THE DISSEMINATION OF CANCER CELLS DURING OPERATIVE PROCEDURES

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by

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SINCE THE DAYS of John Hunter, there have been great advances in the methods of research, due to the technical progress made by modern science. In Hunter's day, the main tool of the research worker was his own ability to observe and record what could be seen with the naked eye and with the limited microscopical instruments then at his disposal. Indeed, it is to such men that science owes its heritage, for many of their observations have been confirmed by the elaborate technical methods now in use. Perhaps one of the most striking differences in such work today may be seen in cancer research, where the vastly increased knowledge of its biochemical and cytological background has resulted in the development of the research team, composed of experts in their own fields. It was my privilege to work with such a team, who have been investigating the dissemination of cancer, with a special interest in the effect of surgical treatment on dissemination and the possible methods of increasing the value of operative techniques with the use of anti-cancer drugs.

With the great advance in modern surgery, the extent of surgical resection in carcinoma has been carried to its anatomical limit. Although in some fields the results of radical surgery have been encouraging, it is clear to most surgeons that, such is the nature of neoplastic dissemination, it is impossible to eliminate the disease from the whole body in a large number of patients by radical surgery.

In recent years, renewed interest has been taken in the mechanism by which dissemination of cancer occurs following operative procedures. The commonest ways in which the spread of malignant cells can occur during surgery are through implantation of the neoplastic cell at the site of operation and through the entrance of cancer cells as emboli into the blood stream.

Implantation of malignant cells

Ryall in 1908 drew attention to the dangers of dissemination of cancer by implantation and used the term " cancer infection ", which describes well the danger of contamination of operative wounds by malignant cells.

The hazards of the free cancer cell were apparent to such great masters of surgery as Sampson Handley and Sir Ernest Miles, and it was these men who first introduced the concept of adjuvant chemotherapy during surgical operations; they both used mercury perchloride to wash the wounds following resection—Miles following his abdomino-perineal resection of the rectum, and Sampson Handley following radical mastectomy.

In a recent survey of cases operated upon for carcinoma of the breast, rectum and colon at St. Bartholomew's Hospital, London, between 1947 and 1952, and subsequently followed up for 5 years, the incidence of local recurrence was found to be 10 per cent. in carcinoma of the breast (Whittle *et al.*, 1960), 10 per cent. in rectum and rectosigmoid and 9 per cent. in carcinoma of the colon (Griffiths *et al.*, 1960) (Table I). In these cases, curative operations were attempted at the time of surgery. This indicates the hazard of local dissemination, although at the time of operation there is no visible evidence of its presence.

TABLE I

LOCAL RECURRENCES AT ST. BARTHOLOMEW'S HOSPITAL (1948–1952)

Five Year Follow-up

Site	*No. of Cases*	*No. of Local Recurrences*	
Breast	472	45	10%
Colon	112	10	9%
Rectum	175	17	10%

One of the major advances in operative technique in carcinoma of the colon and rectum is recognition of possible implantation of malignant cells into the anastomosis following a resection. Naunton Morgan and Lloyd-Davies (1950), in this country, drew attention to the possible cause of local recurrence following anterior resection of the rectum being due to implantation of malignant cells in the anastomosis and described a technique using mercury perchloride 1 in 500 to swab out the proximal colon and wash out the rectal stump from below before the anastomosis is performed. In America, Cole (1952) drew attention to a local recurrence rate of 16 per cent. in 55 consecutive patients having resection for carcinoma of the colon and proximal rectum over a five-year period at the Illinois Research Hospital; of these, 10.9 per cent. were recurrences by implantation at the site of anastomosis. Cole suggested that intraluminar dissemination of the growth during surgical procedures could be prevented by placing ties above and below the tumour in the colon before any manipulation was performed, the clamps being placed beyond the ties during the resection. McGrew *et al.* (1954) demonstrated by cytological technique that manipulation of the growth disseminates free cancer

cells along the lumen of the bowel and that the procedure described by Cole was effective in preventing contamination of the distal and proximal ends of the colon by malignant cells at the site of the anastomosis. Cole also advocates washing the proximal and distal loops with distilled water before performing the anastomosis. The effectiveness of these principles can be gauged by the successful diminution of local recurrences that has occurred in the operation of anterior resection for carcinoma of the rectum. Goligher *et al.* (1951) reported 23 local recurrences in 162 cases of resection of the rectum with anastomosis or Hartmann's procedure; in half of these recurrences there was strong evidence that implantation had occurred at the site of anastomosis during the operation. Morgan (1958), in a personal series of 136 cases of anterior resections in which the rectum had been washed out with 1 in 500 mercury perchloride, reported a local recurrence rate of only 1.5 per cent.

TABLE II

LOCAL RECURRENCES IN CARCINOMA OF THE RECTUM

Number of Cases	175	
Number of Recurrences	17	10%
Number of Anterior Resections	23	
Number of Recurrences	1	4%
Number of Excisions	152	
Number of Recurrences	16	11%
Extra Rectal Spread found at Operation	8	5%

In 112 cases of carcinoma of the colon operated upon at St. Bartholomew's Hospital between 1947 and 1952 (Table II) 10 developed local recurrences within five years. Of these, three were found to be at the site of the anastomosis. Over the same period, 23 cases of carcinoma of the rectum were treated by anterior resection and one (4 per cent.) developed local recurrence at the anastomosis. In 13 of the 23 anterior resections, the distal rectum was washed out with 1 in 500 mercury perchloride, but the case in which the local recurrence occurred had not been treated in this way.

Excision of the rectum was performed in 152 patients and local perineal recurrences occurred in 16. Of these, eight were shown to have perirectal spread at the time of operation and seven had no demonstrable extra-rectal involvement. In one case of local recurrence the rectum was perforated during the operation with consequent contamination of the perineum by faeces and malignant cells. At the period during which these excisions were performed no " anti-cancer cell " measures were routinely used in the perineal wound.

It is often difficult to separate implantation from local recurrence, but where adequate normal tissue has been removed at operation, as proved by histological examination of the specimen, it can be assumed that a recurrence at the site of operation is due to implantation of malignant cells. This is even more certain when the recurrence occurs at a suture-line or in the scar.

Circulating cancer cells

The dissemination of the cancer cell through venous channels has for a long time been postulated and venous invasion by growth often demonstrated histologically. Ashworth in 1896 reported cells in the blood after death which resembled the cells seen at post-mortem in a patient dying of a malignant skin tumour, and Ward in 1913 reported numerous large cells in the peripheral blood of a patient a few hours before dying of a carcinoma of the stomach. Pool and Dunlop (1934) examined the peripheral blood of 40 patients having malignant disease, and although they describe atypical cells in 17 of the cases they did not positively identify these cells as cancer cells. Isolation of the circulating cancer cell in the general circulation of a live patient with malignant disease was first performed by Engell (1955). This was followed by Roberts and his co-workers (1958), who developed a technique for the separation of the elements of blood, which allows the use of the Papanicalaou stain to identify malignant cells. Long *et al.* (1959) have further simplified their techniques and have in all, using both methods, examined the blood of 506 subjects for circulating cells. Of these, 178 were investigated by Long's method and 328 by Roberts'.

In 328 patients investigated using the original technique described by Roberts *et al.*, and reported by Long *et al.* (1960), the patients were divided into " curable " and " incurable ". This is an arbitrary classification based on a surgical assessment at the time of operation: if the patient's tumour was completely removed he was classified as " curable "; if growth was known to have been left, including distant metastasis, the classification was " incurable ". In the curable group—representing all types of carcinoma—20 per cent. had positive cancer cells demonstrable in the blood obtained from the anti-cubital vein, while 29 per cent. of the incurables showed cells. In reporting the results of the simplified isolation technique, Long *et al.* (1960) gives the percentage of " curable " with malignant cells in peripheral venous blood as 25 per cent. and " incurable " 39 per cent., in 178 patients examined. In 69 patients in which blood was obtained from veins draining the site of the tumour, 28 per cent. were found to have circulating malignant cells in the " curable " cases and 37 per cent. in those classified as " incurable ".

It can be assumed that the percentage of patients who have in fact circulating cells is much higher than those reported above, as the technique

for their isolation is difficult and there is a possible loss of some cells during the separation technique. The significance to be attached to the presence of the circulating cell is not yet known. Engell recently reported the follow-up for five to nine years of his original cases and concluded that there was no overall difference in the survival figures of patients in whom cancer cells were found and those in whom they were not demonstrated.

