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EFFECT OF SELECTIVE TUMOR HEATING ON THE LOCALIZATION OF ^{131}I FIBRINOGEN IN THE WALKER CARCINOMA 256

I. Heating by immersion in warm water

by

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Intravenously administered ^{131}I-labeled fibrinogen or antibody to fibrin localizes preferentially enough in some transplantable rat tumors that it can be used for effective tumor radiation therapy (DAY et coll. 1959, BALE et coll. 1960). Localization also occurs quite commonly in human tumors, sometimes to an extent that the use of this method for therapy seems feasible. However, attempts at therapy have not produced prolonged remissions (MCCARDLE et coll. 1966). In many human tumors and in some transplantable rat tumors, in particular the Walker carcinoma 256, the localization of radioiodinated fibrinogen is not remarkable.

The purpose of the present work is to investigate methods by which the localization of ^{131}I-fibrinogen in the Walker carcinoma 256 may be enhanced, with the hope that successful techniques might serve as useful adjuncts to human cancer therapy with radioiodinated fibrinogen. It has been suggested that deposition of fibrin results from the invasive growth of the tumor and is due perhaps to

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an inflammatory reaction of the host tissues or to simple injury of blood vessels of supporting stroma or invaded tissues (BALE et coll. 1960).

In this laboratory, the localization of ^{131}I-fibrinogen in two transplantable rat tumors has been studied extensively. In the course of this investigation, 'localization' will refer to the amount of ^{131}I activity found in a specific site and presumably represents the result of a steady deposition and destruction of fibrin. SHAEFFER (1962) has shown that about 20 % of the injected radioactivity localizes in a 2.5 gram Murphy-Sturm lymphosarcoma 18 hours after intravenous administration of ^{131}I-fibrinogen. A 3 gram Walker tumor, on the other hand, as reported later, localizes only 1 % of the injected dose 3 days after ^{131}I-fibrinogen injection. If the percentages of the remaining whole body activity found in the tumors at these times are calculated for these two tumors, one obtains 30 % and 8 % for the Murphy-Sturm and Walker tumors, respectively. According to HIRAMOTO et coll. (1960), the small amount of localization in the Walker tumor might be explained in part on the basis of its slower growth rate as compared to the Murphy tumor. However, studies by SOARDI (1959) suggest that rats bearing the Walker tumor have increased plasma fibrinolytic activity and slightly reduced blood coagulability. VARON & SPAR (1965) have demonstrated that enhancement of the coagulation mechanism of the host increases fibrinogen localization in the Murphy-Sturm lymphosarcoma. BALE et coll. (1962) have shown that when rats bearing the Walker tumor are given epsilon aminocaproic acid (EACA) in their drinking water, a marked increase in tumor fibrin localization occurs. MUTSCHLER (1963, 1964) has extensively investigated the effect of EACA as a fibrinolysis inhibitor. Her results were corroborated in this investigation and are discussed in detail below.

A simple model can serve as the basis for experimentation: tumor fibrin localization equals depositions minus removal. Its removal from the tumor site involves fibrinolytic processes; thus systemic inhibition of fibrinolysis should result in increased tumor fibrin localization. Tumor fibrin deposition may be hypothetically divided into two phases: fibrinogen extravasation due to a localized inflammatory reaction or to invasive damage to vasculature, and fibrinogen transformation to fibrin by the coagulation mechanism. It seems reasonable then that at least three means could be employed to increase the amount of fibrin localized in the Walker and other tumors: (1) inhibition of the fibrinolytic system of the host, (2) enhancement of any inflammatory reaction occurring at the tumor site, and (3) selective damage to tumor tissue.

If tumor cells and cells of the tumor stroma, such as mast cells and capillary endothelial cells, could be selectively damaged in some way, mediators of the inflammatory reaction such as histamine might be released at the site of the insult and increased fibrinogen extravasation would occur. If tissue thromoplastin

were also released from the damaged cells, this extravasated fibrinogen should clot at the site of injury. It is also possible that selective tumor damage might directly injure the vasculature of the supporting stroma and in this way increase fibrinogen extravasation.

In 1935, WARREN studied the effects of artificially induced fever upon hopeless tumor cases. Infrared heat lamps were used to induce fever (rectal temperature 41.5° C) for periods up to 21 hours. After such treatment, tumor growth was temporarily retarded and the patient's life was prolonged. His work shows that tumor tissue is more susceptible to destruction by heat than are the tissues in which it grows. CRILE JR (1963) has reported that heating certain melanomas implanted in the feet of mice to 44° C for from 30 to 40 minutes destroyed a high proportion of the tumors without damage to the feet.

Materials and Methods

Rat fibrinogen. Fraction 1 was obtained from fresh oxalated rat plasma by alcohol addition in the cold (ice-salt bath) according to method 6 of COHN et coll. (1946) modified by the omission of acetate buffer. The procedure is essentially that reported by SHAEFFER (1964).

Labeling of fibrinogen with ^{131}I. The protein iodination method of HELMKAMP et coll. (1960) using iodine monochloride, as modified by BALE et coll. (1966) to include the use of catalase to eliminate H_2O_2 was followed throughout these studies.

^{131}I-labeled rat albumin. Rat albumin was isolated from fresh rat serum according to a modified version of the acidified methanol technique of MICHAEL (1962). This twice recrystallized albumin was then radioiodinated using the technique described for fibrinogen iodination. The purity of the ^{131}I albumin preparation was then tested by several methods. Only 1.5 % of the radioactivity of an aliquot was bound to proteins precipitable with thrombin. More than 97 % of the radioactivity of an aliquot precipitated in 20 % trichloracetic acid. Nearly 93 % of the radioactivity of ^{131}I albumin-spiked plasma or serum samples remained in solution when one-third volume saturated ammonium sulfate was added. Electrophoresis studies indicated that not more than 5 % of the sample radioactivity could be fibrinogen bound.

