JCI The Journal of Clinical Investigation

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J Clin Invest. 1943;22(2):161-168. https://doi.org/10.1172/JCI101379.

Research Article

STUDIES WITH RADIOACTIVE DI-AZO DYES. III. THE DISTRIBUTON OF RADIOACTIVE DYES IN TUMOR-BEARING MICE [1]

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(Received for publication August 3, 1942)

In a previous communication (1), the concentration of radioactive di-brom trypan blue in animal abscesses was described. The study was undertaken with the underlying concept that such a radioactive substance might provide a method for diagnosing the presence and exact location of inflammatory lesions.

An investigation of the potential usefulness of these radioactive colloids as a means of internally radiating tumors will be described in the present paper. In so far as such usefulness will primarily depend on differential uptake of the radioactive substance by tumor tissue, our initial approach to this subject has been the study of uptake, rather than any attempt to gain a therapeutic effect. The dosage of radioactivity necessary to study tissue uptake of a radioactive substance is considerably below that necessary to gain a therapeutic effect in tumors.

As early as 1909, when Goldmann (2) undertook a study of the biological reactions of certain colloidal dyes, it was noted that tumors appeared to take up the dyes in some concentration. At that time, interest was centered chiefly on histological staining by the "vital stains" (of which the di-azo dyes are examples), and the dyes were administered subcutaneously in large and repeated doses. These circumstances, as is well known, are not conducive to the observation of maximal *selective* staining of any organ or tissue because the animal is so flooded with the dye that all tissues which take it up to any extent show maximal coloration.

Weil (3), Marsh and Simpson (4), and Ludford (5) continued research in this field, and described selective staining of tumor tissue, especially the periphery of necrotic zones, with trypan blue. Ludford was of the opinion that the dye caused necrosis of the cells which excreted it, and was therefore interested in the possibility of producing regression in the tumors. He observed no such effect, however. Most observers agreed that healthy growing tumor cells did not take up the dye, but that cells of diminished viability or cells in frankly necrotic areas were diffusely stained. In the growing zones of the tumor, only the phagocytic cells of the stroma showed droplets of intracellular dye.

These observations lead to two conclusions about the mechanism of uptake of these dyes which are important for such a study as this. First, that tumors with large amounts of connective tissue stroma might be expected to take up more dye than others, due to the presence of more phagocytes. Secondly that tumor-uptake may be determined by a balance between blood-supply and necrosis in so far as necrotic cells are stained, yet the central areas of necrosis, out of touch with the blood supply, cannot take up the blood-borne colloid.

In 1939, Duran-Reynals (6) revived interest in this subject by showing that under the proper conditions, the tumor may appear by coloration to have taken up the majority of the dye in a highly selective fashion. He was also the first to experiment in tumors with Evans blue, which he found accumulated in the tumors to a greater extent than trypan blue. He observed no therapeutic effect even in large doses, and found maximal selective staining with doses around 0.1 to 0.5 mgm. in mice. Hess (7) corroborated this work and experimented with a number of other dyes.

The concept of treating human tumors with colloidal dyes dates back to the work of Roosen (8) and Bernhardt (9) who injected them into human patients. In 1939, stimulated by interest

[1] This is reprint No. 564 of the Harvard Cancer Commission.

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in the more selective concentration described by Duran-Reynals (6) for Evans blue, Brunschwig, Schmitz, and Clarke (10) did a careful study of the accumulation of this dye in human tumors. They reported selective staining of the tumor tissue in 20 out of 30 malignant neoplasms, with non-staining of benign tumors and chronic inflammation. They report no correlation between histological type and ability to localize the dye. Their doses were in the neighborhood of 1 mgm. per kgm. An attempt to increase the permeation of dye into tumor by locally increasing capillary permeability through the use of histamine gave indifferent results; they report very little staining of internal viscera. No therapeutic effect was observed or, indeed, expected, as these authors were aware of the non-toxic nature of Evans blue for tissues.

