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EFFECT OF 5-HYDROXYTRYPTAMINE AND CYPROHEPTADINE ON TUMOUR BLOOD FLOW

Estimation by rate of cooling after microwave diathermy

by

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The radioprotector 5-hydroxytryptamine (5-HT) was found to reduce the oxygen tension in tumours more rapidly than the oxygen tension of bone marrow (CATER, GRIGSON & WATKINSON 1962). After 5-HT, the oxygen tension in tumour does not rise during inhalation of oxygen at atmospheric pressure or even at a pressure of 5 atmospheres absolute (CATER, SCHOENIGER & WATKINSON 1962, 1963). 'The 5-HT effect' was reversed or prevented by the antihistamine, anti-5-HT drug cyproheptadine. A reasonable interpretation of these findings was that 5-HT produced a considerable degree of circulatory stasis in the tumour.

It is important to collect evidence for or against a special relationship of 5-HT to tumour circulation because some observers have suggested that 5-HT assists the implantation and spread of tumour cells (SCOTT, SCHELINE & STONE 1958; SCOTT & STONE 1959; COMVALIUS 1960; COMVALIUS, HOWARD & STRAWITZ 1963) and is involved in the reaction to carcinogens (COUPLAND &

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RILEY 1960; CSABA, HORVATH & MOLD 1961). CRILE (1963) has suggested that the therapeutic effect of heat on tumours can be increased by 5-HT.

A knowledge of tumour blood flow is important in radiotherapy because of the oxygen effect, and in chemotherapy because access of the therapeutic agent to the tumour cells is dependent upon blood flow. In fact, certain therapeutic agents are thought to destroy a tumour by damaging its vessels (e. g. the polysaccharide of SHEAR 1941, SHEAR & PERRAULT 1944). Tumour vessels are known to be abnormal in arrangement and structure: a deeper knowledge of their anatomical peculiarities, and of their function might facilitate new ways of therapeutic attack upon cancer through this 'Achilles heel' of tumour organisation.

During a study of tumour therapy with combined microwave heating and radiation (CATER, SILVER & WATKINSON 1964) a technique was developed for following the tissue temperature of the tumour, even during the microwave heating by the aid of very fine thermocouples. It was argued that after the temperature of a tumour in the leg of an animal had been raised by 3° or 4° C above rectal temperature, the rate of cooling (apart from the effect of metabolism) would depend, partly on cooling through the skin to the ambient, partly on conduction from the heated leg to the cooler body, and partly on cooling of the heated tumour by its blood flow. It was further argued that if injection of 5-HT markedly reduced tumour blood flow, then the rate of cooling of the tumour after its temperature had been raised to 40° or 41° C by microwave heating would be slower after 5-HT; and it should be possible, given the right conditions, to reverse this effect with the anti-histamine, anti-5-HT drug cyproheptadine.

Materials and Methods

All rats had tumours implanted in the left leg. The material included August strain rats with hepatoma 223, and white Wistar rats with Jensen sarcoma or Yoshida carcinoma. A few C+ mice with spontaneous mammary carcinomas were also used.

The animals were anaesthetised with 25 % w/v urethane, 0.6 ml/100 g body weight. The tumour-bearing leg was suspended by an adhesive plaster stocking at the mouth of the wave guide of the 10 cm microwave diathermy apparatus (PERKINS 1955). The animal lay on its side and its body was out of the beam. The rectal temperature was taken by a fine mercury thermometer. Two thermocouples were made of 0.5 mm diameter stainless steel, hypodermic, dental needles by threading insulated constantan wire through the needle and

soldering this at the pointed end of the needle. These thermocouples were inserted into the tumour so that they were parallel with the horizontal axis of the mouth of the wave guide. The other junctions of the thermocouples were insulated and placed in a constant-temperature water bath held at 37° C. The thermocouple currents were measured by two (Cambridge Instrument Co) spot galvanometers shunted by suitable resistances. The thermocouples were calibrated frequently and remained constant. Experiments with water phantoms and living and dead tissues indicated that power was not picked up by the thermocouples direct from the microwave beam, even at considerably greater power outputs than were used in the experiments.

In the experiments of the first series, the temperature of the tumour was recorded, and then the tumour was heated to 40 or 41° C for 1 min, the diathermy turned off and the fall of temperature followed for some minutes. This procedure was repeated and gave normal runs 1 and 2 (N_1, N_2); 5-HT 5 mg/kg (of base) was injected intraperitoneally, and after 10 min the heating-cooling cycle was repeated. Observations were made on another cycle of heating and cooling 20 to 30 min after the injection of 5-HT (S_1 and S_2). Cyproheptadine, 2 mg/kg, was then injected intravenously (occasionally 4 mg/kg intraperitoneally) and after an interval of 10 min two more heating and cooling cycles were observed (C_1 and C_2). The rectal temperature was noted and this was kept as constant as possible. The animal was killed with $CHCl_3$, and the tumour dissected to assess the state of the tumour in which the thermocouples were situated.

In a second series of experiments (because the heating cycle might be damaging the tumour and producing 5-HT and histamine), only one heating-cooling cycle was used for each type of treatment and the temperature was raised only to 40° C. Occasionally a second dose of cyproheptadine was injected followed by an additional cycle of observations. Heating and cooling curves were also observed after death.