Experimental animals. Sprague-Dawley female rats (Holtzmann Farms, Wisconsin) weighing 100—200 grams each, were used. Potassium iodide drinking water (6×10^{-4} M) was given to all experimental animals to reduce the uptake of inorganic ^{131}I by the thyroid. Animals were given checkers of Purina Labora-

tory Chow (for rats) ad libitum. Two rats were housed per cage. In all experiments in which EACA was employed, a 5 % solution of EACA in the drinking water was given to the rats at least 18 hours prior to fibrinogen injection.

Walker carcinoma 256. A line of Walker carcinoma 256 (EARLE 1935, FISHER & FISHER 1961) which for some years has been carried in this laboratory and was originally obtained from Dr Florence Millar, National Institutes of Health, Bethesda, Maryland, was transplanted by trocar into Sprague-Dawley rats. A single fragment (about 15 mg) from vascular non-necrotic tumor areas was placed subcutaneously on the right side approximately opposite the lowest rib. The transplant site had a barely palpable tumor on the seventh day after inoculation, which grew to a 10 to 14 gram tumor in six more days.

^{131}I fibrinogen injection and radioactivity measurements. Immediately prior to its injection, the previously iodinated frozen fibrinogen was thawed and diluted with fresh oxalated rat plasma to an activity concentration of about 200 μCi per ml. Diluted fibrinogen was assayed by a clottability test within 2 hours prior to injection and was found to contain 82 to 92 % clottable protein. Diluted fibrinogen solution was injected via the saphenous vein in a volume of 0.5 ml. Immediately after the injection, a whole body radioactivity measurement was made by using MUTSCHLER's technique (1963, 1964). Counts were compared to those from an injection standard prepared by adding 0.5 ml of injection solution to 100 ml of distilled water made basic with several NaOH pellets in a 250 ml flask. (The standard was made basic to keep any non-protein bound ^{131}I in the reduced, non-vaporizable iodide form.) Additional whole body counts were made prior to sacrifice. Immediately after sacrifice in ether, the rats were weighed. The tumor was then carefully removed, weighed, and counted. The remainder of the animal (the 'carcass') was similarly counted.

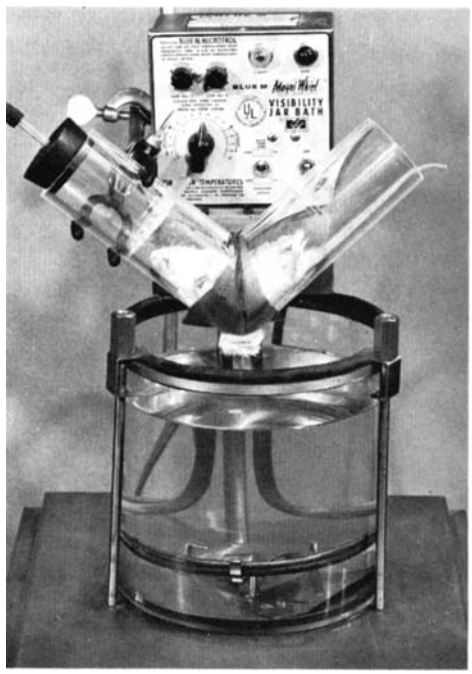

Fig. 1. The tumour heating apparatus.

Tumor-heating technique. For selective tumor heating a Blue M Magni Whirl Jar Bath (Blue M Electric Company, Blue Island, Illinois) was used. In order to heat the tumor alone, the rat was placed in an L-shaped lucite holder which had a one-inch diameter hole in the outside wall of the angle, through which the subcutaneous tumor could be pulled. The tumor was then pulled through a small hole in a thin rubber sheet placed outside the lucite container to prevent entrance of warm water (see Fig. 1).

In order to keep the rats immobile during treatment, they were anesthetized with a fluothane-oxygen anesthetic. Fluothane, the Ayerst Laboratories brand of halothane, was kindly supplied by Dr J. B. Jewell of Ayerst Laboratories, 685 Third Avenue, New York, New York 10017. Oxygen loaded with fluothane was allowed to enter the anesthesia chamber at 0.2 cubic feet per hour and 100 % oxygen entered the chamber at 2.0 cubic feet per hour.

Radioautography studies. For radioautographic and further histologic study, tumors were fixed in a buffered 10 % formalin solution, exposed to no-screen roentgen film as paraffin sections, and later stained with hematoxylin and eosin. The film was exposed for 48 to 96 hours, depending on the activity of the ^{131}I in the sections.

Experimental results

Quantitative evaluation of the effect of the various treatments required parameters measuring ^{131}I localization. The parameters used in this study are as follows.

Percentage of injected dose per gram tumor normalized (% ID/g TN). This parameter thus involves a normalization of localized radioactivity to correct for the differences in body weights of the individual experimental rats. The parameter is calculated as follows:

$$\%\ \text{ID/g TN} = \frac{\text{net counts per min from tumor} \times \text{whole body weight}}{\text{net counts per min from injection standard} \times \text{tumor wt}}$$

Therapeutic ratio. The value of radiation treatment of a cancerous growth is usually judged according to the magnitude of the ratio:

$$\frac{\text{rad absorbed by malignant tissue}}{\text{rad absorbed by normal tissue}}$$

Table 1

Effect of mechanical traumatization on tumor fibrinogen localization

Group	Number of rats	%ID/gTN	Therapeutic ratio	3-day whole body radioactivity retained (%)
No treatment	28	1.06±0.07	7.9±0.7	19.5±1.1
Daily mechanical traumatization	5	2.36±0.42*	22.2±5.2*	30.4±4.1*

* Indicates a statistically significant difference from the control value at the 1 % significance level. In this and following table, each parameter is followed by ± standard error of the mean.

This ratio can be approximated for the case of a rat bearing the Walker tumor and having been administered a certain injected dose of ^{131}I fibrinogen:

$$\frac{\text{percentage injected dose per gram tumor tissue}}{\text{percentage injected dose per gram normal tissue}} = \frac{\%\ \text{ID/g tumor}}{\%\ \text{ID/g carcass}}$$

This latter approximate calculation will be referred to as the therapeutic ratio for the purposes of this study.