Since 1940, Zahl and his co-workers (11 to 14) have focussed interest on the possibility of using such dyes as a means of localizing slow neutron-capturing elements (boron, lithium) in tumors and thus radiating the tissue. They used lithiated dyes, derivatives of trypan blue and others of the di-azo acid group. These dyes were evidently made simply by substituting lithium for sodium as the base in the sulfonic-acid-salt, *i.e.* the dyes were lithium salts rather than sodium salts of their respective sulfonic acids. These authors report accumulation of lithium ion alone (injected as lithium chloride) in tumors to the same order of magnitude as the lithium of the lithium-dye complex. This defect in the method probably arises from the fact that the lithiated dye is merely a highly dissociated salt, the dye molecule thus not being a determinant of the lithium distribution. Indeed, the authors find no correlation whatever, after a few hours, between the color-content and the lithium content of the tumor tissue. This is clear evidence in itself that the dye is not determining the distribution of the lithium, but instead the two are assuming distributions of their own. Because of these facts, we have taken care to see that the radioactive isotope in the molecule of the dye is firmly attached to the rest of the molecule and does not dissociate or ionize in solution. Zahl and his group speculate on the usefulness of dyes made radioactive by the inclusion of active isotopes in the molecule, but they do not report work with such dyes.

It is our desire to report, in this paper, work on mouse tumors using radioactive di-azo dyes, made active by the introduction of two atoms of radiobromine into the molecule. A description of the synthesis is given in a previous publication (15).

EXPERIMENTAL PROCEDURE

These studies on the distribution of radioactive di-brom trypan blue and radioactive di-brom Evans blue are based on the injection of these dyes into approximately 65 mice and the measurement of the concentration of the dye in organs and tissues by radioactivity detection methods. The mice used were of various pure strains, C_3H, C_{57}, Strain A and BBC.[3] The tumors studied were spontaneous mammary carcinomas in the C_3H, and transplanted tumors of a wide variety of types: neurofibrosarcoma, ovarian embryoma (16), rhabdomyosarcoma, and sarcomata induced by carcinogens. The tumors varied widely in size and degree of necrosis. An effort was made to study a variety of tumors with the idea that certain tumors might be found which would accumulate the radioactive colloid to a greater extent than others.

The dose of dye used was in the range of 1 to 5 mgm. per mouse. The radioactivity contained in this amount of dye varied widely from 0.07 μc.[4] to 1.2 μc. This quantity of radioactivity is undoubtedly below the therapeutic level except for the most sensitive of tumors, and it must be emphasized that our studies were an attempt to study the uptake of the dye rather than to achieve a therapeutic effect. The amount of radioactivity per unit of dye is a function not only of the original peak strength of the radiobromine as produced on the cyclotron but also of the length of time during which the Br^{82} has had an opportunity to decay, at the rate of 50 per cent over 34 hours.

The dye was injected intravenously, either in the tail vein or in the femoral vein, in most animals. Other routes of administration were studied but offered no advantages over the intravenous route. The mice were then killed at intervals from 3 to 78 hours. The tissues were excised, weighed, and aliquots, weighing around 0.5 gram, oxidized with concentrated nitric acid in a Coors porcelain ashing capsule, on the steam bath. One-half cc. of acid is used for each 0.5 gram of tissue, and 0.5 cc. of 0.15 M $AgNO_3$ is added to guard against the loss of activity by volatilization of Br_2 or HBr. The radioactivity in the resultant residue at the bottom of the porcelain capsule is then measured by means of a Geiger counter.

[3] The "BBC" is an inbred descendant of the hybrid BBC mice obtained from Roscoe B. Jackson Laboratories.

[4] The unit μc. (micro-curie) as used here represents an arbitrary correlation with the radiation emitted from a uranium standard. One μc. of Br^{82} is defined as that amount giving the same number of counts per minute as a standard amount of uranium under the standard geometrical conditions used on our counter. This amounts to about 1.5×10^5 counts per minute.

This reading is expressed in μc. (micro-curie) or fractions thereof; it is corrected for decay of the Br^{82} and for self-absorption according to the amount of tissue in the sample. To make a standard method for expression which will permit comparison between various experiments, the result is expressed as per cent of the injected dose found per gram of tissue, and corrected for the weight of the mouse. This corrected "per cent per gram" figure is used in Tables II, III, and IV.