In a third series of experiments after one cycle, the effect of noradrenaline 50μg/mg S.C. was studied and then 5-HT and cyproheptadine.

A fourth series of experiments was made on the spontaneous mammary carcinomas in mice. The mice were too small to make it easy to suspend the tumour in the microwave beam, and the tumours were frequently in sites which made this difficult. The results were rather unsatisfactory because several times the thermocouples were found to be in fluid-filled cysts in the tumours.

In a fifth series of experiments, using rats, after two control cycles, cyproheptadine was given and the effect studied before 5-HT was given, in order to ascertain whether cyproheptadine would block the effect of 5-HT.

Theoretical considerations

If we consider 1 cm³ of tissue surrounding the tip of the thermocouple, after being heated to temperature T this will lose heat as a result of cooling by the blood. It will also lose heat by conduction down the limb and through the skin but will gain heat as a result of its metabolic activity.

A strict mathematical analysis of the situation is not possible, but, assuming that the amount of heat gained by the blood flowing through the test volume of tissue is proportional to the total amount of heat gained by this blood during its journey from the arteries to the tissue, then the rate of loss of heat due to cooling by the blood will be equal to

$$K_1 S_b f(T)\ (T-T_r)$$

where S_b equals the specific heat of the blood, $f(T)$ is the rate of flow of the blood in g/cm³/min at temperature T, T_r is rectal temperature and K_1 is a constant of proportionality. The rate of loss of heat down the limb will be approximately equal to $K_2(T-T_r)$ where K_2 is a constant depending on the conductivity of the limb, and the rate of loss of heat through the skin will be equal to $K_3(T-T_a)$ where K_3 is a combined conduction and radiation constant, and T_a is a measure of the mean ambient temperature. The rate of gain of heat will be equal to $m(T)$ where $m(T)$ is the metabolic rate expressed as cal/cm³/min.

Since the rate of cooling of a volume of tissue is equal to its rate of loss of heat divided by its thermal capacity, we can combine the results above into the following differential equation.

$$-\frac{dT}{dt} = \frac{1}{S_c}\left(K_1 S_b f(T)(T-T_r) + K_2(T-T_r) + K_3(T-T_a) - m(T)\right) \qquad (1)$$

where t represents time, and S_c is the thermal capacity of 1 cm³ of tissue. (It should be noted that $\frac{dT}{dt}$ means rate of increase of temperature with time.)

If one assumes that the rate of blood flow and metabolism are independent of temperature and equal to f and m, respectively, (see discussion) eq. (1) may be written more conveniently

$$\frac{dT}{dt} = \alpha - \beta T \qquad (2)$$

$$\text{where } \alpha = (K_1 S_b f T_r + K_2 T_r + K_3 T_a + m)/S_c \qquad (3)$$

$$\text{and } \beta = (K_1 S_b f + K_2 + K_3)/S_c \qquad (4)$$

both being positive constants, and β may be referred to as the temperature-dependent rate of cooling.

Eq. (2) implies that the cooling curves have the form

$$T=A+\exp\ (-\beta t+C) \tag{5}$$

where $A = \alpha/\beta$, and C is a constant depending on where the time origin is taken.

In other words if the correct value for A is chosen, then the logarithm of $(T - A)$ plotted against time is a straight line of slope $-\beta$. The value of β may be estimated for each cooling run by fitting curves of the type represented by eq. (5) to the experimental data. If K_1, K_2, K_3, and m can be assumed to be constant for two cooling runs (on the same rat), then differences between values of β for these two runs will be proportional to differences in blood flow. $A = \alpha/\beta$ may also be expected to change with blood flow, but the value of A is in fact much less sensitive to change in blood flow than the value of β.

The basis of the analysis was that changes in the estimated values of β (which are of course subject to some degree of variation) correspond to changes in blood flow, although no attempt was made to estimate this flow; β was estimated for each cooling run and the results interpreted in terms of blood flow.

Results

If one attempts to estimate β by plotting log $(T—A)$ against time, the slope, $-\beta$, of the line is dependent on a proper choice of the value A. For this reason the best curve of the theoretical type was fitted to the experimental data by the method of least squares. The values of A, β, and C, were so chosen that the sum of the squares of the expression $(T—A—\exp\ (-\beta t + C))$ over all the experimental points (T, t) was as small as possible. A considerable amount of computation is involved in the fitting of this model by least squares. Suitable programmes were therefore developed, and the computation was carried out on the Cambridge University electronic computer EDSAC. The value of the minimized sum of squares was found to agree well with that which could be expected on the basis of the experimental error in reading the instruments. In the majority of cases, A was found to be very near to rectal temperature (usually one or two degrees below).

Post mortem examination occasionally showed that the end of a thermocouple did not lie in the tumour. Observations made on such thermocouples were discarded from the analysis. In cases where both thermocouples lay in tumour, the behaviour of the estimated values of β was remarkably similar. Indeed, this was to be expected, and had there been no correlation between the pair of thermocouples in one tumour, the validity of the results would have been open to grave doubt. Due note of this correlation had to be taken when calculating standard errors of the mean response to the various treatments.