Percentage of whole body radioactivity retained. If one animal of an experimental group had a radioactivity retention which was greater than two standard deviation units higher than the mean of the group, it was usually an indication that some of the ^{131}I fibrinogen had been injected into the tissue surrounding the saphenous vein rather than intravenously and was clotted there under the action of tissue thromboplastin. The data from such experimental animals was discarded. The percentage of whole body retention was calculated as follows:

$$\text{percentage whole body retention on day X} = \frac{\dfrac{\text{whole body counts on day X}}{\text{standard counts on day X}}}{\dfrac{\text{whole body counts at injection}}{\text{standard count at injection}}} \times 100$$

Thus, by comparison with an injection standard, the whole body counts on experimental animals were corrected for the physical decay of ^{131}I.

Effect of epsilon aminocaproic acid (EACA) on the localization of ^{131}I fibrinogen in the Walker carcinoma 256. The data now reported were gathered from experiments in which control rats received either KI or EACA plus KI in their drinking water but no additional treatment. In each case, Walker tumor-

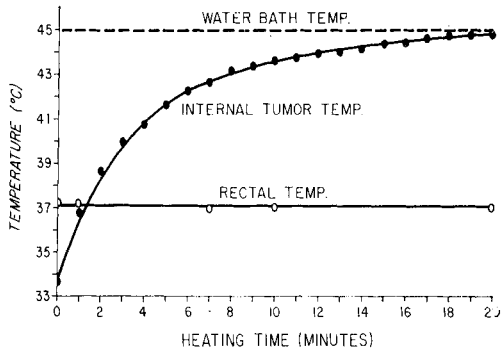

Fig. 2. Effect of selective warm water tumor heating on tumor and rectal temperature.

bearing rats were placed on either KI or 5 % EACA-KI nine days after tumor transplant and one day prior to ^{131}I injection. After three days the tumor was carefully excised. The 23 control rats which had only KI in their drinking water showed a % ID/g TN of 0.44 ± 0.03, whereas this parameter for the 27 rats drinking 5 % EACA-KI was 1.48 ± 0.17. Thus, an increase of tumor fibrinogen localization by a factor of 3.4 was induced by EACA. This figure is within the range previously reported (BALE et coll. 1962, MUTSCHLER 1963, 1964).

Effect of mechanical traumatization on tumor fibrinogen localization. Preliminary experiments indicated that room temperature heating of control tumors caused considerably more tumor fibrinogen localization than nontreatment of controls. That this increased localization could be due to mechanical traumatization during positioning is shown by the results in Table 1.

These results indicate that rough handling of the Walker tumor can lead to marked increase of ^{131}I fibrinogen localization there and that mock treatment of control animals is necessary in order that the effect of tumor heating per se can be studied.

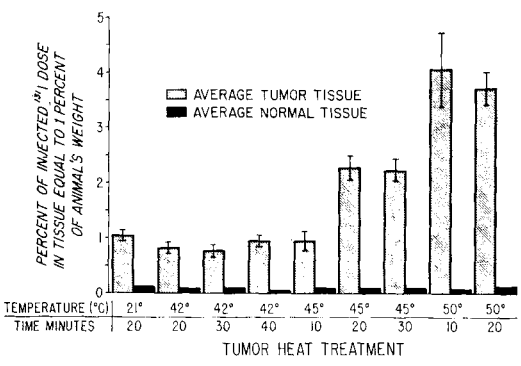

Fig. 3. Effect of warm water tumor heating on localization of ^{131}I fibrinogen in Walker carcinoma 256. The percentage of injected radioactivity dose remaining in tumor and non-tumor portions of rats, whose tumors were subjected to heat treatment for the time and temperature indicated, is plotted here in the form of a bar graph. Values are expressed as percentage of injected ^{131}I dose in an amount of tissue equal to 1 % of animal's total weight. Vertical line at top of each bar indicates standard error of mean. (Standard error of the mean values for average normal tissue were not large enough to be reproduced.)

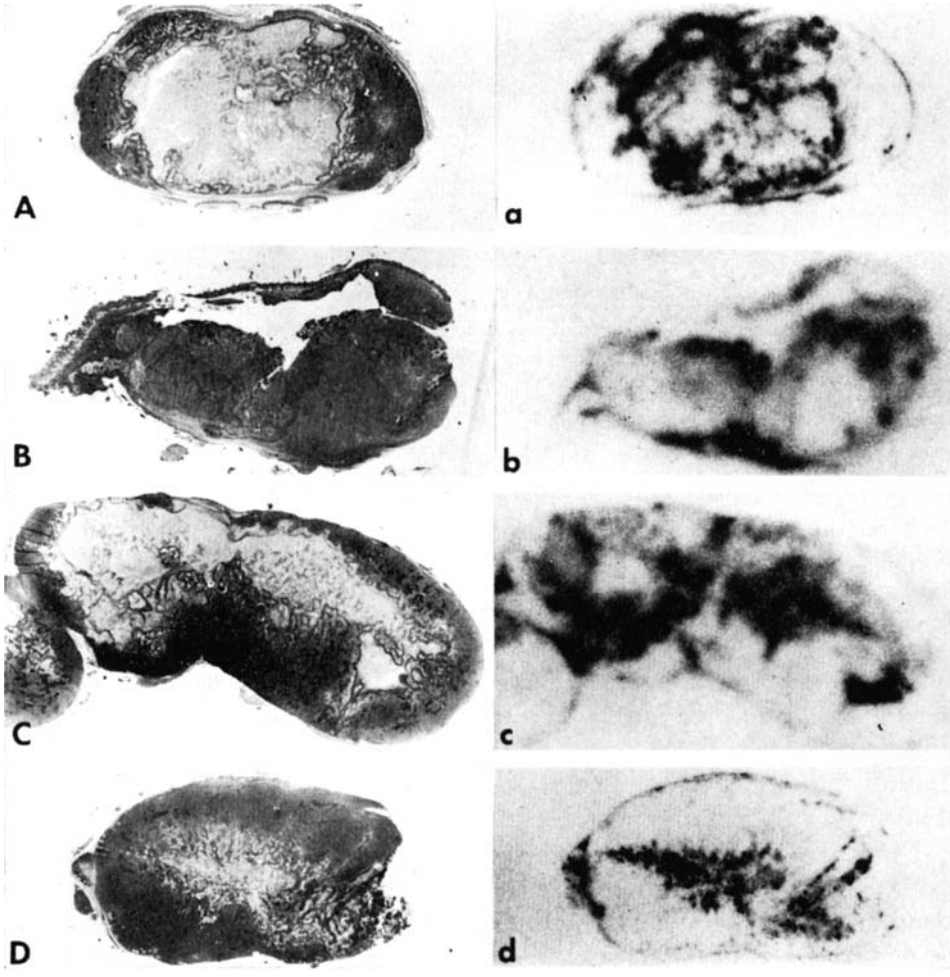

Fig. 4. Photomacrographs and radioautographs from selective tumor heating study (warm water), 3-day uptake, magnification $\times 1.4$.