There is in the body a resistance to the implantation of the malignant cell, which has been termed " host resistance " by the older pathologist. An interesting observation has been made by Roberts and Long that in all the cases of carcinoma of the gastrointestinal tract, i.e. drained by the portal vein into the liver, positive peripheral circulating cancer cells have only been demonstrated in cases where there has been spread of the tumour outside the organ of origin, i.e. there would be drainage into the systemic circulation as well. It has been previously shown by Cole *et al.* (1954) and later by Fisher and Turnbull (1955) that, in carcinoma of the colon, blood from veins draining the site of the tumour often contains cancer cells (Fisher and Turnbull report 31.4 per cent.). This implies that the liver in some ways plays a part in destroying or removing the cell from the portal circulation before it enters the systemic vein; the fate of the cell then depends on whether it can implant and grow or be destroyed.

Circulating cancer cells during surgery

The possibility of dissemination of cancer cells via the bloodstream during operative procedures has been considered for many years. Long *et al.* (1960) investigated the response of 57 patients with malignant disease to operative procedures by examining their blood before, during, and after operations involving resection of the neoplasm. They describe three responses to operative procedures which may occur. In the first group of patients, the pre-operative blood samples taken showed no circulating cancer cells, but during the operation cancer cells were recovered from the circulation, only to disappear after removal of the tumour. This occurred in eight (14 per cent.) of the 57 patients. The second group of five patients (9 per cent.) were those in whom pre-operatively malignant cells had been detected in the circulation; at operation, there was an increase in the number of cells in each sample, but following removal of the neoplasm no circulating cells could be obtained. Three patients (5 per cent.) in the third group showed cancer cells in the blood before and during the operation, and a subsequent fall in the number of cells found immediately post-operatively, which again increased during the first and second post-operative days. In this last group the patients were classed as " incurable ", having non-resectable or inadequately removed disease. In the remaining 41 (72 per cent.) patients examined, no cancer cells were found in the blood at any time. This does not mean that they were not present in the circulation, as the techniques for detection are still limited in their accuracy.

It has therefore been well substantiated that cancer cells appear in the general circulation in a number of patients with neoplastic disease, and that surgical procedures and manipulation of the primary growth tend to produce showers of cells in the blood stream. What of the fate of the cancer cell? Earlier pathologists concluded that there was a resistance in the body to vascular dissemination. Goldman (1897) concluded that since the invasion of veins was a common finding in post-mortem, but yet a number of organs remained free from metastasis, there existed in the body some " protecting substance " giving a resistance to the cancer cell. The nature of this mechanism has eluded the research workers in this field for over sixty years, but the conclusions of Goldman have recently been re-amplified by Engell (1959), as stated previously, who followed up cases from whose blood he had isolated cancer cells five to nine years previously and found no difference in the survival rate compared with similar groups of patients in which he had failed to demonstrate circulating cancer cells. But cancer cells have been known to lie dormant for many years, as pointed out by Hadfield (1954), who maintains that secondaries which develop two or more years after adequate excision of the primary growth cannot be due to malignant emboli or circulating cancer cells. The period of dormancy can be from 6 to 20 years, as demonstrated by Gordon Taylor (1959), who quotes from his own long experience a personal case of one recurrence which occurred 17 years after radical mastectomy. It therefore cannot be assumed that circulating cancer cells are all destroyed, but it must be assumed that they are all potentially capable of forming secondaries. Any surgical procedure which will increase the number of circulating cells is therefore increasing the danger of dissemination and cannot be ignored.

The fate of the circulating cancer cell

The fate of the circulating cancer cell has been investigated by many. Schmidt (1903) studied the lungs and pulmonary vessels of 41 cases of carcinoma of the abdominal viscera and found malignant nodules in 15 cases. He stressed that few of these gave rise to pulmonary secondaries since the fibrous reaction, which occurs in the vessel wall, results in the death of the neoplastic cell. He also drew attention to the importance of organization of the thrombus in providing new vascular connections for the developing metastasis. Levin and Sittenfield (1911) showed that the number of lung metastases found after intravenous injection of transmittable tumours in rats and mice was low, although the tumour grew satisfactorily following subcutaneous injection. They confirmed Schmidt's finding that many tumour emboli had been destroyed in the pulmonary vessels. Takahashi (1915) emphasized the effectiveness of the vessel endothelium as a barrier against extravascular spread and demonstrated, using mouse tumours, that before the cells disappeared from the blood, they became vacuolated and surrounded by leucocytes. The

histogenesis of metastasis produced by the T150 tumour in C57 black mice has been well described by Baserga *et al.* (1955), who showed that the cancer cells form small emboli of five to six cells, which adhere to the wall of the pulmonary arterioles. They postulate that there is an intravascular growth of the cells before extravascular spread occurs.

In all previous reports on intravascular spread, great emphasis has been laid on the occurrence and importance of the tumour emboli, but the fate of the single circulating cell has not been investigated. Experiments have been performed to demonstrate this, using intravenous injection of Walker 256 tumour in rats. The Walker tumour was made into a sterile suspension of single cells by passing through a cyto-sieve, as described by Snell (1953). The number of cells in the suspension was counted in a

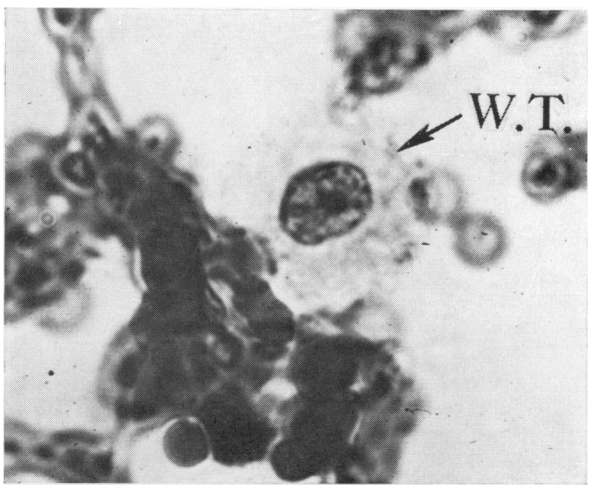


Fig. 1. Walker 256 carcinosarcoma cell (W.T.) isolated from rat's blood using Long's modified streptolysin technique 60 minutes following intravenous injection of tumour suspension.

counting chamber and diluted until 1 c.c. contained 100,000 viable cells. Adult female rats were then taken, the back leg shaved and 1 c.c. of the suspension injected into the saphenous vein, care being taken to prevent any extravascular spill.

In one group of rats, cardiac puncture was performed to obtain blood from the left ventricle at 5, 10, 15 minutes and then every 15 minutes after injection for two-and-a-half hours. One c.c. of blood was obtained at each cardiac puncture, but rarely did one rat survive more than three punctures. It was therefore necessary for a number of rats to be injected with the same suspension simultaneously. The blood samples were then treated by the method described by Long *et al.* (1959) and examined for

circulating Walker tumour. (One modification of the technique as described for human blood is that 9 c.c. of streptolysin must be used for 1 c.c. of rat's blood to produce haemolysis of the red cells.) In this way it was possible to trace the number of circulating cells over the period of the experiment. During the earlier samples up to one hour, only a few circulating cells could be found, but following this the number increased and was maximal at 90 minutes. An examination of blood taken 24 hours after intravenous injection revealed no circulating tumour cells (Fig. 1).

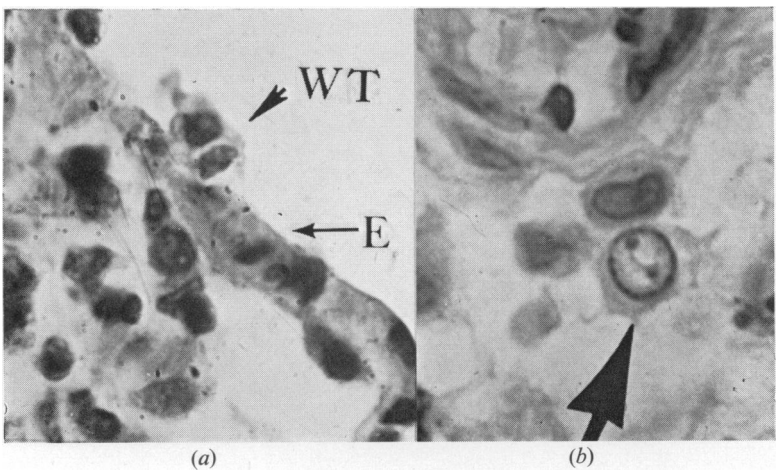
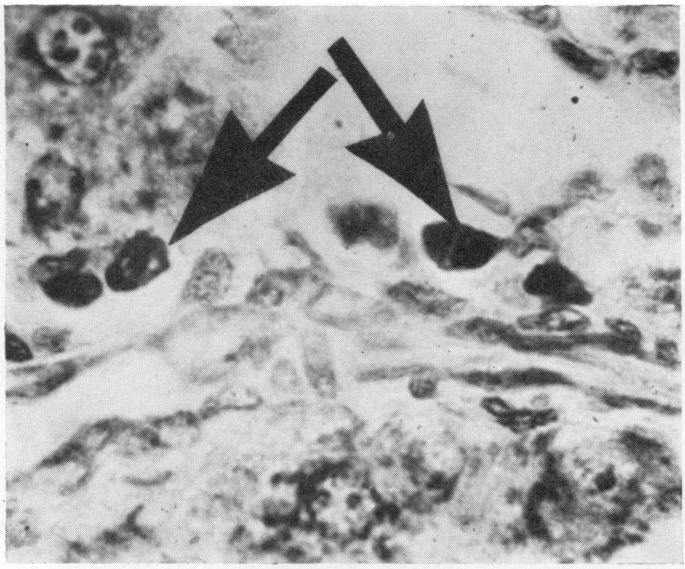