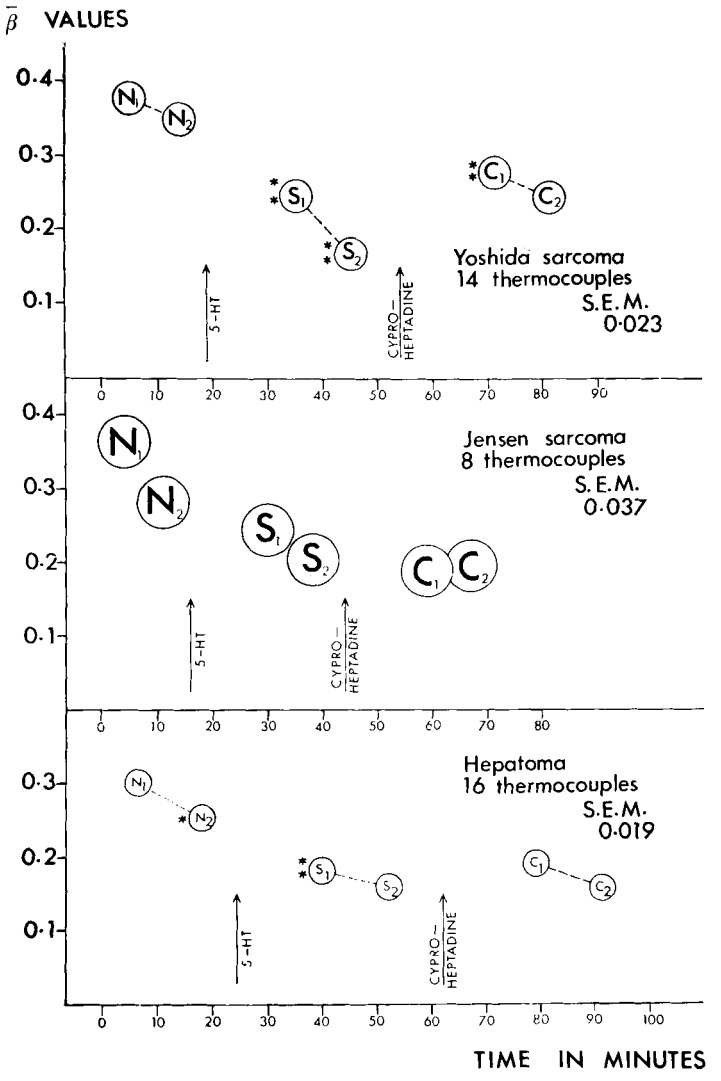

Fig. 1. Experimental series 1. The mean values of β (temperature-dependent rate of cooling) for Yoshida carcinoma, Jensen sarcoma, and hepatoma 223, plotted against time. N_1 and N_2 indicate control heating and cooling, S_1 and S_2 represent values after administration of 5-HT, while C_1 and C_2 are values after cyproheptadine.

Within each experimental series, the results from different types of tumour were analysed separately, and for each cooling cycle the mean value of β together with its standard error were calculated. (The standard error quoted is that to be used when comparing repeated experiments, using the same

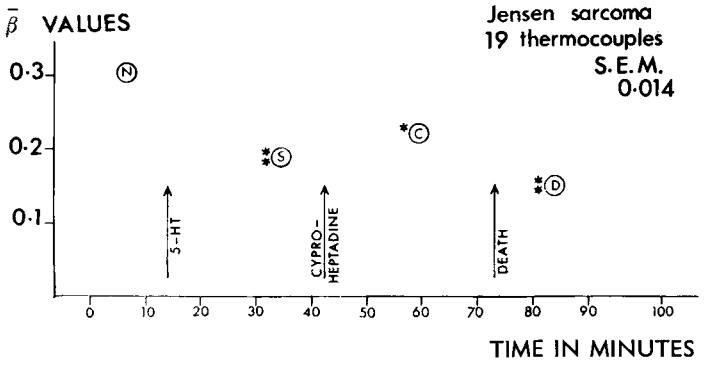

Fig. 2. Experimental series 2. Mean values of β, plotted against time, for Jensen sarcoma. One heating-cooling cycle for each treatment. D indicates cooling after death.

animals and thermocouples, and differs from that to be used when comparing repeated experiments using different animals and thermocouples. The mean for each run has been given the same standard error, which is a pooled estimate based on all the cooling runs. There was no evidence that the standard errors varied significantly within an experimental series.)

The experimental results are presented graphically: the mean value of β, i. e. $\bar{\beta}$, for a particular cooling cycle, is plotted against the mean of the times at which the cooling cycle was carried out. Examination of the results however showed the change in the value of $\bar{\beta}$, from one cooling cycle to the next, did not depend on the time between them. The mean value of β for each cycle is surrounded by a circle of radius equal to the standard error of the mean.