A—a) Control tumor (R.T.—20′). Central area of tumor section composed of light-pink cytoplasmic material with low nuclear density and empty space region thought to be necrotic. Outer margins of tumor (cortex) composed of cells with large, dark-blue nuclei and little cytoplasm. Most radioactivity in transition zone between these two areas.

B—b) Treated tumor (42° C—20′). There is a large acellular cavity just below skin surface, probably filled with bloody fluid at sacrifice, and surrounded by narrow band of predominately cytoplasmic cells. Tumor surrounded by considerable amount of muscle and connective tissue. Radioactivity in area around cavity and in connective tissue.

C—c) Treated tumor (42° C—40′). Tumor is considerably larger than others in the series and has a large lightly stained medulla. Radioactivity in transition zone between medulla and cortex.

D—d) Treated tumor (42.5° C—30′). Tumor similar in structure to tumor in A—a but has a much smaller medulla. Radioactivity in the lightly stained medulla.

Effect of selective tumor heating on the localization of ^{131}I fibrinogen in the Walker carcinoma 256. Actual tumor temperature during a typical heat treatment was measured by a thermistor placed in the center of the tumor with precautions taken to prevent water seepage into the tumor (Fig. 2). A rapid temperature rise occurred for about 8 minutes until 43° C was reached, and then the temperature gradually increased 1.8° C in the remaining 12 minutes. The water bath was kept at 45° C and the tumor temperature was monitored for 20 minutes.

Results of a treatment temperature series are compiled in Fig. 3. Only in the last four treatment groups was a statistically significant difference from the control values noted in fibrinogen localization.

Representative stained sections and the corresponding radioautographs from each group in the above study are illustrated in Figs 4 and 5. These radioautographs suggest that ^{131}I fibrinogen localization took place in necrotic tumor tissue and that heat treatment increased tumor necrosis.

At least 20 minutes at 45° C was necessary to enhance fibrinogen localization in the tumor. Treatment at 50° C produced too much injury to normal tissue for it to be a feasible means of fibrinogen localization enhancement.

In an additional study, the hind legs of several animals were heated at 50° C-10′, 50° C-20′, 45° C-20′ and 45° C-30′. The hind legs which were heated at 50° C were badly injured. Hind legs which were heated at 45° C swelled somewhat at the time of treatment but did not show subsequent visible damage. When the parameter, % ID/g TN, is compared for tumor and hind leg muscle heated at 45° C for 20 minutes, one finds 3.87 ± 0.62 and 0.17 ± 0.05, for tumor and muscle, respectively. At this temperature, then, tumor tissue is much more susceptible to heat damage than is normal muscle tissue as evidenced by the degree of fibrinogen localization.

Kinetics of tumor fibrinogen localization after heating at 45° C-30′. Control and experimental animals were sacrificed at the following times after initiation of the 30 minute heat treatment: 0.5, 24, 42, and 61 hours. ^{131}I fibrinogen was injected intravenously just before beginning heat treatment. The variation of control and experimental therapeutic ratios with time is plotted in Fig. 6. This parameter of fibrinogen localization appears to increase exponentially with time in both groups after 20 hours.

In the second kinetics of uptake study, control and experimental rats were injected just before, just after, or 24 hours after treatment and sacrificed 24 hours later. The results are tabulated in Table 2. The greatest 24-hour localization took place in the group injected immediately after being treated.