Fig. 2. Histological sections from rats following intravenous or intraportal injection of cell suspension from Walker 256 carcinosarcoma. (*a*) Section of lung taken immediately following intravenous injection of cell suspension, showing the attachment of the Walker cell (W.T.) to the endothelium of the pulmonary vessel. (*b*) Section of lung five minutes following intravenous injection, showing Walker tumour cell having become extra-vascular. (*c*) (*see page* 22) Section of liver five minutes following intraportal injection of tumour suspension, showing extra-vascular tumour cells. (*d*) Section of lung 10 days following intravenous injection, showing development of metastasis in the interstitial tissue of the alveolar wall. (*e*) (*see page* 24) Section of liver from same animal as (*d*) showing extra-vascular metastasis (M) around a hepatic vessel (V). (*f*) Section of lung 30 days after intravenous injection of Walker tumour, showing a large metastasis having developed around a pulmonary vessel (V).

The second group of rats was injected as described with the same number of Walker tumour cells intravenously, but they were sacrificed at set times and their lungs and liver removed, placed immediately in a fixative and then submitted for serial histological section. In this way the fate of the cells in the tissues could be followed by sections taken at intervals varying from five minutes to five weeks following after injection. A third group of anaesthetized rats was injected intraportally following coeliotomy, the lungs and liver being removed after 5, 10, 15 and 30 minutes and immediately fixed and submitted for serial histological section.



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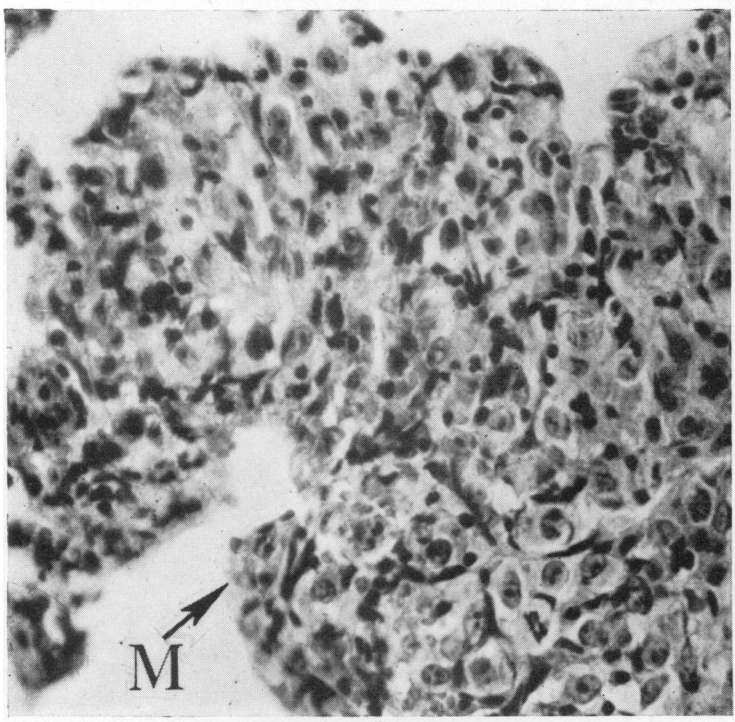


(*d*)

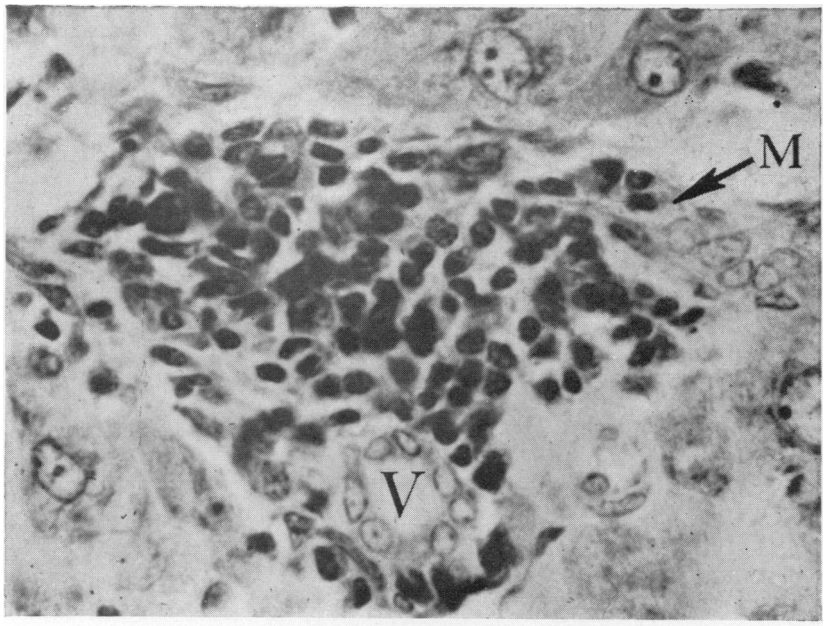
From the examination of the histological sections a number of interesting findings were noted. It was demonstrated that the tumour cells attached themselves to the endothelium of the walls of the arterioles in the lung and soon passed through to become extravascular, being identified in the interstitial tissues within 5 minutes. Examination of sections of the liver in animals having had intravenous injection of the tumour suspension also revealed extravascular tumour cells, indicating that the cells had passed through the pulmonary capillary bed, entered the portal system and then passed into the liver tissue. In some sections of the lungs, tumour cells could be seen passing through the capillaries and arterioles. It is difficult to determine the exact difference between the arteriolar and capillary plexuses in the lung tissue, except in the alveoli where the capillaries are very thin. Following intraportal injection of the tumour suspension, cells could be seen in the liver vessels but the majority were extravascular, lying in the centre of the liver lobules. Sections of lung of the same animal show the cells having passed into the interstitial tissue of the lungs (Fig. 2).

If the rats are injected intravenously with Walker tumour suspension and allowed to live for one month and then sacrificed, multiple tumour metastases can be seen in the lungs. The liver, however, appears normal on naked eye examination, but tumour cells can be seen microscopically.

Histological sections of both lungs and liver taken from animals sacrificed between six and 14 days following injection showed that the development of tumour in the lung is much more advanced and the secondaries are bigger than in the liver. This is contrary to what was found by Lucke *et al.* (1952), who injected V2 carcinoma cells into rabbits intraportally and intravenously simultaneously, and found that the liver developed tumour five times larger than the lung. In the case of intraportal injection of Walker tumour in rats, the lungs rarely show metastases although, as shown previously, malignant cells can be demonstrated in the lung tissue.

It must be assumed that the cancer cell has, in some way not yet understood, lost some of its virulence during its passage through the pulmonary capillary plexus, although the circulating cancer cells which have been demonstrated in the blood 90 minutes following intravenous injection are still capable of producing tumours. This has been proved by subcutaneous inoculation of blood from one of these rats into a control, a tumour being produced at the inoculation site in three weeks.