Experimental series 1: two cycles of heating and cooling for each treatment. The results are summarized in Fig. 1, in which the mean values of β (temperature-dependent rate of cooling) for Yoshida carcinoma, Jensen sarcoma and hepatoma 223 are plotted against time. The different heating-cooling cycles are indicated as follows: with no treatment: N_1 and N_2; after 5-HT: S_1 and S_2; after the anti-5-HT drug cyproheptadine: C_1 and C_2. The size of the circle indicates the standard error of the mean in the vertical direction. When the mean values of β are significantly different, at the 5 % level, from the preceding value, assuming the direction of change has been predicted, one asterisk is placed against it. Two asterisks indicate that the difference is significant, at the 5 % level, when no assumptions regarding the expected direction of the change have been made. In Yoshida carcinoma (7 pairs of thermocouples) and hepatoma (7 pairs and 2 single thermocouples) there was a significant fall of $\bar{\beta}$ after injection of 5-HT, and a rise of $\bar{\beta}$ after injection of the anti-

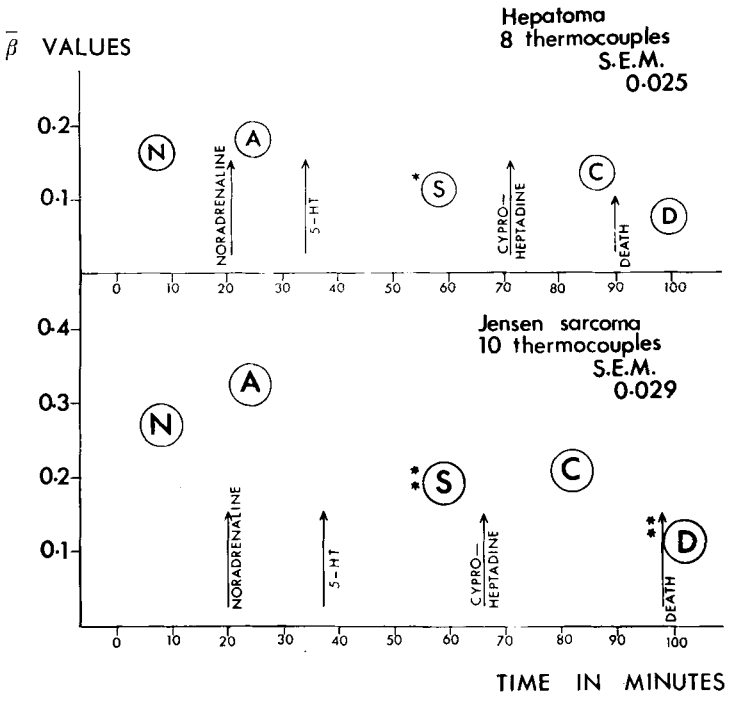

Fig. 3. Experimental series 3. Mean values of β, plotted against time, for hepatoma and Jensen sarcoma. In this series, the control cycle, denoted N, was followed by injection of noradrenaline, denoted with A.

5-HT drug cyproheptadine. This rise was significant in the Yoshida carcinoma. In Jensen sarcoma the values of $\bar{\beta}$ show a general drift downwards, except for C_2, but the number of thermocouples (4 pairs) were too few for the changes to be of statistical significance.

Experimental series 2: one heating-cooling cycle for each treatment. The results in Jensen sarcoma (1 single and 9 pairs of thermocouples) are given in Fig. 2. The mean value of β is significantly lowered after 5-HT and raised after cyproheptadine. After death there is a significant fall in the mean value of β.

Experimental series 3, with noradrenaline as one of the treatments. These experiments were similar to those of series 2, except that, after the normal cycle, noradrenaline 50 μg/kg was injected subcutaneously and immediately afterwards the heating cycle was begun. The results are summarized in Fig. 3 for hepatoma (4 pairs of thermocouples) and Jensen sarcoma (5 pairs of thermocouples).

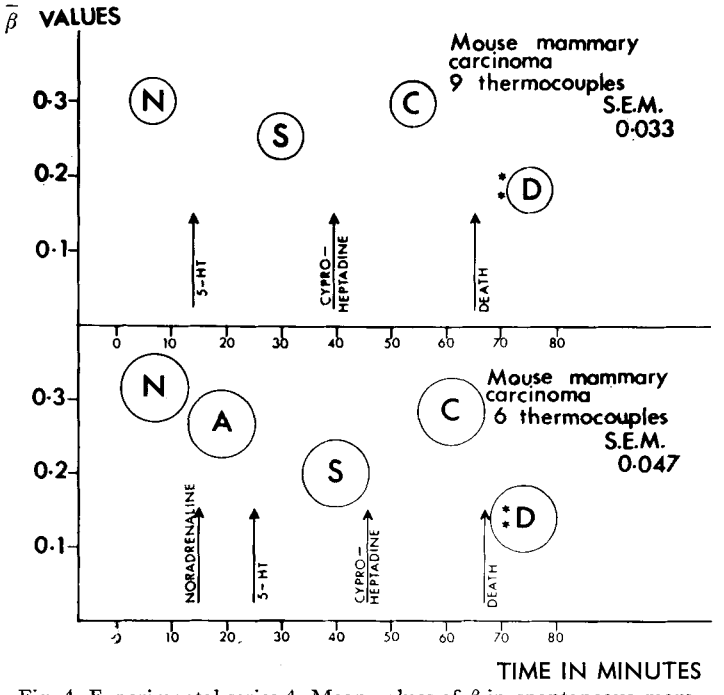

Fig. 4. Experimental series 4. Mean values of β in spontaneous mammary carcinoma in mice. The symbols denote the same as in previous figures.

