gauze square and handed to the room nurse who, in turn, holds it under the ultraviolet lamp. Thus the surgeon can turn, examine the tissue, and confirm the presence or absence of fluorescence without moving from his operating position. It has not been found necessary to darken the room in order to examine the tissue adequately.

The technic is of further value in that if only small segments of the aspirated material fluoresce, these pieces can be removed separately for section or smear by the pathologist and thus afford him a better chance of establishing a definite diagnosis.

In general, the degree of fluorescence was correlated with the grade of malignancy. Acoustic neuromas and slow-growing astrocytomas took up the least amount of dye, while astroblastoma and glioblastomas appeared to fluoresce the most. Necrotic tumor material failed to take up the dye. Meningioma differed widely as regards fluorescence. While some exhibited only slight fluorescence, others were a brilliant yellow. Metastatic tumors were observed to exhibit a definite fluorescence in every case. In addition, spinal

cord tumors were also found to take up appreciable amounts of the dye in contrast to the surrounding normal nervous tissue.

In several cases, after the tumor tissue was believed to be completely removed, residual remnants of the tumor were disclosed by examining the residual cavity directly under ultraviolet light.

A summary of 141 consecutive cases in which the fluorescein technic was used and in which a biopsy was obtained is presented in Table II.

Many other fluorescent and highly colored dyes have been tested on mice with experimentally induced brain tumors. The majority were rejected because of their toxicity, lack of solubility, or instability. Of the several dyes given clinical trial, all were discarded because of their tendency to color deeply the patient's skin.

TABLE II.—*Diagnosis of Central Nervous System Tumors with Sodium Fluorescein.*

Diagnosis	Fluorescence	Cases
Tumor present.....	+	105
Tumor present.....	—	7
Tumor absent.....	+	2
Tumor absent.....	—	27
Total cases.....	..	141

Attempts have been made to combine fluorescein and similar dyes with several of the heavier elements in the hope of rendering brain tumors radio-opaque to the ordinary roentgenologic examination. To date, no success has been obtained in this particular project. One compound studied, tetraiodophthalic fluorescein, proved useful for intravenous cholecystography.¹¹ Other derivatives were found to be too toxic, insoluble, unstable, or highly colored. At present, additional radio-opaque compounds possessing chemical characteristics consistent with the theory of the blood-brain-barrier are being synthesized.

The unsuccessful attempt to synthesize radio-opaque dyes which would aid in the localization of brain tumors prompted efforts to tag fluorescein with radioactive elements. For this purpose, I₁₃₁ is eminently suited, since it has the requisites of relatively rapid decay (half life of eight days), and it emits a penetrating gamma ray (0.367 M.E.V.) which can be detected at a distance. The synthesis of radioactive diiodofluorescein and the initial studies have been previously reported.^{6, 10}

At present, the following technic is being employed. Radioactive diiodofluorescein containing 1 M.C. of I₁₃₁ is injected intravenously. One-half to four hours later, the activity over symmetrical areas of the head are measured by a specially shielded Geiger-Mueller counter.

The counting rates for each area per unit time can then be compared for localization of the tumor. Care must be exercised that areas over the longitudinal sinus, for example, are not included, since high counts result from the

radioactive dye still present in the blood. In every case, the radioactive localization tests have been done without knowledge of the patient's history or physical findings. The results of these tests have been evaluated independently by members of the neurosurgical staff. The summary (Table III) includes only those patients in whom a lesion was later demonstrated to be present or proved to be absent by operation, ventriculogram, or angiogram.

It should be remembered that among those patients who were later proved to have a tumor, but in whom no tumor was localized by the radioactive dye technic, several had tumors too small (mainly cerebellar tumors), or of such a type (acoustic neuroma) as to contain less than the minimum amount of dye known to be necessary for detection. Other tumors that were not localized were usually found to be deeply situated. The easiest tumors to localize were those situated close to the cortex, malignant glial tumors, and those tumors accompanied by an appreciable amount of edema.

TABLE III.—*Localization of Intracranial Lesions with Radioactive Diiodofluorescein.*

Tumors correctly localized	22
No tumor localized, none demonstrated by angiogram, ventriculogram, or craniotomy	21
Correct localization (but vascular or demyelination rather than tumor)	4
Close to, but not exact localization	3
Located as to hemisphere only	1
Tumor present but not located	20
Tumor localized but not found at operation, ventriculogram or angiogram	2
Incorrectly localized	2
Abscess not localized	1
Cholesteatoma not localized	1
Total	77

In order to study this technic more completely, tracer doses of radioactive diiodofluorescein were injected preoperatively, and biopsies taken of tumor and adjacent normal brain tissue at operation. Equal samples were then measured for their radioactivity. The activity found in human brain tumors ranged from 1.64 to 29 times that of adjacent normal brain.

In general, the highest ratios of activity were found in the more malignant tumors (glioblastoma). Some ratios were probably low because of difficulties in obtaining satisfactory "normal" brain tissue for comparison. For example, there is reason to believe that traumatized tissue or tissue momentarily deprived of its blood supply takes up an appreciable amount of dye. In other instances, a considerable time elapsed between obtaining the normal brain biopsy and the exposure of the tumor and the subsequent removal of a portion for measurement.

For further evaluation of the physical limitations of the radioactive dye technic, measurements were made of a human calvarium containing phantom tumors.⁶ It was concluded that if the concentration of the dye in the brain tumor was twice that of the surrounding tissue, the minimal volume of a tumor that could be detected would be about 40 cc. If greater concentration ratios were present, smaller tumors could be detected.