The full derivation of this figure is as follows:

$$\text{"per cent per gram"} = \frac{\text{Corrected counter reading}}{\text{Weight of sample}} \times \frac{100}{\text{Injected radioactive dose}} \times \frac{\text{Weight of mouse}}{25}$$

Twenty-five grams is the mean weight of the mice used, and is thus a reference point for weight-corrections resulting in a small correction in most cases. Thus, in a mouse weighing 25 grams, one might expect to find 4 per cent of the injected dose in each gram of the mouse if the radioactivity were evenly distributed throughout the animal. However, about 50 per cent of the dose is found in liver, colon, and feces (see below) in the first 24 hours, and other organs such as kidney and spleen take up differentially high amounts, so that in general it can be stated that any tumor reading of 1.5 per cent per gram, or higher, indicates uptake to a degree greater than one would predict from weight alone. However, as shall be pointed out below, uptake to an extent greater than this does not necessarily indicate differential accumulation of radioactivity to a significant degree; rather, it is the tumor-uptake relative to uptake by other organs which is important.

Using this technique of measurement, the radioactive dye may be measured in body fluids with an accuracy of 2 to 5 per cent, and in tissues with an accuracy of 5 to 8 per cent.

RESULTS

Distribution of dye in animal as a whole

The total distribution of an injected dose of radioactive di-brom Evans blue 24 hours after injection is shown in Table I. Radioactive di-brom trypan blue adopts the same general distribution save for a somewhat higher concentration in the liver (15 to 20 per cent per gram). The experiment shown in Table I was undertaken as a normal control, and a small tumor on the diaphragm was found at autopsy. The lesion was sufficiently small so that it did not affect the distribution of dye as a whole; the table, therefore, illustrates the distribution of the dye in an essentially normal animal.

It will be noted that liver, colon, intestine, bile, and feces, in combination, take up about 50 per cent of the dose. It is our finding that this represents the chief excretory route of the dye. Urine figures are low; in this particular animal, the finding of 1.61 per cent of the dose in the urine at 24 hours was rather higher than usual. In contrast to this, the feces had accounted for the excretion of 15.2 per cent of the dose, at a concentration higher than that of any other organ or tissue. The high figures for intestine and colon represent both contents and staining of mucosa by dye. We do not know to what extent dye is excreted through mucosa because dye in the lumen dyes the mucosa and cannot be washed out. Dye present in feces is not visible by its color, due to the dark color of the intestinal content; the color cannot be washed clear of feces as it has stained "fast" the cellulose and proteins of the feces. These facts doubtless explain the fact that past

TABLE I

Total distribution of radioactive di-brom Evans blue in mouse

Breed of Mouse: Strain A. Weight: 21.5 grams. Dose of dye: 1.25 mgm., totalling 0.297 μc. of radioactivity. Duration: 24 hours.

Tissue	Concentration *per cent of dose per gram of tissue*	Total uptake *per cent of dose in entire organ or tissue*
Colon	18.6	13.0
Liver	12.5	16.2
Spleen	4.98	1.37
Intestine (small)	4.85	7.30
Stomach	4.81	1.57
Kidneys	4.60	1.72
Tumor*	3.12	2.07
Skin	3.12	11.2
Heart and lungs	2.68	1.39
Testis	1.88	0.70
Brain	0.69	0.28
Bladder	0.26	0.40
Carcass:		
Forelegs and thorax	2.27	5.90
Hind legs	1.72	4.30
Thorax	1.86	3.66
Lumbar region	1.23	2.08
Tail	2.67†	1.87
Head	2.08	4.16
Blood	2.74 per cent per cc.	5.45
Bile	4.63 per cent per cc.	0.46
Urine	2.61 per cent per cc.	1.61
Feces	30.4	15.2
Total		101.9 per cent

Figures in this table are uncorrected for weight of mouse.

* This tumor was unsuspected at the time of injection; it was a small firm tumor on the diaphragm; every appearance of a slowly growing lesion of low malignancy.

† The tail concentration figure is high because of slight spillage around the tail vein at the time of injection.

studies based on color alone have not ascertained the large excretion by this route.

By the end of 24 hours, the blood concentration is quite low as compared with determinations at a shorter interval after injection. Because of this fact, the animals described in the tumor experiments were not killed by bleeding, but the tissues were measured with the blood content in them, the blood concentration altering the tissue reading very little.

The central nervous system shows the least concentration of dye in any tissue and cerebrospinal fluid likewise shows little or no dye, as observed in other experiments in dogs (17). These measurements corroborate previous observations based on color.