In both cases, there was a rise of $\bar{\beta}$ after noradrenaline, and a significant fall of $\bar{\beta}$ after 5-HT; then a slight rise of $\bar{\beta}$ after cyproheptadine, and a fall after death. Noradrenaline would be expected to have only a transient effect, and it will be seen that the subsequent changes of $\bar{\beta}$ follow the same pattern of behaviour as in the experimental series 2. The values of $\bar{\beta}$ for the hepatoma were low; this can probably be explained by its very poor circulation.

Experimental series 4, with spontaneous mammary carcinomas in mice. The results are summarized in Fig. 4. One series of mice (one single and 4 pairs of thermocouples) underwent the same sequence of treatment as in experimental series 2. The mean value of β follows the same pattern as previously. Another series of mice (3 pairs of thermocouples) underwent the same sequence of treatments as in experimental series 3. Excepting a fall in $\bar{\beta}$ between the normal and noradrenaline run, the results are the same as previously. The tumours concerned in this experiment were however large, and approximately half the thermocouples were found to be in a cyst, a blood clot or near to the capsule of the tumour.

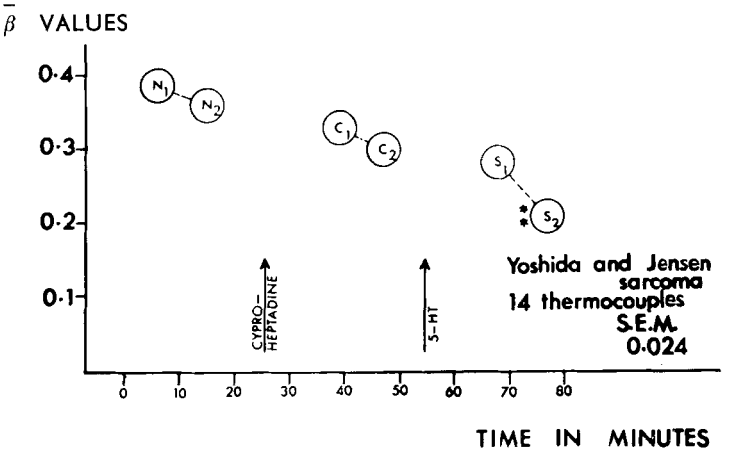

Fig. 5. Experimental series 5. Mean values of β in Yoshida carcinoma and Jensen sarcoma in rats. The cyproheptadine was given before the 5-HT in this series; there is a tendency for $\bar{\beta}$ to fall during the course of the experiments, and the cyproheptadine has not prevented the fall of $\bar{\beta}$ in the second 5-HT cycle, denoted S_2. (The technique used in these early experiments of heating the tumour to 41° C, and having two heating-cooling cycles for each treatment, was not good and may be partly responsible for the progressive failure of tumour blood flow.)

Experimental series 5, in which cyproheptadine was given before 5-hydroxytryptamine. There were two cycles of heating and cooling after each treatment. The results are summarized in Fig. 5. It is seen that $\bar{\beta}$ falls throughout the experiment. The experiments of series 5, like those of series 1, belong to the earlier part of the investigation, in which the temperature of the tumour was raised to 41° C during the heating cycle.

Combined analysis of experiments 1 to 4. Examination of the results of the various experimental series shows that while the direction of the change in $\bar{\beta}$ after a particular treatment is the same, the magnitude of this change is not always so. Therefore, in order to combine results from different experimental series only the direction of changes in $\bar{\beta}$ were taken into account.

Thermocouples in the same tumour behave very similarly. Results were therefore considered in terms of pairs of thermocouples, and only tumours with readings from two thermocouples were included in this combined analysis. Observations were scored as follows. If in the run after a certain treatment both thermocouples showed a decrease in β, i. e. a decrease in blood flow compared with the preceding run, the observation was scored 1 under the heading — —. If both thermocouples showed an increase in β, 1 was

scored under the heading ++. If one thermocouple showed an increase, and one a decrease in β, 1 was scored under +—. The results of the 1st experimental series scored in this way are recorded in Table 1 and in Table 2 the results of the experimental series 2 to 4.

Neglecting pairs of thermocouples for which the difference is of opposite sign, if β is as likely to increase as to decrease between consecutive runs (this would happen for instance if differences in β corresponded to random variation) then one would expect equal numbers of pairs of thermocouples to score (++) or (— —). On this hypothesis, the probability of the observed result, or a more extreme result, can be calculated, and this is the figure given at the foot of each column of the table. A 'more extreme' result is one in which the ratio of pairs of thermocouples scoring (++) to those scoring (— —) is greater if the observed ratio is greater than 1, and smaller if the observed ratio is less than 1.

Examination of the two tables showed that, with exception of the difference between a normal and a noradrenaline cycle, these probabilities are all less than 0.05, the largest being 0.0385, and the results are consequently significant at the 5 % level. One can conclude that β is more likely to go down than to go up between successive treatments except after cyproheptadine, when β shows a highly significant tendency to increase.