Not all the cancer cells which circulate in the blood stream produce metastases (Fig. 3). Some are undoubtedly destroyed in the circulation by the elements of blood, as described by Takahashi, although Warren and Gates (1936) disagree with this and have shown that blood does not inactivate tumour suspension. The larger emboli of tumour cells will get trapped in the capillary system of either the lungs in the systemic circu-



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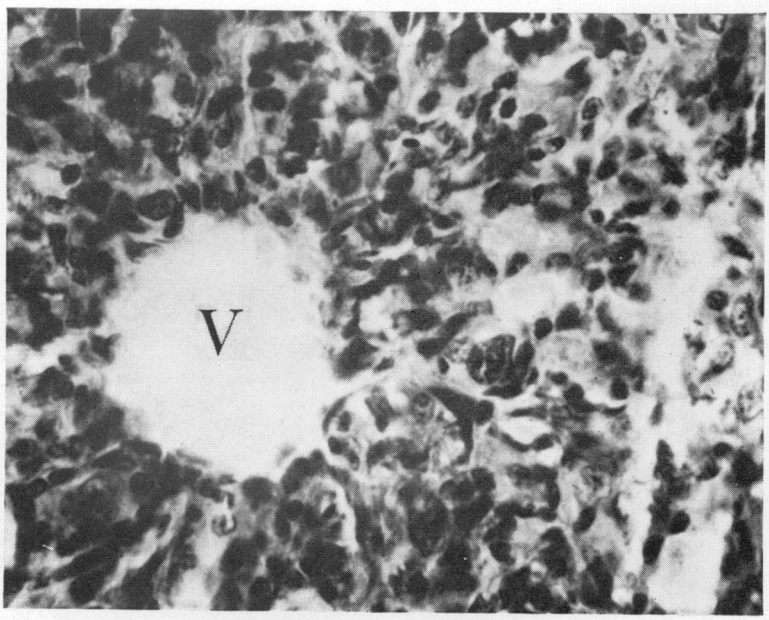

(*f*)

lation or the liver in the portal system; these either grow or are destroyed and form hyaline thrombi. Other cells become attached to the endothelial lining of the capillaries or arterioles and then either pass through the vessel wall and become extravascular, or become free again and rejoin the circulation. Of the cells that become extravascular, some will form secondary metastases, the rate of growth depending on the nutrition available and the host resistance; others are undoubtedly prevented from dividing and die, and are dealt with by the endothelial system.

Many unknown factors are involved in the fate of the circulating cancer cell, one of the most important being its own virulence. This may be affected by the antigen-antibody reaction between the cell and its host,

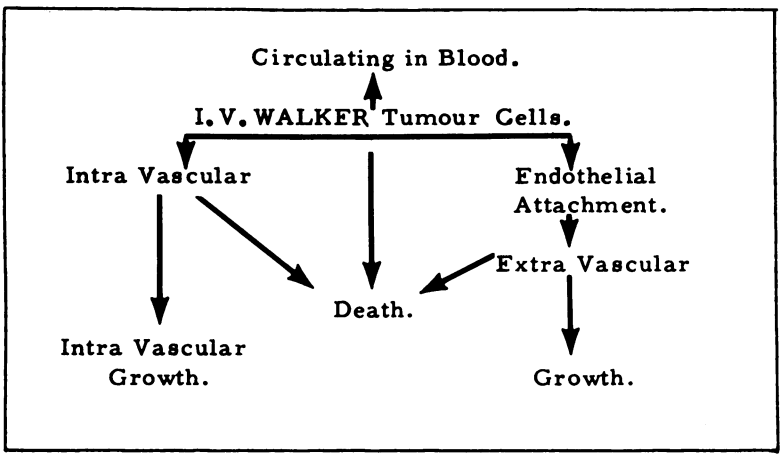

Fig. 3. Diagram illustrating the fate of cells injected into the circulation.

and the factors which ultimately decide whether or not a tumour cell becomes established may well be governed by this reaction, of which very little is known as yet.

Host resistance

In recent years, the energies of the pathologists, cytologists and biochemists have been directed towards investigation of the nature and causation of the change in the normal cell which produces malignancy. The ultimate treatment and cure of cancer may well be found in the laboratory, but for those who deal with patients who have the disease there is another important consideration. It is the effect of the host on the neoplastic cell, and I feel this side of the " cancer " problem has been neglected for many years. Sir Gordon Gordon Taylor (1959), in his Mitchell Banks lecture, drew attention to the variation which exists in the resistance of patients to disease and the way in which carcinoma cells can be dormant for many

years and then be brought back to activity by some disturbance of the patient's general health, such as operation or trauma, causing a general reaction which we have come to look upon as a " stress " reaction.

In the following experiments, I will endeavour to present some basic physiological responses which affect the host resistance and which may have a bearing on the surgical treatment of the patient suffering from neoplastic disease.

The influence of surgical procedures on the increased susceptibility of animals to the implantation of malignant cells has been demonstrated by Buinauskas *et al.* (1958), Lewis *et al.* (1958) and Fisher *et al.* (1959a). They have demonstrated that surgical procedures produce an increased susceptibility in the rat to the inoculation of transmittable Walker tumour.

In repeating these experiments in a modified form, the effect of surgical operation on the development of pulmonary metastasis following intravenous injection of tumour cells has been demonstrated. Two groups of mature female rats were taken and kept under identical environmental conditions. One group was then anaesthetized and a laparotomy performed, the intestines being left outside the peritoneal cavity for 30 minutes and traumatized by rough handling before being replaced. During the period of the experiment, 100,000 fresh Walker tumour cells were injected intravenously into the saphenous vein of both the operated group and a control group of anaesthetized rats which had not been subjected to surgical trauma. Both groups were sacrificed after 21 days and their lungs removed and examined macroscopically for secondaries. In the control group, metastatic nodules could only be seen in 15 per cent.; in the operated group, metastases were visible in 65 per cent. of the specimens (Table III). Histological section of the lungs in both groups showed evidence of growing cancer cells, but the rate of growth in the operated rats was more rapid and hence the higher percentage of visible macroscopic secondaries.

TABLE III

EFFECT OF OPERATIVE STRESS ON METASTASIS IN LUNG

Groups	*No. of Rats*	*% Macroscopic Secondaries*
Operative Stress	26	65
Control	26	15

I.V. 100,000 Walker 256 Tumour Cells.
Rats sacrificed after 21 days.

In further investigating this response, which can be considered to be due to surgical " stress ", experiments were performed using non-traumatic forms of stress induced by subjecting rats to extreme temperatures, to which they had not been acclimatized, for a period compatible with their survival. These types of " stress " can be considered to be metabolic in

nature, as they alter the metabolic rate of the animal by inducing physiological changes due to the normal adaptation mechanisms which act during variations in the environmental temperature.

The experiments which have been performed can be divided into two main groups. In the first group the rats were anaesthetized and in the second group they received no anaesthetic or any other form of medication. The physiological reaction in these two groups is entirely different. In the unanaesthetized rat (in Group 2), the mechanism of adaptation occurs, which enables the body to adjust its metabolism within certain limits of the environmental temperatures. In the first group the anaesthetized animal loses all of its adaptation mechanism and tends to assume the temperature of its surroundings, thus, when subjected to low temperatures, hypothermia ensues, and when subjected to high temperatures, hyperthermia is produced. Another difference between these two groups is that in the anaesthetized animal the metabolism is more constant, but during exposure of the normal animal to cold and heat the mechanism varies within the body's needs to maintain its normal temperature. Both groups have been subjected to cold and heat sufficient to produce maximum " stress " reaction compatible with survival.

Rats were subjected to cold and heat " stress ", also to hypothermia and hyperthermia before being inoculated subcutaneously with Walker 256 carcinosarcoma cells. By " cold stress " is meant the response of the unanaesthetized animal to temperatures lower than its normal habitat, but compatible with life, which in most cases did not lower the body temperature. In the hypothermia used in these experiments, the animal was anaesthetized and the body temperature was lowered by external cooling. Similarly, in the unanaesthetized animals subjected to higher than normal environmental temperatures (whose rise in body temperature was only very small) the term " heat stress " is applied; in animals anaesthetized and subjected to temperatures which gave a considerable rise in body temperature, compatible with life, the term hyperthermia is used.

Influences of thermal stress on host resistance

The method used for assessing the host resistance to the malignant cell in these experiments is based on the " takes " of the transplantable Walker tumour in the rats following " stress " as compared with a normal animal (Griffiths *et al.*, 1960). A suspension of Walker 256 carcinosarcoma cells " aged " from 6 to 12 hours at room temperature is prepared by the method described by Lucke *et al.* (1952) and modified by Chan *et al.* (1960). Ageing of the tumour suspension is necessary to diminish the percentage " takes " obtained, since the Walker tumour at the time the experiments were being prepared was so virulent that inoculation of a fresh

suspension of cells would yield " takes " in 100 per cent. of animals. The suspension of Walker cells was injected subcutaneously on the abdomen of the rats, which had previously been shaved. It must be emphasized that during these procedures of making and injecting the " aged " suspensions a full sterile technique must be observed, as infection can cause erroneous results. One half of the rats were inoculated immediately before being subjected to the " stress " and the other half following the thermal stress. The percentage " takes " in each group was calculated after six weeks; the rats not having developed tumours by this time were kept for three months to detect delayed " takes ".

Cold stress hypothermia

The maximum cold " stress " to which adult rats could be subjected was determined by placing the animals in a constant temperature cold room in individual cages. The temperature at which a maximum " stress " was produced for a given time, without producing death through cardiac arrest, was determined. Rats left for six hours at a temperature of 21° F. survived satisfactorily without mortality; if left for eight hours at this temperature there was a mortality of 10 per cent. and at 24 hours it was 100 per cent. A temperature of 32° F. for 24 hours was well tolerated by the rats and it was possible to acclimatize them to this temperature.