Distribution with respect to tumors

The results of tumor experiments are shown in Tables II and III. Only experiments in which the dye was administered intravenously are included in these tables. All figures are shown in the corrected "per cent per gram" mode of expression, permitting direct comparison between the tumor and tissue concentrations of various mice. Only figures for concentration are given in the tables and not the total uptake; because it is the concentration of a radioactive substance in tissue which determines the intensity of the radiation to the cells. The relationship of total uptake in tumors to total uptake by other tissues is shown in Figure 1.

The weight of the tumor, in per cent of the animal's body weight, is included in the tables because, in general, any tumor over 7 per cent of the animal's body weight may be expected to show areas of necrosis; any tumor over 15 per cent will have large areas of necrosis which may contain some fluid. There is no observable correlation between size of tumor and acute hemorrhagic necrosis, however. Mice with very large tumors are usually cachectic in appearance and may show anomalous distribution of dye, frequently a low liver concentration suggesting impaired liver function.

The duration of the experiment is indicated in hours from injection of the dye to the death of the animal. In most cases, the animals were killed; a few mice with large tumors died in from 3 to 36 hours and in that case, this interval is considered the duration of the experiment. Mice dying rapidly from too large injections of dye are not included in the protocol.

The ratio of tumor concentration to liver con-

TABLE II

Concentration of radioactive di-brom trypan blue in tissues of tumor-bearing mice

Breed of mouse	Type of tumor†	Size of tumor	Dose	Duration	Concentration of dye					
					Tumor	Liver	Kidney	Spleen	Muscle	Tumor/liver ratio
		per cent	*mgm.*	*hours*	*per cent per gram**					
C$_3$H	SMC	4.7	5.0	3	2.37	16.7	7.67	3.12	3.00	0.14
C$_3$H	SMC	8.2	5.0	31	2.80	20.4	9.40	6.50	2.17	0.13
C$_3$H	SMC	4.3	3.0	30	2.91	18.1	7.10	7.45	1.07	0.16
Average tumor concentration in SMC = 2.69 per cent per gram. Average tumor/liver ratio = 0.14										
C$_3$H	OVT	8.7	2.5	12	3.61	21.2	6.26	5.77	3.94	0.17
C$_3$H	OVT	1.0	3.0	27	3.02	19.7	4.32	5.16	1.67	0.15
C$_3$H	OVT	10.5	2.5	29	5.06	14.9	7.14	5.57		0.34
C$_3$H	OVT	4.8	2.0	31	3.06	12.0	5.75	6.00	3.07	0.25
C$_3$H	OVT	1.3	5.0	78	1.46	18.5	6.25	3.34	0.38	0.07
Average tumor concentration in OVT = 3.24 per cent per gram. Average tumor/liver ratio = 0.20										
C$_3$H	NFS	8.1	3.0	12	12.7	20.3	19.6	10.9	2.13	0.62

† Tumor types are indicated as follows: SMC, spontaneous mammary carcinoma; OVT, transplanted ovarian embryoma; NFS, transplanted neurofibrosarcoma. The size of the tumor is indicated in per cent of the animal's body weight.

* Per cent of injected dose per gram of tissue.