Discussion

Critique of the theoretical assumptions

Constancy of blood flow and metabolic rate. It is quite possible that the metabolic rate of the tumour might rise with temperature and that this would induce increased blood flow. If this were the case, then eq. (1) for the rate of cooling would not reduce to eq. (2) with α and β constant. The extremely good fit to the experimental data of the curve given in eq. (5) indicates that for all practical purposes the rate of cooling has the form given by eq. (2). This implies, to a first approximation at least, that variations in the blood flow and metabolism during a cooling run can be neglected.

Constancy of K_1 between different cooling cycles. This has been tacitly assumed in our comparison of β from one cooling cycle to another. This is a fairly reasonable assumption but there is no obvious means of checking it experimentally.

Constancy of K_2 between different cooling cycles. K_2 is a constant depending on the conductivity of the limb, and it would not be expected to change between cooling cycles. However, in approximating the rate of loss of heat up the limb by $K_2 (T—T_r)$, a uniform temperature gradient was assumed. This might not

Table 1

Experimental series 1 Combined results for all tumours with 2 thermocouples, showing the number of of blood flow) be-

Treatment between runs:	Control N_1 and N_2			5-HT N_2 and S_1		
Change in β (blood flow):	−−	−+	++	−−	−+	++
Type of tumour						
Yoshida	5	2	0	5	0	2
Jensen	4	0	0	2	0	2
Hepatoma	6	0	1	5	2	0
Total	15	2	1	12	2	4
Probability of the observed or more extreme ratio of (− −) to (++)	$p = 0.003$			$p = 0.0384$		

be the case, and the temperature gradient might well be affected by the blood flow. This difficulty can be surmounted and still leave eq. (1) and hence eqs (2)—(4) in the same form, if, instead of assuming that K_2 is constant, we assume that K_2 depends on the blood flow f. Differences in β (eq. 4) will still reflect changes in blood flow but not in such a simple manner as previously.

Constancy of K_3 between experimental runs. The same remarks as given under the preceding heading apply also here.

The spatial resolution of the thermocouples was poor. Tests indicated that 3 mm of the tip was the zone of most rapid response but when this zone was in pus or blood clot, β still showed some change with treatment. This was probably because the cooling of such a pool, although it had no blood flow, was dependent on the blood flow of the surrounding tissue. Some conduction would also occur along the thermocouple, so that the rate of cooling would be affected to some extent by the temperature of tissue surrounding the rest of its inserted length.

The *cooling by blood flow* was responsible for only a part of β. If the other part, due to conduction from the hot limb to the body and the heat loss from the skin to the surroundings, was large compared with the cooling by blood flow, then changes in blood flow might be lost in random variations due to inaccuracies in the determination of the value of β. It should be noted that $\bar{\beta}$ was not equal to zero after death (see Figs 2 and 3).

Readings of temperature. Those made during the first half minute after switching the power off the magnetron were discarded from the analysis. This was done

Table 1 *(cont.)*

tumours in which one or both thermocouples measured a decrease or increase in β (indicating change tween succesive runs

S_1 and S_2			Anti-5-HT S_2 and C_1			C_1 and C_2			Number of pairs of thermocouples
— —	—+	++	— —	—+	++	— —	—+	++	
4	3	0	0	1	6	5	2	0	7
3	0	1	3	0	1	1	0	3	4
5	0	2	0	2	5	6	0	1	7
12	3	3	3	3	12	12	2	4	18
$p = 0.0176$			$p = 0.0176$			$p = 0.0384$			

because the precise time when the power was cut off from the beam was uncertain. If there was by chance any pick-up of power out of the beam by the thermocouples, this artefact would have disappeared during the first half minute. Also, during the first half minute, when temperature was changing rapidly, small inaccuracies in timing would be relatively important.

In spite of all these theoretical and experimental difficulties, significant results were obtained, indicating that a reduction in tumour blood flow occurred after the tumour had been heated, and after injection of 5-HT, but that cyproheptadine (an antihistamine, anti-5-HT agent) increased the tumour blood flow.

Difficulties inherent in measuring tumour blood flow

A more serious criticism of the technique used in these experiments for measuring changes in tumour blood flow is that it is indirect and time-consuming. With such criticism the authors would be the first to agree were it not for the fact that all other methods at present available for measuring tumour blood flow are either indirect, time-consuming, inaccurate, or of limited application.

Injection techniques during life will show the anatomy of the vessels but only with great difficulty can information about the physiologic control of tumour vessels be obtained by such methods. Tumour vascular patterns have been extensively investigated by roentgen arteriography, but the technique has been used for diagnosis rather than for the investigation of the functions of tumour vessels. For example, DOS SANTOS (1950) studied bone tumours by

Table 2

Experimental series 2 to 4 Combined results for all tumours with 2 thermocouples showing the number of tumours in which one or both thermocouples measured a decrease or increase in β (indicating change of blood flow) between sucessive runs