Hypothermia was induced by anaesthetizing rats with nembutal (2 mgm. per kilo body weight, intra-peritoneally) and chlorpromazine (1 mgm. intra-muscularly). The animals were then placed in a cold chamber at 32° F. for half-an-hour. The fall of their body temperature was recorded every five minutes by means of a rectal thermometer, the temperature drop being rapid at first, but becoming slower at the end of half-an-hour. When the rectal temperature had fallen to 85° F. the rats were removed and placed in a chamber at 58° F. to prevent the body temperature dropping below 80° F. They were maintained at a body temperature in the region of 80° F., but the temperature was not allowed to fall below 75° F., because cardiac arrest is produced at temperatures below this level. The period of hypothermia lasted for two to three hours, the body temperature then gradually rose and a normal temperature was regained as the effect of the anaesthetic wore off, normally in four to six hours.

Rats subjected to cold for various lengths of time respond by shivering and increasing their intake of food. For the purpose of these experiments convenient temperatures, which would produce maximum stress without producing mortality in the animals, were taken as 32° F. for 24 hours and 21° F. for six hours. Rats can be kept at 32° F. indefinitely without a lowering of their body temperature, but when kept at 21° F. for more than six hours a drop of 2° to 3° in their body temperatures occurs, and hypothermia, with consequent cardiac arrest, ensues. At 21° F. for eight hours

there was a mortality rate of 10 per cent., and when the animals were left in the room at this temperature for 18 hours there was a mortality of 90 per cent.

In 41 rats subjected to a temperature of 32° F. for 24 hours 85 per cent. developed tumours following subcutaneous inoculation of 25,000 Walker 256 cells " aged " 12 hours at room temperature, compared to 53 per cent. in 34 rats kept at room temperature (78° F.). Of 96 rats exposed to a temperature of 21° F. for six hours, 96 per cent. developed a tumour following inoculation subcutaneously of 20,000 cells " aged " six hours, compared to 72 per cent. of 61 rats inoculated with the same suspension but kept at room temperature (see Table IV). In the rats subjected to

TABLE IV

" TAKES " OF WALKER 256 CARCINOSARCOMA IN RATS FOLLOWING COLD STRESS

Type of Stress	*Average rectal temperatures*	*Number of rats*	% *" Takes "*
24 hours at 32° F.	101° F.	41	85%
Controls (Room temp. 78° F.)	101° F.	34	53%
Subcutaneous inoculation of 25,000 cells " aged " for 12 hours			
6 hours at 21° F.	100° F.	96	96%
Controls (Room temp. 78° F.)	101° F.	61	72%
Subcutaneous inoculation of 20,000 cells " aged " for 6 hours			

hypothermia, 92 per cent. of 61 animals developed tumour as compared with 58 per cent. in 29 controls, following subcutaneous inoculation of 25,000 Walker cells " aged " 12 hours (see Table V).

TABLE V

" TAKES " OF WALKER 256 CARCINOSARCOMA IN RATS FOLLOWING " HYPOTHERMIA " (UNDER NEMBUTAL ANAESTHESIA)

Types of Stress	*Average rectal temperatures*	*Number of rats*	% *" Takes "*
Hypothermia for 2 hours	80° F.	61	92%
Controls (Unanaesthetized at room temp. 78° F.)	101° F.	29	58%
Subcutaneous inoculation of 25,000 cells " aged " for 12 hours			

The tumours in the hypothermic animals appeared five to six days earlier and were consequently bigger than those in the control group. Half the rats in the " cold stress " and hypothermia groups were inoculated

before the commencement of the exposure and the other half at the end, but there was no difference in the percentage " takes " in these two groups.

The result of coeliotomy in hypothermic animals was a 100 per cent. " take " in 12 animals, as compared with a 39 per cent. " take " in the 21 control animals. This shows that there was an additive effect of the two types of stress reaction.

Another group of 59 rats of the same age and weight was divided into three groups. The first group of 15 rats was acclimatized at 32° F. and kept under these conditions for two weeks; the second group was placed in the cold room at 32° F. for 24 hours, and 21 rats were kept as a control in a room at normal temperature (78° F.). All three groups were inoculated subcutaneously at the same time, i.e. at the end of two weeks' acclimatization of the first group, with 25,000 cells " aged " 12 hours. The first group was kept in the cold room at 32° F. for an additional three weeks, the second group being taken back to its normal room temperature following its 24 hours' " cold stress ". As can be seen in Table VI, in the

TABLE VI

" TAKES " OF WALKER 256 CARCINOSARCOMA FOLLOWING ACCLIMATIZATION OF RATS TO COLD

Type of Stress	*Average rectal temperatures*	*Number of rats*	*% " Takes "*
32° F. for 24 hours	101° F'	24	100%
32° F. for 2 weeks pre- and 3 weeks post-inoculation	101° F.	15	93%
Control at room temp. (78° F.)	101° F.	21	62%

first group " takes " occurred in 93 per cent. of 15 rats; in the second group, having had the " acute stress ", " takes " occurred in 100 per cent. of 24 animals, the controls revealing " takes " in 62 per cent. of 21 rats. This shows that it is not necessarily the acute nature of the stress which produces the increased susceptibility to the inoculated tumour cell, but the general response of the body to cold, which through increasing the metabolism has also created a more favourable " culture media " for the cancer cell to grow.

The " cold stress " experiment was repeated in 25 rats kept at 32° F. for 24 hours and then kept at normal room temperature for 48 hours, before being inoculated subcutaneously with 25,000 Walker 256 cells " aged " 12 hours, as described above. At the same time, 30 control animals kept at room temperature were inoculated. At the end of three weeks, 20 per cent. of the control animals developed tumours and 25 per cent. of the

" stressed " rats (Table VII). This indicates that the increased susceptibility of the animal to the implantation of the tumour cell has disappeared within 48 hours of the termination of the cold stress. As will be shown later, this corresponds to the fall of the total plasma 17-hydroxycorticoids, indicating that the adrenal response has returned to normal after 48 hours.

TABLE VII

" TAKES " OF WALKER 256 CARCINOSARCOMA IN RATS INOCULATED 48 HOURS FOLLOWING COLD STRESS

Type of Stress	*Number of Rats*	% " *Takes* "
24 hours at 32° F.	25	25%
Controls (Room temp. 78° F.)	30	20%

In the rats subjected to " cold stress " there was no weight loss, but their consumption of food increased from 75 grams per kilo per day at room temperature to 165 grams per kilo per day during " cold stress ", indicating an increased metabolic activity to maintain body temperature. During hypothermia, the weight loss was minimal with an average loss of 3.5 grams per rat. This is comparable to the loss in the unanaesthetized rats deprived of food and water during the period of time the animal was under hypothermia.

Heat stress and hyperthermia

In the heat stress experiments the rats were placed in a chamber kept at 110° F. at a humidity of 60 per cent. for six hours, in individual cages. It was found that if the body temperature was maintained higher than at this level, death usually ensued.

Rats were anaesthetized as in the hypothermia experiment and placed in a chamber kept at 105° F., with a humidity of 65 per cent., for half-an-hour. Their body temperature rose to 104° F.; they were then removed to a chamber kept at 98° F., with a lower humidity of 40 per cent., for three hours. The body temperature remained at 104° F. They were then left to recover from the anaesthetic at room temperature. Another group of rats was anaesthetized and kept in a constant temperature chamber at 88° F. with a humidity of 30 per cent.; this maintained their body temperature at the normal of 101° F., thus preventing the fall in body temperature of 5° to 10° F., which was found to occur when anaesthetized rats were left at room temperature (78° F.), following nembutal anaesthesia. These rats were kept at this temperature until they fully recovered from their anaesthetic, which lasted for 3 to 4 hours.

It is important in the hyperthermia experiments to anaesthetize the animals at the temperature at which they are to be subjected, since if

subjected to room temperature for a short period of time they tend to drop their body temperature and develop an initial degree of hypothermia.

The unanaesthetized animals were fed in the normal way with water and a standard laboratory " chow ", except for those rats subjected to a temperature of 21° F. and given a special mixture of sustagen and glucose in water, which did not freeze at this temperature.

All the rats were weighed before the experiment started, again following the " stress ", and subsequently for two days thereafter; following this they were weighed at weekly intervals. The consumption of food by the stressed animals was measured and compared with the intake of control groups of rats kept at room temperature (78° F.).

During the experiments, the conscious rats subjected to a temperature of 110° F. for six hours were given water and their normal diet of laboratory " chow ". The average loss of weight was 5 gm. per rat, but the consumption of water had risen from an average of 5 c.c. per six hours to 55 c.c. per six hours. In the hyperthermic rats, there was a weight loss of only 6 gm., despite their inability to drink during the three to four hour period when they were anaesthetized.

