that cholesterol plays a rôle in the fat metabolism of the liver.

Summary. Under the stress of increased metabolic rate, due to dinitrophenol injection, liver phospholipid and cholesterol are decreased. These changes are consistent with the belief that at least part of the phospholipid and cholesterol of the liver are important in fat metabolism.

The authors wish to express their thanks to Dr. W. R. Bloor for his advice on this problem.

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Localization of Colloidal Dyes in Animal Tumors.

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Introduction. It has long been known that certain colloidal dyes when injected intravenously exhibit properties of selective accumulation in tumor tissue.* The possible use of such dyes or other similarly behaving colloids as "carriers" for localizing radioactive and slow neutron capturing materials in neoplastic tissue for internal radiation therapy was suggested recently by Zahl and Cooper.[3, 4] With the radiation properties of these materials in mind (that is, type of emitted particle, penetration path, ionization density, etc.), it seemed of value, in an effort to probe the usefulness of such a concept, to reëxamine the localization features of such "carriers" in tumor tissue. This seemed of particular importance in relation to (a) the gross distribution within the body and (b) the distribution pattern in the tumor mass.

The localization of certain dyes in tumors has been described in some detail by Ludford[5] and Duran-Reynals[1] on animal material, and grossly by Brunschwig, Schmitz and Clarke[2] on human material. Some of the points presented here are in general confirmatory of

* For recent appraisal of this subject, *vid.*:1,2

1 Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

2 Brunschwig, A., Schmitz, R. L., and Clarke, T. Howard, *Arch. Path.*, 1940, **30**, 902.

3 Zahl, Paul A., and Cooper, F. S., *Science*, 1941, **93**, 64.

4 Zahl, Paul A., and Cooper, F. S., *Radiology*, 1941, in press.

5 Ludford, R. J., *Proc. Roy. Soc. London*, Ser. B, 1929, **104**, 493.

this earlier work, but are of renewed interest when considered from the standpoint of radiation possibilities.

Procedure. Although a number of newly prepared dyestuffs were tested as to gross tumor-localizing properties, representative detailed study was made only on the 2 well-known vital dyes, Evans Blue and Lithium Carmine.† The dyes were injected intravenously into somewhat more than 150 tumor-bearing mice in amounts of 0.1 to 0.5 cc of a 1.0-2.0% aqueous solution. Mice used were an inbred strain implanted with Sarcoma 180 or Mammary Carcinoma 15091a. Mice with spontaneous mammary carcinoma were also used.

Following injection of the dye solutions, the animals were sacrificed at varying intervals and their tissues studied histologically, with special reference to the tumor sites. Ordinary methods of fixation and dehydration do not remove the dyes. The usual procedure was to clear and mount one section unstained, stain the duplicate very slightly with hematoxylin so as not to mask the dye, and to stain the triplicate routinely for cellular detail. By comparing such sections, the exact location of the dye and roughly the quantity present could be easily ascertained.

No essential differences were noted in the behavior of Evans

† Other dyes tested for tumor localization properties were:

(1) Copper azo-dye (No. R-1124-25, Amer. Cyan.)—too acutely toxic for localization observations.

(2) Uranium azo-dye (No. R-1203-99, Amer. Cyan.)—decolorized soon after injection, preventing observation as to tinctorial localization.

(3) Lithium Pontamine Sky Blue 6B (No. L-2300, Dupont).

(4) Lithium Salt of Trypan Blue (Nat. Aniline).

(5) Lithium azo-dye (No. R-1209-68,18, Amer. Cyan.).

Dyes (3), (4) and (5) are probably isomeric or at least similar to Evans Blue (Eastman) and grossly similar in localizing properties.

(6) Pontacyl Fast Blue Conc. 5R (Dupont)—little or no localization.

(7) Pontacyl Fast Blue Conc. GR (Dupont)—little or no localization.

(8) Pontamine Fast Scarlet 4Ba (Dupont)—similar to Evans Blue and Lithium Carmine in localizing properties, although somewhat more toxic.

(9) Pontamine Sky Blue 5B—similar to Pontamine Sky Blue 6B.

A discussion of the chemistry of these dyes cannot be presented here. In a later communication, certain theoretical questions involved in the structure and preparation of localizing dyes will be considered.