If the two experiments are considered as a whole, several trends become

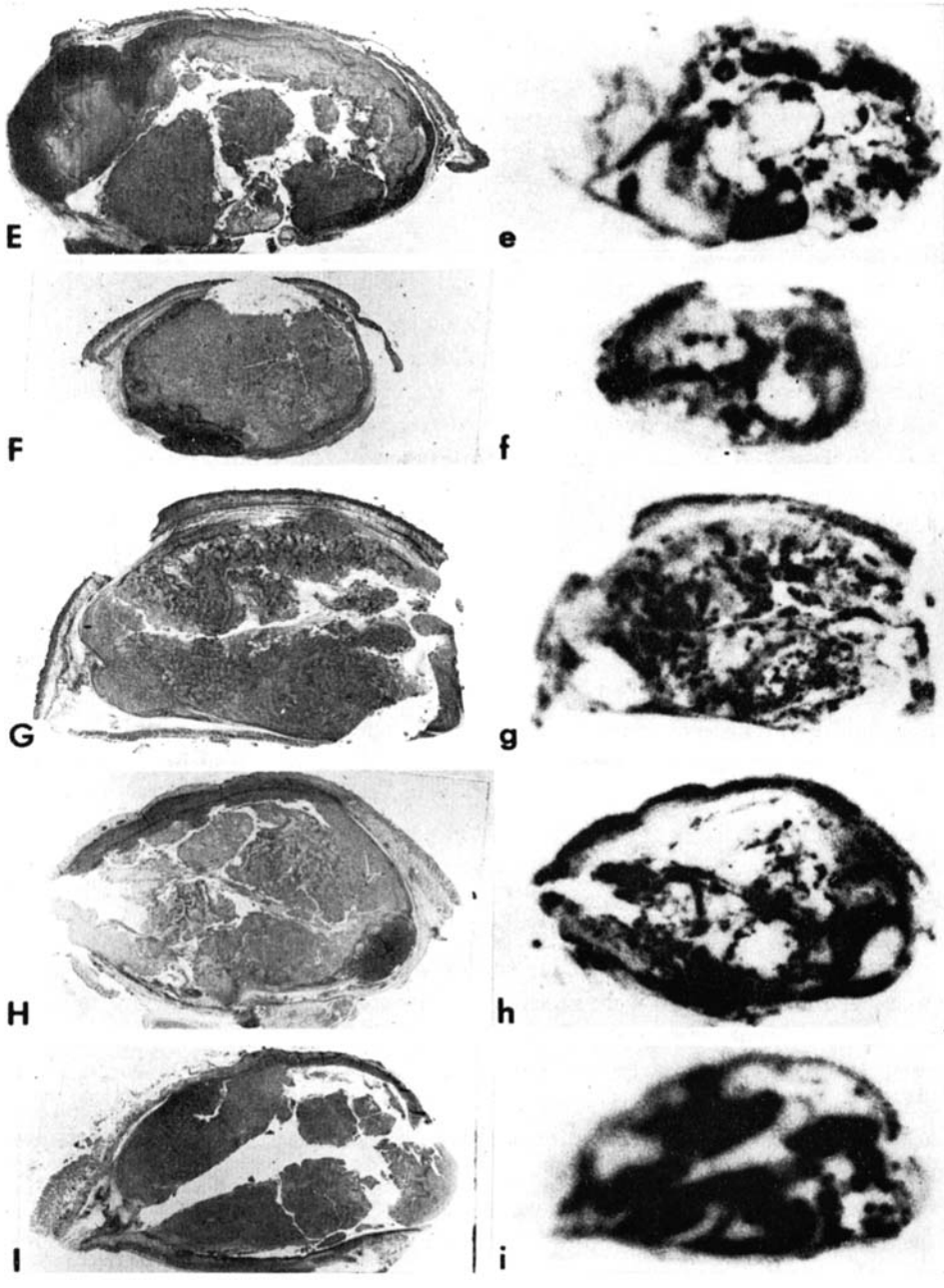

Fig. 5 (for legend, see opposite page).

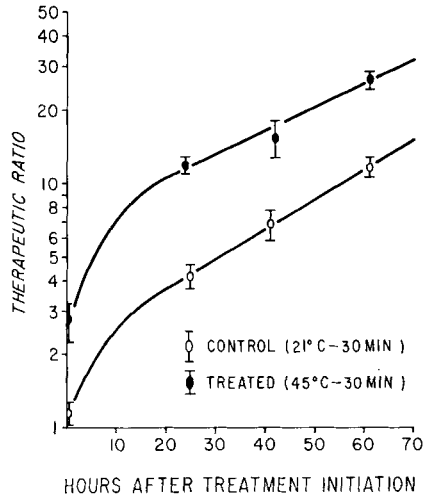

Fig. 6. Kinetics of ^{131}I fibrinogen localization in Walker carcinoma 256 after warm water tumor heating at 45° C—30′. Therapeutic ratio plotted against time in hours which elapsed between beginning of heat treatment and sacrifice. Vertical bars at each point indicate variation allowed by the standard error of mean. The therapeutic ratios of both the heated and control groups seem to increase exponentially with time after 20 hours.

evident. The greatest amount of radioactive fibrinogen is deposited in the tumor during the first day after treatment. A smaller amount is deposited in the tumor during treatment. The exponential increase of therapeutic ratio with time suggested by Fig. 6 probably results from a greater biologic half-time of ^{131}I fibrin(ogen) in the tumor than in the remaining body tissues (see Fig. 8).

Representative stained tumor sections and the corresponding radioautographs are presented in Fig. 7. There did not seem to be any change with time in the pattern of ^{131}I fixation in either the control or heated tumors. In the control tumors, radioactivity was found predominately in the center of the tumor in an

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Fig. 5 (see opposite page). Photomacrographs and radioautographs from selective tumor heating study (warm water), 3-day uptake, magnification × 1.7.

E—e) Treated tumor (45° C—10′). First tumor in series to show some effect of heat treatment, composed almost entirely of light-staining regions. Radioactivity distributed irregularly throughout this region. Area of highest density composed of cells almost completely devoid of nuclei.

F—f) Treated tumor (45° C—20′). Most of this tumor composed of lightly stained area; cells loosely packed, with large nuclei and little cytoplasm, surrounded by pink filaments (fibrin?). Radioactivity throughout tumor and most dense in the lightly stained areas.

G—g) Treated tumor (45° C—30′). Tumor is similar to the one in F—f. It is almost completely composed of loosely packed cells imbedded in filamentous material; entire section lightly stained and radioactivity spread throughout the section.

H—h) Tumor similar to both the two preceding ones. Some erosion of tumor can be seen. There are: a small region of normal tumor cells of cortex type which has almost no radioactivity, and a long blood-filled cavity which is not especially radioactive. Radioactivity is greatest in areas immediately surrounding blood cavities and in the area bordering the cortex-type cells.

I—i) Treated tumor (50° C—20′). There is considerable tumor erosion. Radioactivity is dense throughout tumor and is minimal in muscle and connective tissue.

Table 2

Kinetics of tumor fibrinogen localization after heating at 45° C—30′ — 24-hour uptakes starting at various times in relation to treatment

Treatment	Number of rats	%ID/gTN	Therapeutic ratio	Whole body radioactivity retained (%)	Average rat weight gram	Average tumor weight gram
Control R.T. 30′ injected prior to treatment	8	1.77±0.29	4.2±0.5	51.1±3.9	150±7	5.55±0.30
Control injected following treatment	5	1.99±0.08	4.0±0.2	52.9±1.3	148±4	2.12±0.33
Control injected 24 hours following treatment	5	1.87±0.23	4.7±0.6	45.1±1.8	154±3	2.06±0.55
45° C—30′ injected prior to treatment	7	4.34±0.48	12.0±0.8	53.6±2.4	147±7	5.69±1.33
45° C—30′ injected following treatment	5	6.94±1.11	12.8±1.7	67.5±3.9	151±3	3.38±0.61
45° C—30′ injected 24 hours following treatment	5	1.65±0.29	3.8±0.8	50.1±1.0	145±5	2.21±0.28

area thought to be necrotic and which stained pink in hematoxylin and eosin. In the heated tumors, radioactivity was also found in areas staining pink in hematoxylin and eosin but this area occupied a greater portion of the tumor and usually included the tumor cortex. Microscopic examination of sections of heated tumors and their radioautographs suggests that ^{131}I radioactivity is fixed in a lightly pink stained filamentous network located outside blood capillaries and sinuses. It is difficult, however, to pin-point the source of radioactivity at a cellular level with the radioautograph procedure here employed.