As in the previous experiments, the term " heat stress " is used to denote the effect of high temperatures on conscious animals and the effect of hyperthermia on anaesthetized rats. Experiments performed to determine the maximum degree of hyperthermia and " heat stress " to which the rats could be subjected revealed that the unanaesthetized rat was able to withstand a temperature of 110° F. at a humidity of 60 per cent. for six hours. When placed at a temperature of 110° F. for 24 hours, all the rats died in four days with pulmonary disease, although all were alive at the end of the experiment. Rectal temperatures showed a rise of 1° to 2° F. at the end of three hours and 3° to 4° F. at the end of six hours, 40 per cent. of the rats having temperatures up to 105° F. In the anaesthetized rats, the body temperature rose very quickly and when kept at 110° F. for one hour the temperature rose to 106° F., with a 100 per cent. mortality. It was found that if the temperature exceeded 105° F. in the hyperthermic rats, they would not regain consciousness from the anaesthetic. To obtain maximum hyperthermia, with a low mortality, it was necessary to anaesthetize the rats in the heat chamber to prevent the initial hypothermia which occurs when they are anaesthetized at room temperature. They were then left in the chamber at 110° F. for half-an-hour; at the end of this time the body temperature was 104° to 105° F.; the temperature of the chamber was then lowered to 98° F.; this maintains their body temperature at 104° F. The rats were kept at this temperature until they recovered consciousness.

In the " heat-stressed " rats, i.e. the unanaesthetized rats subjected to temperatures of 110° F. for six hours, there were " takes " in 62 per cent.

of the animals following subcutaneous inoculation of 25,000 Walker cells " aged " for 12 hours at room temperature; in a control group, which was not anaesthetized and kept at normal room temperature of 78° F., there were " takes " in 64 per cent. of 47 rats. The hyperthermic anaesthetized rats inoculated with the same cell suspensions sustained " takes " in 100 per cent. of 46 animals (see Table VIII).

TABLE VIII

" TAKES " OF WALKER 256 CARCINOSARCOMA IN RATS FOLLOWING " HYPERTHERMIA " AND " HEAT STRESS "

Type of Stress	*Average rectal temperatures*	*Number of rats*	*% " Takes "*
6 hours at 110° F. Humidity 60%	103° F.	45	62%
Hyperthermia (anaesthetized)	105° F.	46	100%
Controls (unanaesthetized at room temp. 78° F.)	101° F.	47	64%

In the 45 rats which were anaesthetized and kept at their normal body temperature of 101° F. throughout the experiment, there were " takes " in 68 per cent., compared with only 22 per cent. in 22 unanaesthetized control animals kept at their normal room temperature of 78° F.; both groups were inoculated with 25,000 Walker cells " aged " 12 hours (see Table IX).

TABLE IX

" TAKES " OF WALKER 256 CARCINOSARCOMA UNDER NEMBUTAL ANAESTHESIA KEPT AT THEIR NORMAL BODY TEMPERATURE

Type of Stress	*Average rectal temperatures*	*Number of rats*	*% " Takes "*
Normothermia (under anaesthesia)	101° F.	45	68%
Controls (unanaesthetized at room temp. 78° F.)	101° F.	22	22%

Measurement of stress by adreno-cortical response

The adreno-cortical response to the experiments was assessed using the estimation of the total 17-hydroxycorticoids in the plasma, using the technique described by Reddy *et al.* (1956). The blood was obtained at varying times during the " stress " by direct cardiac puncture, without anaesthetic in the unanaesthetized rats. The estimation was carried out on 2 ml. of the rat's plasma, to obtain which 4 to 5 c.c. of blood was necessary. Consequently, it was not possible to use the same rat more than

twice; in fact, the rats usually succumbed after the first cardiac puncture. Three or more rats were bled at the same time to give more accuracy to the sampling and to account for the individual variations from one rat to another. In the " cold stress " experiments the urinary steroids were estimated also and found to be comparable with values obtained from plasma.

An increase in the total plasma 17-hydroxycorticoids was found following cold stress and hypothermia (Fig. 4). In cold stress, the plasma levels returned to normal 48 hours from the beginning of the exposure to cold, but in hypothermia the main response was delayed and was not at its

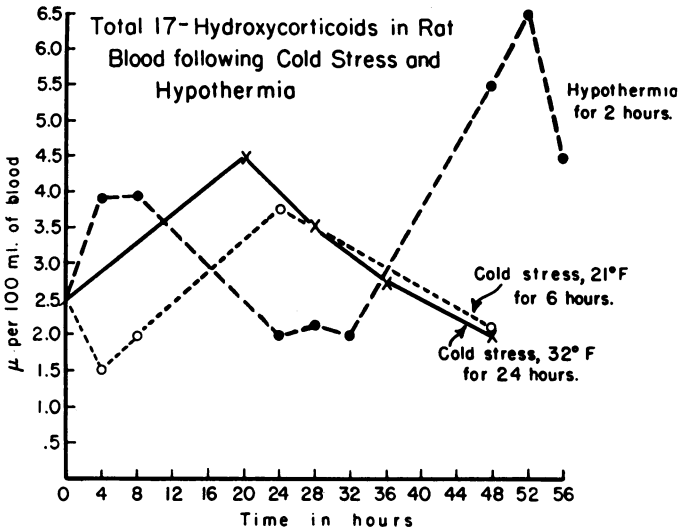

Fig. 4. Adrenocortical response to cold stress and hypothermia as measured by the estimation of the total 17-hydroxycorticoids in the rat's plasma.

height until 48 hours after induction of anaesthesia. This response is similar to that reported by MacPhee and associates (1958) in dogs undergoing hypothermia. The increased adrenocortical activity following cold stress and hypothermia which has been demonstrated is similar to the effect produced on the adrenal cortex by operative stress.

The estimation of the total plasma 17-hydroxycorticoids in both " heat stress " and hyperthermia shows a rise in adrenocortical activity during the first 48 hours following exposure to high temperatures (Fig. 5), but hyperthermia did not show the late rise in the plasma levels demonstrated in hypothermia (Fig. 4).

It has been demonstrated in the above experiments that other forms of physical stress can produce the same type of increased susceptibility to the subcutaneous inoculation of a transmittable tumour in rats. In the first group of rats in which the unanaesthetized animal was subjected to " cold stress " or " heat stress ", it will be noted that in the former there was an increase in the percentage " takes " over the the control group, but in the latter there was no significant difference. The adrenocortical response in both cases, as recorded by the total plasma 17-hydroxycorticoids, shows a stress reaction. Rats undergoing " cold stress " differed from those who were subjected to " heat stress " in that their metabolism was greatly increased, as shown by the increased intake of food required to maintain their body weight during the period of stress. In the second group of rats,

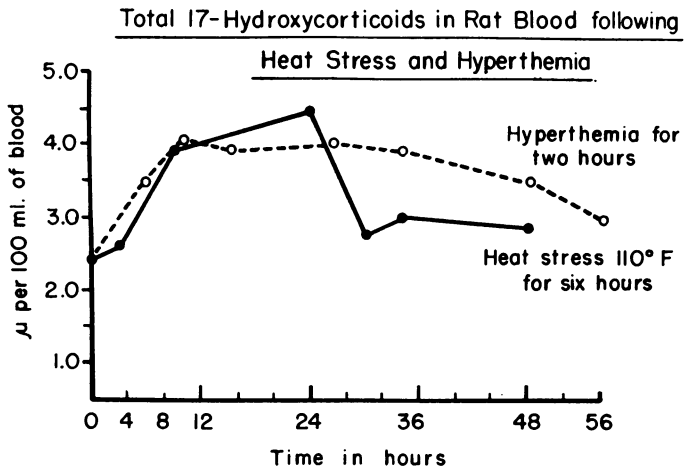

Fig. 5. Adrenocortical response to heat stress and hyperthermia as measured by the estimation of the total 17-hydroxycorticoids in rat's plasma.

which includes those subjected to hypothermia, hyperthermia and normothermia, there is a common factor, namely nembutal anaesthesia; in all these rats there was an increase in the percentage " takes " compared to the " takes " in non-anaesthetized rats kept at room temperature. It has been shown by Travino and associates (1960) that rats anaesthetized and left for two hours at room temperature show an increase in " takes " of Walker tumour injected subcutaneously over non-anaesthetized animals kept under the same conditions. The experiment reported in this paper in which the rats were kept at their normal temperature throughout the period of anaesthesia, demonstrates that although hypo- and hyperthermia probably increase the " stress " reaction, anaesthesia itself can also produce an increased " take " of inoculated Walker cells.