Treatment between runs:	Noradrenaline N and A			5-HT A and S			5-HT N and S			Anti 5-HT S and C			No of pairs of thermocouples
Change in β (blood flow):	−−	−+	++	−−	−+	++	−−	−+	++	−−	−+	++	
Exp. series 2 Jensen sarcoma							9	0	0	1	2	6	9
Exp. series 3 Hepatoma	2	1	1	3	1	0				0	2	2	4
Exp. series 3 Jensen sarcoma	1	0	4	4	1	0				1	1	3	5
Exp. series 4 Mammary carcinoma mouse							2	2	0	1	1	2	4
Exp. series 4 Mammary carcinoma mouse	1	0	2	2	1	0				0	0	3	3
Total	4	1	7	9	3	0	11	2	0	3	6	16	25
Probability of the observed or a more extreme ratio of (— —) to (++)	p=0.2744			p = 0.0020			p = 0.0005			p = 0.0022			

radiographic arteriography, using Thorotrast, and BIERMAN, BYRON, KELLY & GRADY (1951) studied hepatic tumours. Intra-arterial injections of fluorescein will outline a superficial vascular neoplasm when the patient is viewed with ultra-violet light (BIERMAN, KELLY, DOD & BYRON, 1950; BELLMAN, 1953). Intravenous injections of lissamine green have been used in rats and mice by GOLDACRE & SYLVÉN (1959), and in mice, rats, dogs and cats by OWEN (1960). GOLDACRE & SYLVÉN (1962) have reviewed the results obtained by injection techniques. An injection technique using radioisotopes can be used for a study of tumour blood flow (SAPIRSTEIN 1958; CATALAND, COHEN & SAPIRSTEIN 1962) but this is neither simple and direct, nor of wide application.

Transparent chamber techniques have been used to study the anatomy,

development and physiologic responses of the blood vessels of transplanted tumours (IDE, BAKER & WARREN 1939; ALGIRE & CHALKLEY 1945; ALGIRE & LEGALLAIS 1949; ALGIRE, CHALKLEY & LEGALLAIS 1951; NATADZE 1959). KERN & ZANDER (1959) watched the development of vessels in tumours induced by methylcholanthrene, in the transparent tissues of the ear of the mouse. Again, the measurement of blood flow is indirect and the techniques are of restricted application.

The same may be said of estimations of blood flow by observing changes of skin temperature in superficial tumours (BIERMAN, GILFILLAN, KELLY, KUZMA & NOBLE 1952; NATADZE 1959).

The oxygen cathode technique has also been used to get an indirect estimate of changes in tumour blood flow. URBACH & NOEL (1958) explained the different response of squamous cell carcinoma and malignant melanoma of the skin to a test period of oxygen inhalation in terms of the known differences in the vessels of these two types of tumour. CATER, GRIGSON & WATKINSON (1962) and CATER, SCHOENIGER & WATKINSON (1962, 1963) used the oxygen inhalation test during tumour oxygen tension measurements as an indication that tumour blood flow was reduced after the injection of 5-HT. CALIGARA & ROOTH (1961) have given formulas for calculating capillary blood flow from the rate of change of arterial P_{O_2}, compared with tissue P_{O_2}, when the subject breathes oxygen, and KUMLIN, ERTAMA, MATTILA & HALONEN (1962) investigated the blood flow in muscles by noting the time when the muscle P_{O_2} rose, following release of a cuff which had occluded the circulation in the leg for 8 minutes. KOLSTAD (1963) has measured tissue P_{O_2}, and capillary blood P_{O_2}, in carcinoma of the cervix, and correlated this with the intercapillary distance measured by colpomicroscopy.

The heated thermocouple principle has been used for measuring changes of blood flow in tissue, and the difficulties have been reviewed by BILL (1962), but absolute measurements of blood flow can only be obtained under special conditions which would be difficult or impossible to attain in tumours.

Finally, direct measurements of blood flow through transplanted tumours have been made by GULLINO & GRANTHAM (1961, 1962). They injected the tumour into the ovary or kidney of rats and wrapped the organ in a paraffin envelope so that the tumour had a single vascular pedicle. Their technique enabled them to measure the tumour venous outflow directly and to investigate the effects of temperature, adrenaline and acetyl β methylcholine upon the tumour blood flow. This valuable technique can only be used with transplantable tumours in experimental animals and is open to the criticism that a spontaneous tumour often develops an extensive collateral circulation from adhesions with adjacent organs and structures.

Most of the recent evidence is against the popular concept that there is considerable blood flow through tumours. GULLINO & GRANTHAM (1961b), by direct measurement of the venous outflow of tumours transplanted into ovary or kidney found an average blood supply of 0.14 ± 0.01 ml per hour per mg N_2, compared with 7.18 ± 0.12 for normal ovaries and 6.9 ± 0.27 for control kidneys. The oxygen tension in tumours is usually low and responds rather slowly to inhalation of oxygen (URBACH & NOEL 1958; CATER & SILVER 1960; CATER, SCHOENIGER & WATKINSON 1962, 1963). The oxygen tension in tumours is much reduced by lowering the blood pressure (CATER, GRIGSON & WATKINSON 1962). KOLSTAD (1963) has correlated low tissue oxygen tension, and slow response to oxygen inhalation, in carcinoma of the cervix with wide intercapillary distance and tortuous vessels detected by colpomicroscopy. The dye injection techniques of GOLDACRE & SYLVÉN (1962), and OWEN (1960) show absence of blood flow in the central portions of many tumours. CATALAND, COHEN & SAPIRSTEIN (1962), using a radioisotope technique, confirmed GULLINO & GRANTHAM's findings of a low blood flow through tumours. On histologic examination, many tumours show areas of necrosis (THOMLINSON & GRAY 1955), and this is in favour of inadequate vascularisation and possibly a low blood flow.