To expedite the screening of various compounds for their usefulness as diagnostic agents, possible chemotherapeutic effects, and for radiation levels, brain tumors were induced in several strains of inbred mice. The technic employed utilized methylcholanthrene in a manner similar to that of Seligman and Shear¹² and Zimmerman and Arnold.¹¹ Since these tumors were induced in inbred mice, subsequent subcutaneous transplants could be carried out with facility. Several glial tumors have been carried for 17 transplant generations.

DISCUSSION

There is no complete explanation at the present time for the increased concentration of fluorescein dyes in brain tumors. That it is not due to a greater vascularity of the tumor is obvious from the fact that maximal fluorescence does not occur immediately after injection of the dye but gradually increases in intensity from one-half to four hours. It has also been demonstrated that tumors with the greatest blood supply (angiomas) are not very fluorescent, and, in fact, have resulted in two diagnostic errors.

Although the so-called blood-brain-barrier undoubtedly is partly responsible for the differential concentration of dye in brain tumors, that it is not wholly so is substantiated by the fact that some gastrointestinal tumors show a similar differential staining. Recent studies (unpublished) would seem to indicate that there is, perhaps, a specific affinity of tumor cells for the dye.

Since the experiments of Goldmann,⁴ the theory of the blood-brain-barrier has occupied the attention of many investigators. Broman^{2, 3} reported that the barrier exists in the intima of the blood vessels in the central nervous system. In experimental animals he was able to damage the barrier by injecting noxious substances into the carotid artery but not by applying them directly to the pia. He also found that the function of the blood-brain-barrier was impaired or absent in brain tumors, encephalomalacia, edema, abscesses, and multiple sclerosis. With this in mind, it is not surprising that we have found a slight fluorescence in areas of edema, and that the more edematous areas contain a greater amount of dye.

In this regard, it would seem that fluorescein and radioactive diiodofluorescein could be expected to be useful tools for further exploration of other diseases of the central nervous system, such as amyotrophic lateral sclerosis,¹ epilepsy, and other convulsive disorders. Fluorescein dyes are excellent for these purposes, since, (1) they are acid chromagen dyes and therefore should not penetrate the normal blood-brain-barrier, and (2) they can be detected and quantitated in extremely small amounts, either by fluometry, or by tagging them with radioactive isotopes.

The clinical use of other acid chromagen dyes such as Disulphine Blue* has been disappointing. Sufficient amounts of dye to produce sharp color differences between neoplastic and normal brain tissue, when viewed in ordinary light, also deeply stain the patient's skin. This circumstance has proved

* Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England.

frightening to the patient, confusing to the anesthetist, and could result in serious medical-legal problems.

It is hoped that some of these compounds can be used clinically by tagging them with radioactive isotopes. By this method, very dilute solutions can be utilized.

SUMMARY

Sodium fluorescein has been shown to concentrate in an unpredictable manner in many tumors. The most consistent results were obtained in mucinous adenocarcinomas of the gastrointestinal tract and carcinomatous peritoneal implants.

In contrast, tumors of the central nervous system concentrate the dye in a consistent and predictable manner. The use of sodium fluorescein as an aid in the localization of brain tumors at operation is a simple and accurate technic which utilizes no special apparatus except a mercury vapor lamp.

Attempts to use radio-opaque dyes to outline brain tumors have not been successful. The clinical value of the radioactive dye method for the detection of brain tumors preoperatively cannot be evaluated completely at the present time. Better equipment, more sensitive Geiger-Mueller tubes, and other improved methods for detecting radiation should increase the accuracy and consistency of this method.

Mention has been made of the advantages of using fluorescein and radioactive dyes to explore further the role played by the blood-brain-barrier in other diseases of the central nervous system.

BIBLIOGRAPHY

- ¹ Aird, Robert B.: *Trans. of Am. Neuro Association*, page 170, 1946.
- ² Broman, T.: *The Possibilities of the Passage of Substances from the Blood to the Central Nervous System. Acta. Psychiat. et Neurol.*, **16**: 1, 1941.
- ³ Broman, T.: *Acta. Med. Scand.*, **118**: 79, 1944.
- ⁴ Goldmann: *Experimentelle untersuchungen uber die Function der plex. choroid. und der Hirnhaute. Arch f. klin. Chir.*, **101**: 735, 1913.
- ⁵ Hubbard, T. Brannon, and G. E. Moore: *The Use of Fluorescein Dyes in the Diagnosis of Malignancy with Special Reference to Tumors of the Central Nervous System. To be published.*
- ⁶ Marvin, J. F., and G. E. Moore: *Chemical and Physical Aspects of the Localization of Brain Tumors with Radiodyes. (Part II) Nucleonics*, **3**: 63, 1948.
- ⁷ Moore, G. E.: *Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues. Science*, **106**: 130, 1947.
- ⁸ Moore, G. E., W. T. Peyton, L. A. French, W. W. Walker: *The Clinical Use of Fluorescein in Neurosurgery; the Localization of Brain Tumors. J. Neurosurg.*, **5**: 392, 1948.
- ⁹ Moore, G. E., M. J. Smith: *Intravenous Cholecystography with Tetraiodophthalic Fluorescein. Surgery*, **24**: 17, 1948.
- ¹⁰ Moore, G. E.: *The Use of Radioactive Diiodofluorescein in Diagnosis and Localization of Brain Tumors. Science*, **107**: 569, 1948.
- ¹¹ Seligman, A. M., and M. J. Shear: *Studies in Carcinogenesis, VIII. Experimental Production of Brain Tumors in Mice with Methylcholanthrene. Am. J. Cancer*, **37**: 364, 1939.
- ¹² Zimmerman, H. M., and H. Arnold: *Experimental Brain Tumors. Tumors Produced with Methylcholanthrene. Cancer Research*, **1**: 919, 1941.