^{131}I albumin localization in the heated Walker carcinoma. Nine days after Walker tumor transplant, 29 rats were given 5 % EACA-KI as drinking water. On the tenth day after transplant, each rat was given about 100 μCi of ^{131}I albumin intravenously. As soon thereafter as possible, the tumors of 14 experimental animals were treated in 45° C water for 30 minutes as previously described. Tu-

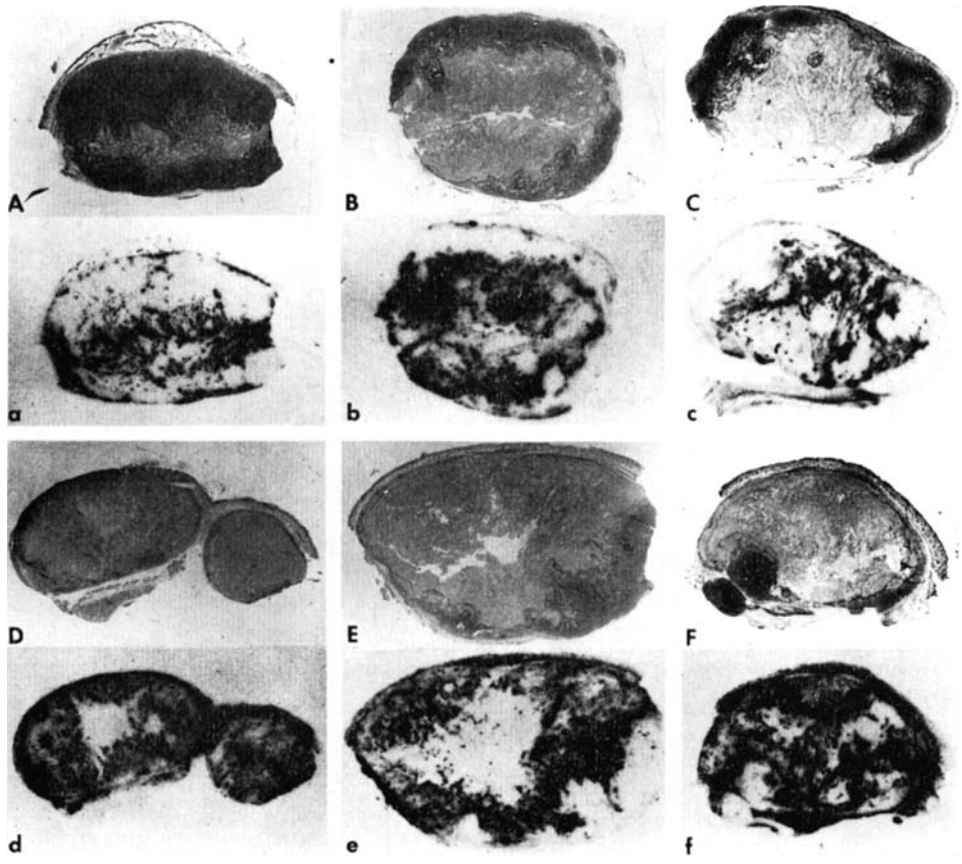

Fig. 7. Photomacrographs and radioautographs from kinetics of uptake study after tumor heating at 45° C—30′. Magnification × 1.1.

A—a), B—b), and C—c) Control tumors (R.T.—30′) 1, 2 and 3 days. The light-pink-staining medullary area expands to occupy more of tumor as time passes. Radioactivity located predominately in this light medullary area and in transition zone immediately surrounding it. Radioactivity becomes more widespread with time as it follows the light-pink-stained area.

D—d), E—e), and F—f) Treated tumors (45° C—30′) 1, 2 and 3 days. Radioactivity widespread throughout tumor on first day after treatment; only a small area of medullary tissue is low in radioactivity. At one day after treatment (D—d) the cortex and medulla are not clearly demarcated and whole tumor stains pinkish-blue. Cellular structure fairly regular, with polygonal tumor cells having moderately large nuclei interspersed in pink fibrinous material. On second and third days after treatment (E—e and F—f) two definite zones appear. Radioactivity is localized mainly in the light-pink-staining regions characteristic of the medulla, and is characteristically absent from the dark, blue-staining areas, that might represent tissue not affected by heat treatment since they usually lie on the side of the tumor which was next to the body.

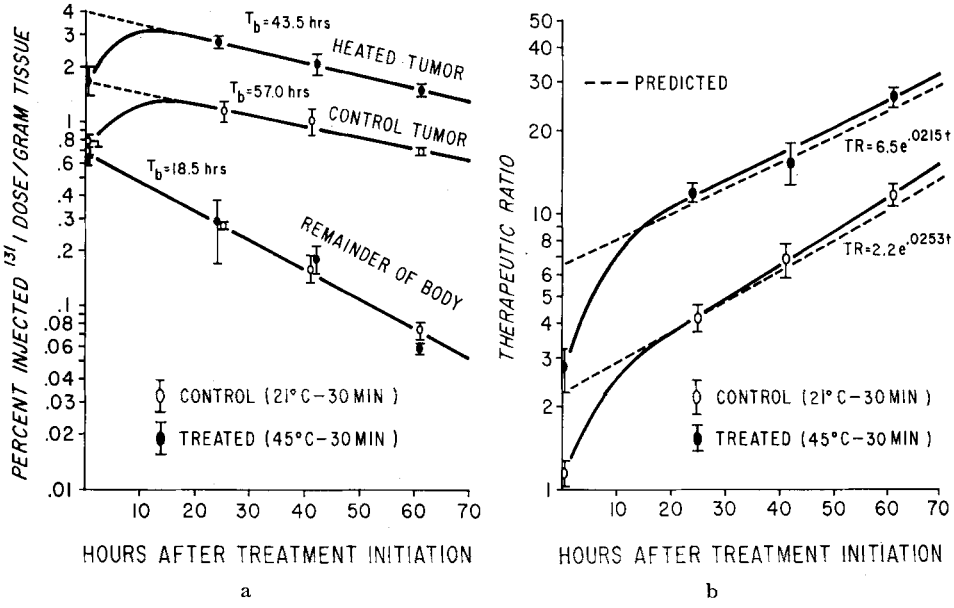

Fig. 8. Specific ^{131}I fibrinogen activtiy of tumor and carcass versus time after heating in 45° C water (a) and therapeutic ratio versus time after treatment (b).