In favour of a large blood flow through tumours may be cited the increased oxygen content of venous blood draining neoplasms (BIERMAN, KELLY & SINGER 1952), although this may be explained by the presence of arterio-venous shunts. The vigorous capillary bleeding, which occurs when a tumour is incised during operation, is in favour of free blood supply, but it is noteworthy that granulation tissue also shows this troublesome type of bleeding which may be explained by a failure of the immature and imperfectly formed vessels to react to trauma by vasospasm.

These special features of tumour blood flow may play an important part in the tumour/host relationship. Tumours are often thought to grow very rapidly, but even those which grow fastest grow in fact more slowly than the human foetus, and in many tumours the rate of growth is very slow by comparison with liver regeneration or epithelial cell replacement in gut and skin. A slow growth rate, in spite of active cell division, can be explained by a high death rate in tumour cells. Some may die from nuclear abnormalities, others because of an inadequate blood supply. In this respect the presence of many dilated capillaries does not necessarily mean a rapid blood flow, for the blood in a dilated capillary may be flowing very slowly or not at all. It is well known that many malignant tumours show abundant evidence of necrosis, but the important part played by toxic absorption from this dead material, in the production of cancer cachexia, does not seem to be sufficiently appreciated.

Toxic absorption from dead cells also sets a limit to the rate at which it is safe to destroy a large tumour. In fact, one of the best features of radiation is that it kills cells slowly, and an important feature of surgical excision of tumours is that it removes the dead tissue from the body.

The tumour circulation is a weak link in the organisation of the tumour and it may be possible to exploit this weakness in cancer therapy. The bacterial polysaccharide, purified by SHEAR and his associates, would appear to work by damaging the vessels and causing haemorrhages in tumour. (It is noteworthy that it is much more toxic to the tumour-bearing than to the normal animal (SHEAR 1941, SHEAR & PERRAULT 1944). Unfortunately, the peripheral portions of the tumours often survived and the tumours regrew. The special absorption of certain drugs in tumours may be due to slow blood flow through tumours or may be increased by this. The action of heat may be greater on tumours because of poor blood flow (CATER, SILVER & WATKINSON 1964). This action of heat is increased by 5-HT (CRILE 1963). In general, agents which cause low blood pressure, or damage endothelium, or increase the inflammatory reaction, will tend to retard the blood flow and tend to cause haemorrhages or thromboses and necrosis in tumours. Deliberate attempts to kill anoxic tumour cells by such methods have obvious dangers with large tumours, but with more knowledge of the physiologic aspects of tumour vascularity it might be possible to exploit these methods in radiotherapy. The evidence presented in this paper that heat or 5-HT can reduce tumour blood flow, and that the effect of the latter can be reversed by the anti-5-HT, antihistamine agent cyproheptadine, may be useful in this respect.

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SUMMARY

Cooling curves of transplanted rat tumours and spontaneous mammary carcinoma in mice, obtained after heating the tumours by 10 cm microwave diathermy, were subjected to mathematical and computer analysis. A formula was devised which accurately fitted the experimental results. Changes in the temperature-dependent rate of cooling were interpreted in terms of changes in tumour blood flow. Heating of the tumour, and injection of 5-hydroxytryptamine, reduced the tumour blood flow and the anti-5HT agent cyproheptadine reversed the effect of 5-HT.

ZUSAMMENFASSUNG

Abkühlungskurven von transplantierten Rattentumoren und spontanen Mammakarzinomen bei Mäusen, nach Erhitzung der Tumore mit 10 cm Kurzwellenbestrahlung, wurden durch Komputationsanalyse matematisch beurteilt. Eine den experimentellen Ergebnissen gut entsprechende Formel wurde erreicht. Die Veränderungen der Temperatur-Abhängigkeit der Abkühlungsgeschwindigkeit wurden als Veränderungen der Tumor-Blutzirkulation aufgefasst. Erhitzung der Tumoren und Injektion von 5-Hydroxytryptamin verursachte eine Reduktion der Blutzirkulation der Tumoren; Cyproheptadin (das Anti-5-HT-Agens) dagegen kehrte die Wirkung von 5-HT um.

RÉSUMÉ

Les courbes de refroidissement de tumeurs transplantées sur des rats et de cancer du sein spontané de la souris, obtenues après échauffement des tumeurs par diathermie à micro-ondes de 10 cm, ont été analysées mathématiquement et par ordinateur. On a établi une formule qui concorde exactement avec les résultats expérimentaux. Les modifications de la vitesse de refroidissement dépendant de la température ont été interprétées comme liées aux modifications du débit sanguin tumoral. L'échauffement de la tumeur et l'injection de 5-hydroxytryptamine réduisent le débit sanguin tumoral et le cyproheptadine, agent anti-5-HT, inverse l'effet du 5-HT.

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