The nature of the physiological mechanism which produces this increased susceptibility (or diminishes the host resistance) to implanted tumour cells must be a central metabolic factor. The difference in the reaction of " cold " and " heat " stress in the percentage " takes " supports the findings of Slawikowski (1960), who showed that the percentage " takes " following coeliotomy in adrenalectomized rats was similar to that following coeliotomy in non-adrenalectomized rats, though somewhat higher in the former (66 per cent. vs. 51 per cent.); the " takes " in the animals having no operation (but an anaesthetic to be consistent with the other two groups) was only 35 per cent. As shown in Table IV the tendency for increased " takes " almost completely disappears when the inoculation of cells is made 48 hours after cold stress. This time element coincides with the fall of the plasma level of the 17-hydroxycorticoids. It may be argued that this physiological reaction is a general response of the body which includes the adrenal glands, but that the essential factor which facilitates the malignant cell to implant is not necessarily the adrenal hormone, although it may play a part. Fisher and Fisher (1959b) have shown that traumatization of the liver is an important factor in the increased growth of tumour in the liver following intraportal injection of Walker tumour. It would appear therefore that any trauma or metabolic disturbance in the normal metabolic processes will create a favourable medium for the growth of the implanted cancer cell, the mechanism of which is as yet unknown.

Effects of starvation and dehydration

The effect of starvation and dehydration on the increased susceptibility to the implantation of Walker tumour in rats has also been investigated. Dietary deficiency in the production of spontaneous tumour has been well described by Tannenbaum and Silverstone (1949). They have demonstrated that the effect of restricted caloric intake and malnutrition in laboratory animals is to diminish the induction and growth of spontaneous and induced tumours. The influence of acute metabolic changes, however, has not previously been investigated in relationship to the increased susceptibility of rats to the inoculation of transmittable malignant tumour cells. In experiments described below the effect of " acute " metabolic changes, such as starvation and dehydration, which can be considered a form of metabolic " stress ", have been studied in relationship to the animals' susceptibility to the inoculation of Walker 256 carcinosarcoma. The effect of acute starvation and dehydration is physiologically different from the slower, more chronic effects of the deficiencies produced by feeding animals for long periods of time on diets deficient in certain essential constituents.

Adult female rats, weighing between 215 and 230 gm., were divided into five groups, which were kept under the same conditions and fed with

the same standard laboratory " chow ",* their weights being recorded every other day. On the 24th day four of the groups were subjected to the following experiments: In *Group* 1 the water bottles were removed for 48 hours but the " chow " food was still made available; in *Group* 2 the food was taken away for 48 hours but the water bottles were left; in *Group* 3 the animals were deprived of food and water for 48 hours; *Group* 4 was kept without food for seven days, but water was still available. The *5th Group* was fed and watered in the normal way and used as control animals. A suspension of Walker 256 carcinosarcoma was made, as described previously, and was injected subcutaneously over the abdomens of half of each group at the commencement of the experiment and the other half at the end of the period of deprivation. Control groups of rats were also inoculated at the same time as the experimental groups with the same tumour suspension and the same number of cells. Following the period of deprivation of food and water, the rats were fed the identical diet given before the experiment. Tumours developed in 14–21 days in rats in which " takes " had occurred. The rats in which tumours did not develop were kept for three months to safeguard against the late development of tumours.

Of the 43 rats subjected to dehydration for 48 hours, tumours developed in 77 per cent., and in a control group of 23 rats kept under normal conditions, 60 per cent. developed " takes ". In 75 rats which were subjected to starvation for 48 hours, 89 per cent. developed tumours, and 76 per cent. of the 50 control animals also showed positive " takes ". The combined effect of dehydration and starvation for 48 hours showed 69 per cent. " takes " in 21 rats and 71 per cent. in 21 control animals. Following starvation for seven days in 44 rats, 80 per cent. developed tumours, as compared to 57 per cent. in 42 control rats. There was no mortality in any group during the period of starvation or dehydration.

Weight charts were kept on each group of rats for 24 days before and 10 days after their starvation or dehydration. The average increase in weight was 3 to 5 gm. a day on their normal diet of laboratory " chow ". During starvation for 48 hours, the average loss was 39 gm. per rat, while starvation for 7 days produced an average loss of 50 gm. per rat. Dehydration for 48 hours caused a loss of weight up to 35 gm. per rat, and in a combination of dehydration and starvation for 48 hours, the loss of weight was 35 gm. In the first three groups the rats' weights returned to the pre-experimental level in 48 hours, and in the fourth group within four days. It is interesting to note that the rats which had suffered starvation for seven days had gained 30 gm. over their pre-starvation

* The food used in our laboratory was the " Purina " Lab. Chow. (Constituents of " Purina " Lab. Chow, as supplied by the manufacturers : crude protein, not less than 23 per cent.; crude fats, not less than 5.0 per cent.; crude fibre, not more than 6.0 per cent.; N.F.E., not less than 44 per cent.)

weight seven days after the end of their period of starvation, whereas the control rats over the same period had maintained a steady weight.

The effect of starvation and dehydration on the experimental rats is to produce a disturbance in the metabolism of the body, which may be considered a metabolic " stress ". Anabolism is replaced by katabolism and there is a mobilization of the body's reserves. In the experiment described above it is interesting to note that the influence of starvation or dehydration, and the combination of both, does not produce an increase in the susceptibility of the animal to the subcutaneous inoculation of Walker tumour, but following extreme starvation for a period of seven days, when there has been a loss of one fifth of their body weight, there is an increase in the percentage " takes " over a normal control group. This would lead us to believe that, following a marked katabolic stress, the conditions in the tissues of the experimental animal are more favourable to the implanted malignant cell than in the normal healthy animal.

Tannenbaum and Silverstone (1949) have shown that malnutrition tends to diminish the incidence of the formation of skin tumours and hepatomas in mice, but they have failed to inhibit the formation of mammary carcinoma in mice by intermittent fasting. There is no contraindication between the influences of malnutrition shown by this investigation and the data presented in the above experiments, as the nature of the physiological response would differ in " acute " starvation from that of chronic malnutrition, as the " takes " following inoculation of Walker carcinosarcoma indicate the susceptibility or " host resistance " to the implantation of the cancer rather than the rate of growth of a spontaneous tumour.

Schneewind (1958) has recently stressed the importance of the nutritional state of patients in the pre-operative period in relationship to the operative and post-operative complications which may ensue. He emphasizes the importance of the patient gaining weight during the immediate pre-operative period, i.e. the patient should be in an anabolic rather than a katabolic state. This may be of even greater importance in patients with malignant disease, especially in patients with carcinoma of the alimentary canal, where there has been a state of " acute " starvation due to intestinal obstruction, if the conditions which have been shown to occur in the experimental animal are true in the human. Animal experiments and clinical observation tend to support the evidence put forward by Buinauskas *et al.* (1958) that operative stress increases the susceptibility of rats to the " takes " of Walker tumour. Any further stress, such as starvation in a pre-operative patient, would increase the " stress " of the surgical procedure and would theoretically diminish the host resistance to the implantation of the disseminating cancer cells and the consequent development of metastasis.

It will therefore be seen in the experiments described that " stress " produced in rats by operative or other means increases the susceptibility of the animal to the implanted malignant cell or, if considered in another way, the stress reaction diminishes the host resistance. What, if any, relationship has this finding in the patient undergoing surgical operation for malignant disease? In the majority of surgical cases with neoplastic disease there is no definite evidence of there being any interference with host resistance, but most surgeons can remember cases in which, following operation, there has been a rapid dissemination of the neoplasm, with widespread appearance of fast-growing secondaries. There is a risk in any operative procedure of spread of malignant cells by direct overflow into the wound, associated with the risk of blood-borne carriage of cells to other organs. Also there is the " katabolic " stress of the operation, which tends to diminish in the post-operative phase the resistance of the tissue to the implantation and growth of the malignant cell.

Factors controlling the development of metastasis

Dissemination of cancer cells during operation is inevitable in most cases of surgery for malignant disease. The body, however, has a resistance to the neoplastic cells and it is conceivable that, as in many infectious processes, the determining factors are the virulence of the neoplasm and the volume of dissemination which occurs.

Fisher *et al.* (1959a) have demonstrated that the development of hepatic metastases in rats, following direct injection of Walker 256 carcinosarcoma cells into the portal vein, is dependent on the number of tumour cells introduced. In discussing the ability of tumour cells to metastasize in mice, Watanabe (1954) postulates that the single cells circulating in the blood stream are of no significance, but that the clumps of tumour cells which enter the blood stream by invasion of the veins are responsible for the development of distant metastasis. Ziedman *et al.* (1950) draw attention to the importance of the presence of the primary on the development and growth of secondary metastasis. Although it has been well recognized that in some cases of neoplastic disease the secondaries become inactive after the removal of the primary, the exact nature of this inhibition has not yet been demonstrated. Evidence presented by Schatten *et al.* (1958) shows that a primary tumour of sufficient size will inhibit the growth of distant metastasis and he argues that secondaries are established before the primary tumour has become large. This is contrary to the general experience of most surgeons.