TABLE III

Concentration of radioactive di-brom Evans blue in tissues of tumor-bearing mice

Breed of mouse	Type of tumor†	Size of tumor	Dose	Duration	Concentration of dye					
					Tumor	Liver	Kidney	Spleen	Muscle	Tumor/liver ratio
		per cent	*mgm.*	*hours*	*per cent per gram*					
C$_3$H	OVT	10.0	2.5	3	2.61	8.71	7.31	5.00		0.30
C$_3$H	OVT	11.0	5.0	19	6.15	6.46	5.10	3.12		0.95
C$_3$H	OVT	10.0	5.0	19	2.92	7.20	8.10	7.86		0.40
C$_3$H	OVT	12.7	2.5	21	2.82	11.6	1.16	4.16	1.00	0.24
C$_3$H	OVT	1.8	2.5	21	2.82	11.6	1.16	4.16	1.00	0.24
C$_3$H	OVT	10.0	2.75	24	2.37	5.33	8.20	3.19	0.43	0.44
C$_3$H	OVT	10.7	2.5	24	4.22	7.95	10.1	8.10	0.50	0.53
Average concentration in OVT = 3.42 per cent per gram. Average tumor/liver ratio = 0.44										
C$_3$H	NFS	5.7	5.0	3	0.85	9.06	6.40	3.58		0.09
C$_3$H	NFS	4.0	5.0	3	1.26	11.3	10.3	4.0		0.11
C$_3$H	NFS	2.0	5.0	3	0.46	14.0	2.5	1.55		0.03
C$_3$H	NFS	14.0	3.0	7.5	2.12	9.85	8.70	2.88	2.16	0.46
C$_3$H	NFS	7.5	5.0	12	3.50	4.15	4.31	1.16	0.44	0.84
C$_3$H	NFS	9.3	2.8	19	6.70	6.90	6.75	2.64		0.97
C$_3$H	NFS	8.6	5.0	19	6.30	2.92	7.91	6.25		2.16
Average concentration in NFS = 3.13 per cent per gram. Average tumor/liver ratio 0.66 Average excluding 3-hour experiments = 4.65 per cent per gram. Average tumor/liver ratio = 1.22										
C$_3$H	RMS	10.5	3.2	12	3.40	5.60	5.30	1.97	0.715	0.60
C$_3$H	RMS	8.0	2.5	12	2.46	7.91	4.20	2.14	1.74	0.31
C$_3$H	RMS	2.5	2.5	12	2.64	7.00	11.0	4.20	1.56	0.37
C$_3$H	RMS	29.2	2.5	24	1.80	4.72	5.10	2.56	0.74	0.38
C$_3$H	RMS	16.0	2.5	25	1.52	4.67	2.92	1.92	0.44	0.32
C$_3$H	RMS	17.0	2.5	29	2.21	7.70	6.36		0.791	0.27
Average concentration in RMS = 2.34 per cent per gram. Average tumor/liver ratio = 0.38										
BBC	TMC	17.0	2.5	24	1.66	4.02	4.04	2.91	1.03	0.41
BBC	TMC	14.0	2.5	24	2.26	7.12	9.84	4.30	1 08	0.32
BBC	TMC	10.0	1.5	25	1.55	10.3	9.90	4.76	0.63	0.15
Average concentration in TMC = 1.82 per cent per gram. Average tumor/liver ratio = 0.29										
C$_3$H	SMC	3.8	3.0	3	2.10	5.12	4.86	3.08	0.68	0.41
C$_3$H	SMC	3.4	5.0	12	2.46	6.10	7.35	3.54	0.86	0.40
C$_3$H	SMC	35.0	3.5	24	1.09					
C$_3$H	SMC	8.1	2.75	24	3.38	5.31	8.50	3.84	0.70	0.63
Average concentration in SMC = 2.26 per cent per gram. Average tumor/liver ratio = 0.48										
C–57	MCAS	2.8	5.0	6	5.60	9.23	8.80	3.83	0.46	0.61
C–57	MCAS	14.0	3.5	8	3.06	10.0	6.70	2.84	0.94	0.30
Average concentration in MCAS = 4.33 per cent per gram. Average tumor/liver ratio = 0.45										

† Tumor types are designated as follows: OVT, transplanted ovarian embryoma; NFS, transplanted neurofibrosarcoma; RMS, transplanted rhabdomyosarcoma; TMC, transplanted mammary carcinoma; SMC, spontaneous mammary carcinoma; MCAS, methyl-cholanthrene induced sarcoma. The size of the tumor is indicated in per cent of the animal's body weight.