In this connection, we wish to express grateful acknowledgment to Dr. W. C. Crossley of American Cyanamid, Dr. R. E. Rose of Dupont, and Mr. F. W. Green of National Aniline for their generous coöperation with us in preparing these various dyestuffs.

We wish also to express our gratitude to Dr. F. S. Cooper of the Haskins Laboratories for his continued and most helpful interest in this work.

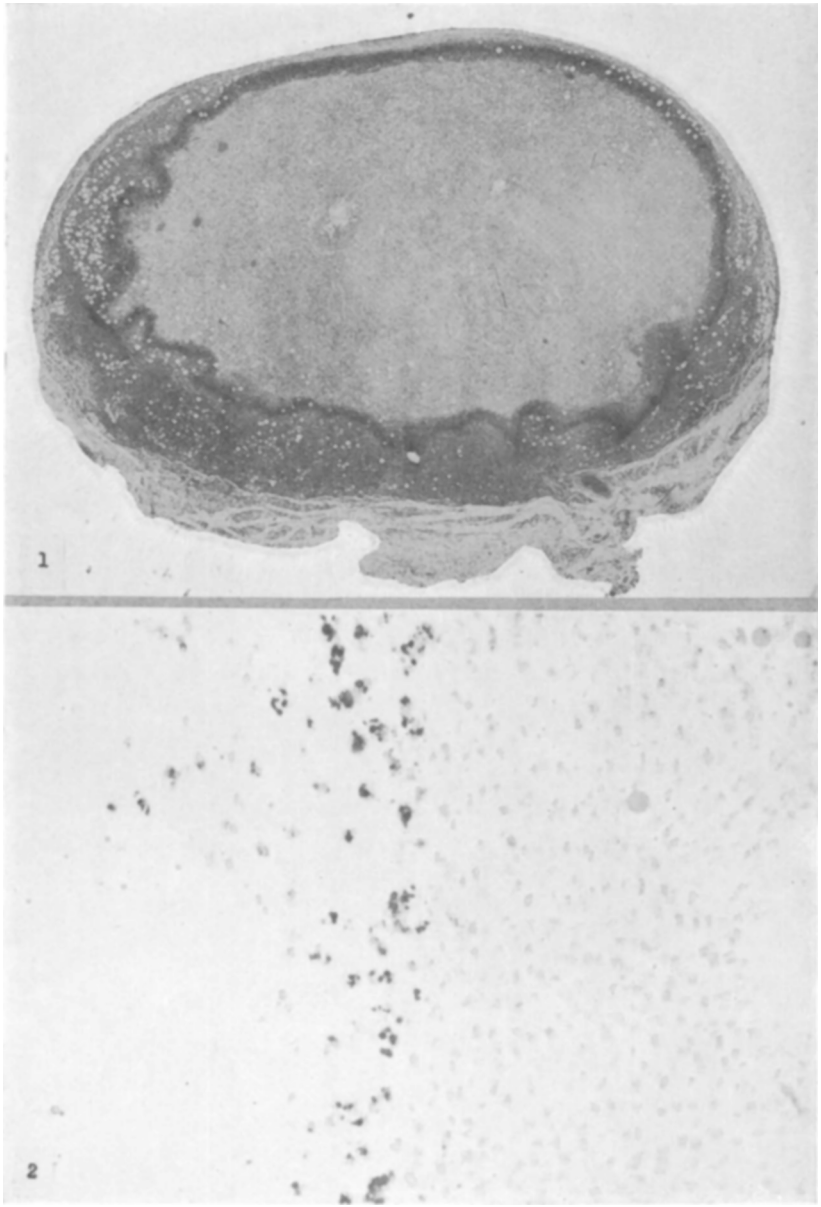

FIG. 1.

Photomicrographs of cross-section of mouse mammary carcinoma to show: (1) Dye localization at viable-necrotic interface. The viable periphery is separated from the necrotic center by a broad dark zone of localized dye. (2) Dye localization at viable-stroma interface. Dye droplets in phagocytes and fixed tissue cells at interface of growing tumor edge (right) and surrounding stroma (left).