mors of 15 control animals were treated for the same length of time but with the water bath at 21° C. Immediately after this treatment, two experimental and three control rats were sacrificed and the previously described parameters were determined. At 24, 45 and 65 hours after treatment initiation, four experimental and four control rats were sacrificed and the usual parameters were calculated. The results of this study are plotted in Figs 9 and 10.

Discussion

The stated object of this series has been to investigate a variety of methods by which the localization of radioiodinated fibrinogen in the Walker carcinoma 256 might be enhanced. Results obtained in the course of this investigation suggest that selective heating of a Walker tumor markedly increases the localization of previously injected ^{131}I fibrinogen in the tumor. The tumor had to be exposed to an environment of about 45° C for at least 20 minutes to obtain significantly greater localization of ^{131}I fibrinogen within the tumor.

It is tempting to apply the findings of Rocha e Silva (1963) to the problem of tumor fibrinogen localization after heat treatment. No significant increase in tumor fibrinogen localization above the control level was noted until heat

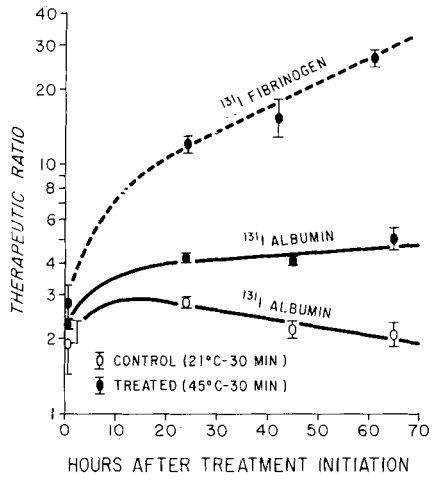

Fig. 9. Comparison of ^{131}I fibrinogen and ^{131}I albumin therapeutic ratios versus time after tumor heating.

treatment was such that the internal tumor temperature reached approximately 44° C and was maintained at that or greater temperatures for several minutes. It is possible that this temperature range must be reached before bradykinin or some other mediator of increased capillary permeability is released in the heated tumor tissue.

Although treatment for 10 or 20 minutes at 50° C caused the most marked increase in tumor fibrinogen localization encountered in the warm water study, this treatment temperature caused considerable injury to normal tissue and was consequently excluded from further investigation.

In general, control tumors exhibited a necrotic region in their center surrounded by a zone in which the cells were starting to undergo necrosis presumably because of an insufficient blood supply. Around this region the tumor cortex, composed of viable tumor cells and stroma, was situated. The radioactivity in control tumors was usually located at interfaces between tumor and normal tissue and in the zone between the viable tumor cortex and the necrotic tumor core. It seems reasonable that materials released from dying cells induced an inflammatory reaction in the neighboring regions and thus blood-borne ^{131}I fibrinogen was deposited there. When heat was applied to tumor tissue, the metabolic demands of the viable tissues were increased and their blood supply, already being under considerable stress, was insufficient for the demand and the cells died. In such regions cellular materials released by autolysis could increase the low-grade inflammatory reaction occurring and induce extravasation and clotting of blood-borne fibrinogen. Thus, an uneven distribution of ^{131}I deposition would be expected and would depend on how close to being overstressed the

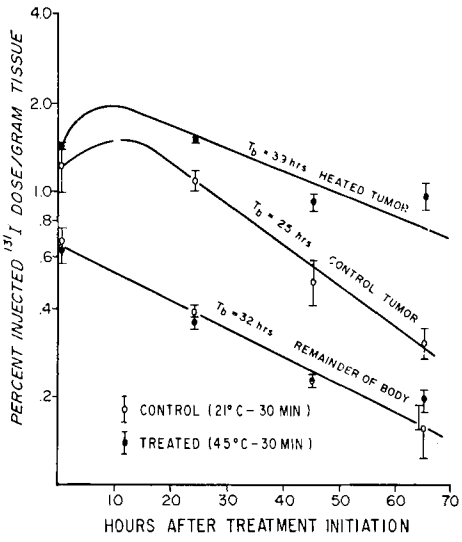

Fig. 10. Specific activity of ^{131}I albumin in tumor and carcass versus time after heating in 45° C water (conditions as in fig. 8).

circulation of particular tumor areas was. Radioautographs of heated tumors evidenced a radioactivity deposition which became more widely distributed as the amount of heat treatment was increased.

A theoretical analysis of tumor fibrinogen localization after treatment in 45° C water for 30 minutes is presented in Fig. 8. On the left of the figure, the logarithms of percentage of injected dose per gram (% ID/g) of heated and control tumor tissue and for the carcasses of heated and control rats are plotted against time. These curves are consistent with the hypothesis that ^{131}I fibrinogen is deposited in the tumor primarily during the first 20 hours after injection whether the tumor is heated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time. Because the % ID/g data contain no component of ^{131}I physical decay, the exponential decrease of tumor radioactivity concentration with time indicates a removal of ^{131}I from the tumor at a rate proportional to the amount present.

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor. The carcass radioactivity concentration is approximately the same initially in the treated and control groups and the carcass biologic half-life of ^{131}I fibrinogen is equal in the treated and control groups.

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation $A_t = A_0 e^{-\lambda t}$ where A_t is tissue radioactivity concentration, % ID/g, at any time t. For the tumors, the

relation holds when t is greater than about 20 hours. A_0 is the hypothetical initial tissue radioactivity concentration, % ID/g; λ is the biological decay constant in hours^{-1}, and is equal to $0.693/T_b$ where T_b is the biologic half-life in hours of ^{131}I fibrinogen in the tissue in question. The time in hours after treatment initiation is represented by t.