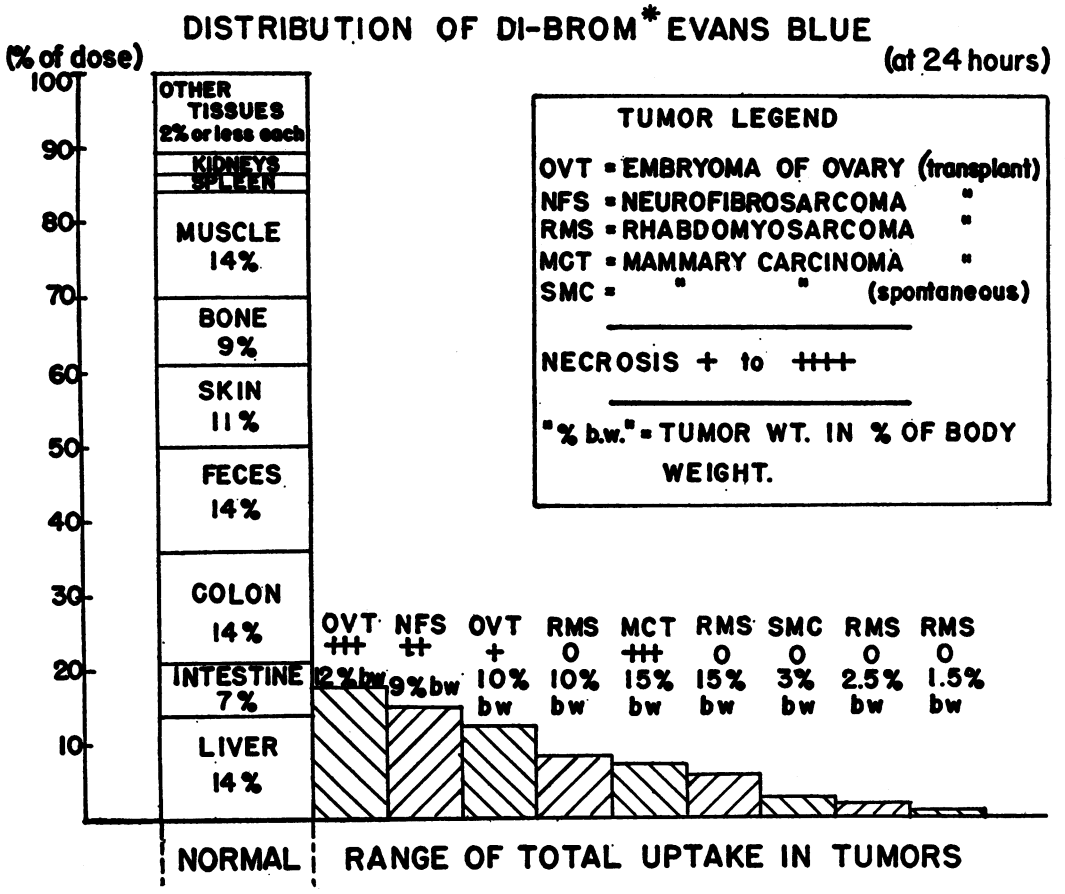

FIG. 1. TOTAL DISTRIBUTION OF DYE AT 24 HOURS, WITH TOTAL UPTAKE OF A REPRESENTATIVE GROUP OF TUMORS FOR COMPARISON

centration is an index of the selectivity of uptake. As will be observed in the table, the relationship between the two is such that were the animal to receive therapeutic radiation by this method, the liver would receive a large dose of radiation and constitute the chief by-effect of the treatment. Any tumor approaching a tumor-liver ratio of 1.0 we consider to have taken up an exceptionally large amount of dye. It will be noted that the two dyes show about the same range of uptake in tumors. There is considerable variation but most of the tumors fall into the 1.5 to 3.5 per cent per gram range, and it can therefore be concluded that the tumor tissue does not actually take up dye to a high order of selectivity. However, the tumor/liver ratios are lower on the average for di-brom trypan blue because of the higher liver concentrations. This indicates greater differential accumulation in tumors for the Evans blue derivative, making it theoretically the more suitable of the two for internal radiation by this method.

This difference between the two dyes, originally suggested by Duran-Reynals (6), together with the higher liver uptake by di-brom trypan blue (which may be correlated with the faster disappearance rate of trypan blue from plasma observed by Gregersen and Gibson (18)), serves to indicate the delicate adjustment which holds between the biological behaviour of these dyes and their chemical structure. The two dyes in question are isomeric, the only difference being the position of the sulfonate radicals on the naphthalene rings. Gusberg, Zamecnik, and Aub (19) report a similar striking difference in distribution between two organic di-selenides, one with and one without mercury in the molecule.

These facts hold out considerable hope that further investigation of radioactive derivatives of

closely related dyes of the same group may reveal a dye possessing a chemical structure which is correlated with a much higher differential uptake by tumor tissue, and therefore more suitable as a carrier of radiation to tumors. Further research should be undertaken in this direction.

Distribution within tumors

With this technique, it is also possible to measure the amount of radioactivity appearing in various zones within the tumor. If the tumor is undergoing caseous necrosis in the central area, considerably less dye is found to have permeated into this zone. The same is true if the necrosis has gone on to clear or straw-colored fluid. If, however, the tumor is undergoing necrosis of an acute type, with hemorrhagic fluid in the central area, this zone tends to "pool" the dye from the plasma, and a higher concentration may be found there than in the surrounding tissue. These findings are summarized in Table IV.