Blue and Lithium Carmine. Nor were any differences noted in the localization of the dyes in spontaneous or transplanted tumors.

Histological Observations. The initial localization of either dye appeared as a diffuse faint staining of the stroma cells and interstices at the periphery of the tumor transplant. This zone was usually 1-2 mm in width. Wherever in the interior of the tumor an interface between viable and necrotic tissue occurred, a band of diffusely dyed cells, largely necrotic tumor cells and debris, was found (Fig. 1). This was especially prominent in the case of spontaneous tumors with numerous ramifying cores of necrotic tissue. Endothelial cells of capillaries at the periphery of the tumor were often seen diffusely dyed, especially if there was frank evidence of inflammation in the region.

Within a few hours after injection, and lasting for many days, macrophages and fixed tissue cells laden with dye granules were found at the periphery of the tumor (Fig. 2). These were present in large numbers after the first 24 hours. The diffuse staining of the capsular stroma gradually disappeared, usually within 48 hours. At the edge of the central necrotic core a few phagocytes containing dye were observed. They were not nearly as numerous, however, as those at the periphery. The necrotic core of the tumor often became slightly and diffusely stained, remaining so many days after injection. An occasional dye-filled phagocyte or a diffusely stained tumor cell could be found in the midst of the viable tumor cords. But the bulk of the malignant tissue remained free of dye throughout the procedure.

The dye substances were also rapidly taken up from the blood stream by the Kupffer cells of the liver, the reticulo-endothelial cells of the spleen and the renal tubule cells. Some localization occurred in the fixed tissue cells and macrophages of the dermis and to a lesser degree in the lungs and intestine. However, several days after injection, the reticulo-endothelial cells of the spleen and liver were found to be relatively clear of dye, although much was still present in phagocytes about the tumor. As long as dye remained in the segregating cells of the tumor mass, which was at least 3 weeks with the doses and tumors used, there was a slow excretion via the kidneys where the dye could be seen in the tubular epithelium, as well as in the urine. A precise quantitative estimate of the comparative concentration of dye in the various organs is not possible from histological preparations.[3, 4]

As far as one could tell a single massive dose resulted in about as much localization of dye in the tumor as the same dose given in

small daily repeated fractions. Furthermore, the reticulo-endothelial system soon excreted the dye of a massive dose, but was continuously blocked when repeated small doses were given.

Collateral Observations. A number of experiments were performed in which 2 tumors were implanted bilaterally in the same animal, and one was irradiated with X-rays, doses varying from 20 to 1000 roentgens.‡ The mice were then injected with dye at various time intervals following irradiation, and the X-rayed tumors compared with the normal. There was no evidence to indicate that external radiations strong enough to kill most of the tumor increased or decreased the localization of dyes in the tumor or other regions of the field. Nor did doses which caused slight cytological changes in the tumor cells appreciably alter the deposition of the dye.

Other experiments were performed in which diluted India ink was injected into tumor-bearing mice. There was no macroscopic or microscopic localization of ink either in the tumor or in the tumor area, although the ink was quickly segregated by the reticulo-endothelial system, the liver, spleen, lungs, and bone marrow taking up various amounts of the substance. This failure of colloidal carbon to localize in or around experimental tumors is almost the reverse of dye localization, and is confirmatory of the observations of Duran-Reynals[1] and Brunschwig, Schmitz and Clarke.[2] In an effort to seek an explanation for this behavior variance, India ink colloid solutions, as well as those of Evans Blue and Lithium Carmine, were subjected to centrifugation of 50,000 to 90,000 times gravity for 30 minutes. Such forces caused no observable sedimentation of the dye materials, but caused a considerable sedimentation of carbon particles from the ink solution. This may indicate a colloidal particle size difference in the two classes of materials, and suggests that their localization properties and passage through capillary membranes may be associated with relative colloid particle size.