The characteristics of the cancer cell itself play a part in its own dissemination, and it is likely that in the near future various neoplastic cells will be shown to possess varying invasive and selective characters similar to those of bacterial organisms. Both animal experiments (Lucke *et al.*, 1952) and clinical observation show that some tissues in the body are more

susceptible to the formation of secondaries than others. Haddow (1934) postulated that in the liver there is a specific mechanism involved and this is the source of a growth-promoting substance of paramount importance in the division of both normal and malignant cells. Further evidence of this is seen in the experimental work of Fisher and Fisher (1959b), in which they show a great number of metastases occurring in the liver following injury during laparotomy. This re-emphasizes the metabolic factors which control the initial implantation of the malignant cell. Much of the work in recent years on the endocrine dependency of tumours indicates that the anabolic or katabolic activity of the malignant cell in the tissues is governed by the hormonal balance of the host.

The effect of surgical operation on these factors may, in certain cases, enhance the development of an environment favourable to the implantation and growth of the neoplastic cell, which has been shown by Wiseman and Ghadially (1958) to be able to assimilate the essential amino acids more readily than normal cells. If animal experiments can be used as an indication for the human subject, then it will be seen from the experiments already described that any " katabolic " phase, such as " acute " starvation or operative stress, has an influence on the resistance of the patient to the cancer cell.

The immunological reaction of the body to neoplastic cells is one of great importance and is at the present time being investigated in many centres. The effect of the antibody reaction on the disseminating cell may be the vital factor determining its viability.

The dormancy of the cancer cell has been mentioned previously, and Hadfield (1954), in the Kettle lecture at this College, put forward the hypothesis that anorexia of the scar tissue is one of the factors which inhibits the development of metastasis, and, consequently, any factor which increases the vascularity of the area, such as infection or trauma, will produce re-activation of the cell and the development of a secondary metastasis after many years of inactivity. This is often demonstrated during palliative operations on cancer patients in whom growth is known to have been left at the time of surgery. In the post-operative period, a severe local infection may occur with subsequent formation of fibrous tissue, and consequent slow dormancy or growth of the neoplasm.

From the animal experiments described in this lecture, factors which may control the development of metastasis during surgical operation can be summarized as follows:

(1) Any procedure which tends to disseminate malignant cells

This will include the unnecessary handling of the neoplasm and procedures which encourage the release of cancer cells, such as incision of the growth or contamination of healthy tissue with exudates or blood from the affected organ.

(2) Forms of stress such as surgical shock

It has been clearly demonstrated in animals that any form of stress will tend to increase the susceptibility of the tissues to the implantation of malignant cells. From this it may be concluded that in the human patient similar stress plays some part in the development of secondary growth.

(3) Starvation

Any metabolic disturbance which can be considered katabolic may enhance the development of metastasis in the tissues. This may be of importance during the post-operative phase of any surgical procedure and the aim of post-operative management should be restore an anabolic metabolism as soon as possible.

Measures to limit the dissemination of cancer cells at operation

In 1907, Ryall described the importance of the dissemination of cancer cells at operation. He coined the term " cancer infection ", comparing the cancer cell with bacteria, and showed conclusively that the neoplastic cell should be treated with the same stringent aseptic techniques as those generally used against micro-organisms. He stressed that the only difference between septic and cancer infection is that the former manifests itself early, whereas the latter shows itself late, after all is thought to be well. With such advances in surgical technique as have been made during the last sixty years, it would be well to re-affirm the principles which Ryall laid down for operative procedures in cancer. It was he who emphasized the error of incision into the cancerous tumour at operation, of piecemeal removal of the growth, of curettage, and, above all, he pointed out the dissemination that could occur at operation either by rough manipulation of the tumour or by rupture of affected lymph nodes and vessels.

These principles have been re-emphasized in recent years by Naunton Morgan, Lloyd-Davies and many others in this country, and by Cole and his associates in America. The concept of the infectivity of the malignant cell led to the introduction of chemotherapy in cancer operations. The results in operations for carcinoma of the colon and rectum, using mercury perchloride, have already been discussed in this lecture. The early reports of the use of nitrogen mustard as a prophylactic and adjuvant in surgical treatment of cancer has been reported by Mrazek *et al.* (1959). Although there is general agreement that there is as yet no satisfactory anti-cancer drug, nitrogen mustard used locally during operation, and intravenously in the immediate post-operative period, has extended the possible cure rate of malignant disease in some patients. However, it will be some years before it is possible to assess fully the results of this therapy.

Blood-borne dissemination during the time of operation can, in many organs, be controlled by early ligature of the vessels, preferably before manipulation and mobilization of the tumour.

Limitation of the " stress " of operation

Work which is still in progress indicates that the thyroid gland may play a very important part in the " stress " reaction and its effect on the implanting cancer cell. There is strong evidence to suggest that rats given propyl thiouracil for some time before being subjected to " stress " do not show the same increased susceptibility to the implanted cancer cell as previously demonstrated in this lecture.

Humphrey *et al.* (1959) have shown that there is no difference in the " takes " of Walker tumour in hyper-, hypo-, and eu-thyroid states. It can be argued that the production of a hypothyroid state may block the increased susceptibility to implanted Walker tumour shown to occur in rats following cold " stress ". The method by which the host resistance has been increased is difficult to determine. The general metabolic response of the body would be diminished by the administration of propyl thiouracil, thus possibly depriving the cancer cell of the necessary nutrition during critical stages of its implantation. This is in contradistinction to the increased metabolic states which occur following operative and cold " stress " in normal rats. Humphrey *et al.* (1960) have shown that nitrogen mustard is much more effective in hypothyroid than hyper- or euthyroid rats. This may be due to a decrease in " stress " which occurs after administration of the drug (Schneewind *et al.*, 1958), thus potentiating its cytotoxic properties. Humphrey has also demonstrated that there is no diminution in the toxicity of the nitrogen mustard in the hypothyroid animal, but a diminished percentage " take " when compared with the euthyroid and hyperthyroid rats.

Much of the work reported in this lecture has, by necessity, been on animal experiments using tumours artificially produced. To what extent the results are valid in the human subject must be left to conjecture. But it is worth noting that John Hunter performed animal experiments which have proved of great benefit in recent years in the field of plastic surgery. It was he who performed the first graft by transplanting a cock's spur into its comb and showing that it continued to grow. He also performed experiments on animals to demonstrate the method and factors controlling bone growth.

It remains for us to apply the general principles of what we can learn from animal experiments in the practice of our surgical treatment of patients suffering from malignant disease. Paget wrote in 1889: " All reasoning from statistics is liable to many errors. The eruption of the specific fevers and of syphilis, the inflammation of typhoid, the lesions of tuberculosis, all show dependence of the seed upon the soil. The best work in the pathology of cancer is now done by those who are studying the nature of the seed. They are like scientific botanists, and he who turns over the records of cases of cancer is only a ploughman, but his observations of the properties of the soil may also be useful."

Those who practise surgery are still " ploughmen " and our observations on the " soil " are still of great importance.

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HONOURS CONFERRED ON FELLOWS AND MEMBERS

In the Birthday Honours List, H.M. The Queen graciously conferred the following Honours on Fellows and Members of the College:

K.B.E. (Military Division)	AIR VICE-MARSHAL C. A. RUMBALL, C.B.E., M.R.C.S., Honorary Physician to The Queen
Knighthood	W. E. CHIESMAN, C.B., M.R.C.S.
	J. S. RICHARDSON, M.V.O., M.R.C.S.
	G. D. ROBB, C.M.G., F.R.C.S.
	B. W. RYCROFT, O.B.E., F.R.C.S.
C.B. (Military Division)	SURGEON VICE-ADMIRAL W. R. S. PANCKRIDGE, M.R.C.S., Honorary Physician to The Queen
C.B. (Civil Division)	L. J. CLAPHAM, M.R.C.S.
	PROFESSOR M. A. RUSHTON, F.D.S.R.C.S., Dean of the Faculty of Dental Surgery
M.V.O.	WING COMMANDER H. B. KELLY, M.R.C.S.
O.B.E. (Military Division)	SURGEON COMMANDER C. V. HARRIES, R.N., M.R.C.S.
	WING COMMANDER J. E. MALCOLM, F.R.C.S.
O.B.E. (Civil Division)	H. R. J. DONALD, M.R.C.S.
	G. D. GORDON, M.R.C.S.
	R. F. H. HINRICHSEN, F.R.C.S.
M.B.E. (Civil Division)	E. H. WILLIAMS, M.R.C.S.