TABLE IV

Distribution of radioactive dye within necrotic tumors

Breed of mouse	Type of tumor	Size of tumor	Concentration of radioactive dye					
			Chronic caseous necrosis			Acute hemorrhagic necrosis		
			Wall	Necrotic zone	Fluid	Wall	Necrotic zone	Bloody fluid
		per cent	*per cent per gram*					
BBC	TMC	17	1.66	0.86				
C3H	SMC	35	1.09	0.75	0.18			
C3H	RMS	29	1.80	1.13				
C3H	RMS	16	1.52	1.11				
C3H	OVT	13	2.82	1.70				
C3H	NFS	6	0.85		0.32			
C3H	NFS	4	1.26		0.35			
C3H	NFS	8				9.7	12.7	
C3H	RMS	8				0.98	2.46	
BBC	TMC	14				1.75		2.26
C3H	OVT	13				1.76		2.37

Designation of tumor types as in previous tables. It will be noted that acutely hemorrhagic tissue or fluid accumulates more dye than the surrounding wall, whereas the reverse is true of chronic caseous necrosis.

These variations in dye concentration produced by necrosis are the end-result of that balance between necrosis and blood supply previously mentioned as the conditioning factor in the accumulation of colloidal dyes in tumors. Chronic necrosis of the type which accumulates clear or slightly colored fluid is the result of long-continued tissue ischemia and as such cannot accumulate blood-borne substances such as the radioactive dye. Acute hemorrhagic necrosis, on the other hand, represents an over-abundance of blood, accompanied by sufficient tissue-destruction to produce increased permeability of the capillaries of the tumor. Such conditions are of course ideal for the accumulation of a blood-borne colloid which leaves the vascular tree at points of increased permeability.

DISCUSSION

It is clear that a technique such as this, which can measure the radioactive dye present in tissues, casts some doubt on previous enthusiastic assertions that tumor uptake is highly selective. The latter observations are influenced by the fact that tumor tissue is light or white in color, with the result that dye in small quantities will appear to have stained it markedly, whereas the reverse is true of kidney, liver, spleen, and feces. Of past workers with the dyes, Hess (7) most closely approximated these results. He stated that with careful microscopic methods he never observed a tumor which had more dye in it than the liver, no matter how selective the gross staining appeared to be.

The significant fact remains, however, that the radioactive colloid permeates into and therefore radiates tumor tissue wherever this tissue may be, and no matter how widespread the metastases are. This fact might render such colloids clinically useful, especially if some way offered itself to increase the uptake of dye by tumor.

A means for increasing this uptake is suggested by our results with acutely hemorrhagic tumors. In addition to administering the radioactive dye, coincident therapy with agents increasing capillary permeability or inducing hemorrhage in the tumor might increase the dye-uptake to the point where clinical application would be feasible. Substances producing an increase in capillary permeability such as bacterial products (20, 21), spreading factor (22), histamine (10), or external radiation, might be useful in this regard and justify further study.

SUMMARY

1. Experiments are described in which radioactive di-brom trypan blue and radioactive di-brom Evans blue have been injected into tumor-bearing mice.

2. Subsequent quantitative measurement of the

distribution of the radioactive colloidal dye demonstrates a widespread gradual uptake from the bloodstream by many tissues and organs. Large amounts of dye are excreted in the bile and feces in the first 24 hours following injection.

3. In the face of this normally widespread distribution, the uptake of dye by tumors does not appear as selective as when judged by tinctorial methods alone.

4. Possible therapeutic effectiveness of a radioactive colloid would be increased by agents producing tumor necrosis.

5. The ideal radioactive colloid for tumor treatment would be one taken up selectively by tumor tissue to a greater degree than those here reported.

The authors wish to express their gratitude to Dr. Baldwin Curtis and the Cyclotron Staff of Harvard University, who supplied most of the radioactive bromine used in this study. Professor Robley D. Evans and the Cyclotron Group at the Massachusets Institute of Technology were also of great assistance in this regard. The technique for measurement of the tissue radioactivity has been worked out with the advice of Dr. Waldo Cohn, which we gratefully acknowledge.

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