When the suitable constants determined in Fig. 6 are substituted into the above relation, the following mathematical descriptions for tissue radioactivity versus time are obtained:

treated tumor	$A_t = 4.0e^{-.0159t}$	(a)
control tumor	$A_t = 1.5e^{-.0122t}$	(b)
treated carcass	$A_t = 0.62e^{-.375t}$	(c)
control carcass	$A_t = 0.68e^{-.375t}$	(d)

The therapeutic ratio as a function of time for control and treated groups can be calculated from relations b, d and a, c, respectively:

$$\text{T.R. control} = \frac{A_t \text{ (control tumor)}}{A_t \text{ (control carcass)}} = 2.2e^{.0253t}$$

$$\text{T.R. treated} = \frac{A_t \text{ (treated tumor)}}{A_t \text{ (treated carcass)}} = 6.5e^{.0215t}$$

These calculated theoretical expressions for the therapeutic rations are plotted as functions of time on the right side of Fig. 8. The variations of the experimental therapeutic ratios with time are also plotted here as they were in Fig. 6. The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference of biologic half-life of ^{131}I fibrinogen in the tumor and carcass.

In order to assess the effect of the warm water treatment on the average total radiation doses to tumor and carcass, the concept of effective half-life must be included:

$$T_{\text{effective}} = \frac{T_b \times T_p}{T_b + T_p}$$

where T_b = biologic half-life (just determined), T_p = physical half-life (192 hours for ^{131}I),

since $\quad D_\beta(\infty) = \text{constant} \times C_0 \times T_{\text{eff}}$ (adapted from LOEVINGER et coll.)

where D_β (∞) is the average total radiation dose from ^{131}I β's absorbed by a tissue in which the initial radioactivity concentration is C_0 and the effective half-life of ^{131}I is T_{eff}. The ratio of total tumor dose to total carcass dose is thus:

$$\frac{D_\beta(\infty)\ \text{tumor}}{D_\beta(\infty)\ \text{carcass}} = \frac{C_0 \times T_{\text{eff}}\ \text{(for tumor)}}{C_0 \times T_{\text{eff}}\ \text{(for carcass)}} = T.R._0 \times \frac{T_{\text{eff(tumor)}}}{T_{\text{eff(carcass)}}}$$

$$\frac{\text{average } \beta \text{ dose to tumor}}{\text{average } \beta \text{ dose to carcass}} = \begin{matrix} 6.5 \times 2.1 = 13.7 \text{ for treated group} \\ 2.2 \times 2.6 = \ 5.7 \text{ for control group} \end{matrix}$$

This theoretical calculation indicates that the warm water treatment improved the ^{131}I fibrinogen radiation therapy of the Walker tumor nearly 250 per cent.

Preliminary experiments by BALE et coll. (1964) have indicated that heating tail-transplanted Walker tumors at 41.5° C for five hours markedly increases tumor localization of ^{131}I-labeled antifibrin antibody.

Several conclusions can be drawn from ^{131}I albumin results. The therapeutic ratio for fibrinogen-injected animals (Fig. 9) is nearly six times greater three days after treatment than that for animals given ^{131}I albumin and identical heat treatment. Data presented in Fig. 10 suggest two reasons for this difference in therapeutic ratios. Although the biologic half-lives of ^{131}I albumin and ^{131}I fibrinogen are approximately the same in the heated Walker tumor, the maximum tumor radioactivity concentration is nearly two times greater after ^{131}I fibrinogen administration. In addition, the biologic half-life of radioalbumin in the non-tumor portion of the rat's body is almost twice as long as that of radiofibrinogen. These factors combine to produce considerably less increase of therapeutic ratio with time in the ^{131}I albumin injected rats.

This study seems to indicate that proteins such as fibrinogen and antifibrin antibody whose tumor localization is associated with the clotting mechanism, are unusual in their susceptibility to be retained in the heated Walker tumor without marked concurrent retention in the non-tumor portions of the host.

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SUMMARY

Heating of the Walker carcinoma 256 by immersion in a water bath at 45° C for 30 minutes led to a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which localized in the tumor. Such heat treatment increased the tumor radiation therapy dose from ^{131}I almost 250 per cent. Heated muscle in the neighborhood of tumor did not localize ^{131}I fibrinogen. Radioactivity deposition coincided with histologically demonstrable areas of tumor damage. ^{131}I albumin was not effectively cleared from non-tumor tissues.

ZUSAMMENFASSUNG

Eine Erwärmung des Walker Carcinoms 256 durch Eintauchen in ein Wasserbad von 45° C 30 Minuten lang führt zu einem kräftigen Anstieg der Menge intravenös injizierten ^{131}I Fibrinogens, die sich im Tumor ansammelt. Eine derartige Wärmebehandlung steigert die therapeutische Strahlendosis des Tumors durch ^{131}I um beinahe 250 %. Die erwärmte Muskulatur der Umgebung des Tumors nimmt nicht ^{131}I Fibrinogen auf. Die Ablagerung der Radioaktivität stimmt mit der histologisch nachweisbaren Ausbreitung der Tumorschädigung überein. ^{131}I Albumin wurde nicht effektiv von den nicht-Tumor-Geweben befreit.

RÉSUMÉ

L'élévation de température dans un carcinome 256 de Walker par immersion dans un bain d'eau à 45° C pendant 30 minutes augmente de façon importante la quantité de fibrinogène marqué par ^{131}I injecté par voie intraveineuse qui se localise dans la tumeur. Ce traitement thermique augmente presque de 250 pour cent la dose d'irradiation fournie par ^{131}I, le muscle chauffé dans le voisinage de la tumeur ne fixant pas le fibrinogène ^{131}I. La fixation de la radio-activité coïncide avec des dommages à la tumeur mis en évidence par l'histologie. L'albumine marquée par ^{131}I n'est pas déplacée hors des tissus non tumoraux par ce traitement thermique.

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