Comment. It seems apparent, as pointed out by Brunschwig, Schmitz and Clarke[2] that the selective localization of dye in neoplastic foci of man, as in the case of animals, is dependent on some factor associated with a local increase in capillary permeability to the dye. According to the same authors, Evans Blue stains malignant neoplasms and their metastases much more heavily than benign neoplasms in rabbits and rats, and there seems to be no correlation between the histologic type of a neoplasm and its ability to localize the dye. The majority, although not all, of human patients with

‡ 190 kv, 30 m.a. Filters: 0.5 mm Cu, 1.6 mm Al. Target distance: 20 cm. 193 r per minute.

various types of carcinoma and sarcoma showed distinctive localization of intravenously injected dye.[2] However, since malignant cells *per se* do not take up injected colloidal materials, it would seem that the localization concept under examination would not be ideal for irradiation usage. On the other hand, there are several considerations which argue against such a conclusion. (a) The penetration paths through tissue of the various available energy particles are in most cases considerably greater than the thickness of a single cell. For electrons from radio-active isotopes the penetration may be as much as a centimeter or even more; for protons from the lithium-neutron reaction it is 50-60 micra; for alpha particles from the boron-neutron reaction it is somewhat less than 10 micra. Therefore, dye or other localizing substances containing materials which emit or can be caused to emit ionizing particles, when lying in juxtaposition with the outside of the growing tumor edge, or at any interface between necrotic and viable tissue, would tend to shower adjacent malignant cells with their ionizing radiations. (b) There is some evidence which indicates that therapeutic radiation effects on neoplasms are not due wholly to damage of the malignant cells *per se*. (c) Animal tumors, as well as most human cancers so far tested[2] not only accumulate a larger quantity of injected colloidal dye material than do normal tissues and vital organs, but in the course of metabolic excretion malignant neoplasms, experimental and spontaneous, retain such accumulations for a longer period than do the normal tissues of the body.

It seems, therefore, that if dye colloids, or other materials acting like them, are used as "carriers" for the selective deposition of either (a) ionizing short-lived radioactive isotopes of such elements as phosphorus, selenium, rubidium, etc., of (b) non-ionizing slow neutron-capturing materials, such as lithium or boron, a substantial internal radiation advantage which may prove of therapeutic value should be achievable.

Summary. (1) As earlier workers have shown, acid dyes do not localize in tumor cells but in the stroma surrounding the tumor. (2) Further localization takes place within the tumor at each interface of viable and necrotic tissue. (3) The initial localization consists of a diffuse staining of the peripheral stroma and the seminecrotic cells within the tumor between viable and necrotic zones. (4) This diffuse dye is progressively phagocytized, and after 24-48 hours, appears in numerous macrophages at the tumor edge. (5) The dye is so located that if it were replaced by a radioactive substance, theoretically effective radiation of the tumor edge should

result. (6) After a single large dose of dye the reticulo-endothelial cells of liver and spleen are rapidly filled with dye droplets. These disappear or diminish substantially after several days. (7) In addition to tumor and reticulo-endothelial systems of liver and spleen, the kidneys take up considerable quantities of dye, as this is the chief channel of excretion. Accumulation in the kidney might prove to be a complicating factor in the use of radioactive materials, but in the case of induced activity, as with lithium by slow neutrons, appropriate shielding during irradiation should overcome this difficulty. (8) Small repeated doses of dye do not localize in greater quantities than does the same amount given at a single injection. Furthermore, the reticulo-endothelial system is blocked by small repeated doses. (9) X-ray treatment does not appreciably alter the amount of dye localizing in tumors, as determined by histological examination. (10) There is a marked difference in behavior, as regards tumor localization, between the colloidal dyes used and colloidal carbon. (11) Brief preliminary report is made on the localizing properties of some heretofore untested dyes.

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Immunological Reaction of the Hamster to Inoculations with the Virus of St. Louis Encephalitis.*

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The immunological response of mice to inoculation with St. Louis encephalitis virus has been rather extensively studied by Hodes and Webster.[1]

These authors[1] observed that mice inoculated subcutaneously with the virus failed to develop clinical evidences of encephalitis, but quickly developed immunity against subsequent intracerebral inoculation. This immunity, however, was not accompanied by the appearance of virus-neutralizing antibodies in the peripheral blood. Such antibodies were noted some 8 weeks after subcutaneous inocu-

* Read before the meeting of the St. Louis Section of the Society for Experimental Biology and Medicine, May 14, 1941.

1 Hodes, H. L., and Webster, L. T., *J. Exp. Med.*, 1938, **68**, 265.