oestrogen excreted by the four patients who failed to benefit from operation (Table II) was 7 μg./24 hours—a value slightly higher than the mean level for all patients in this category, and higher than the mean pre-operative level of patients obtaining remission at hypophysectomy (Table I). There are not enough patients in the series for these findings to have any statistical significance, but there may be a tendency for patients with a low pre-operative oestrogen level to benefit from hypophysectomy while those with a high level do not. Further work is needed to check the validity of this suggestion.

In two instances patients who had been previously treated by oophorectomy and adrenalectomy were subjected to hypophysectomy. In neither case was oestrogen detected in the urine either before or after hypophysectomy, yet in both patients their objective regressions were short. Unless they were excreting amounts of oestrogen not detectable by the chemical method, these results suggest a direct participation of pituitary hormones in the maintenance of human breast cancer, as opposed to an indirect trophic effect on target organs. Similarly, in the patient already referred to (Case 7) objective regression occurred in spite of continued oestrogen excretion. Again, this result is explicable on the assumption that the levels of growth hormone, prolactin, or both had been reduced below a critical level.

In the four patients referred to in Table II the most simple explanation for the failure of hypophysectomy to influence the growth of the breast cancer is the continued excretion of oestrogens due to incomplete hypophysectomy. We have, as yet, been unable to study the transition from remission to relapse in this series. However, Hortling et al. (1957), using the vaginal-smear technique, found that oestrogen secretion increased simultaneously with the deterioration of the patient's clinical condition.

The explanation of failure or success following endocrine ablation for breast cancer must be largely speculative until we are able to assess directly the functional capacity of the human hypophysis with a reasonable degree of precision. The results of the investigations on the small number of patients in the present series suggest that repeated biochemical estimation of oestrogens before hypophysectomy may prove to be of some value in forecasting the clinical outcome of this operation.

Continued post-operative excretion of oestrogen, by indicating incomplete removal, suggests that no significant clinical palliation is likely to occur, while cessation of oestrogen excretion would suggest the reverse. The exceptions to these generalizations provide cogent reasons for supposing that the growth of some human breast cancers is maintained by the direct action on the tumour cells of anterior pituitary hormones.

Summary

The effect of hypophysectomy on the clinical state and on oestrogen excretion has been studied in 15 women and one man with metastatic breast cancer.

Patients obtaining regression following hypophysectomy tended to have a low pre-operative level of urinary oestrogen, and in the majority oestrogen excretion virtually ceased after operation. On the other hand, continued oestrogen excretion was not always exclusive of objective regression.

Patients deriving no benefit from operation tended to have a high pre-operative oestrogen excretion and continued to excrete oestrogen after operation.

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A BIOCHEMICAL CONCEPT OF TUMOUR GROWTH, INFILTRATION, AND CACHEXIA

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Among the many perplexing properties of a growing tumour is its ability to build up protein and increase in weight in a host (experimental animal or man) which is fast losing weight. This ability to obtain the precursors of its protein from the metabolic pool at the expense and to the detriment of the host has been investigated in some detail by Mider, Tesluk, and Morton (1948) (see Greenstein, 1954), and from the results they obtained with the transplantable Walker 256 tumour in growing rats they have been able to show three distinct phases in the growth history of the tumour and tumour-bearing animal. During the first stage (while the tumour is relatively small) both the tumour and the host gain weight. In the second stage the tumour alone continues to gain weight, and at an increasing rate. The host tissues in this second stage at first fail to gain weight and later may even lose weight. In the third and terminal stage (often very brief) the rate of growth of the tumour falls, and eventually the tumour itself may lose weight, although not as markedly as that of the rapidly wasting tissues of the host.

Such observations in the experimental animal, and the well-known clinical fact that tumours continue to grow even in cachectic subjects, suggest that the tumour is an autonomous entity capable of thriving irrespective of the metabolic status of the tissues of the host. It is obvious, however, that even the most autonomous neoplasm must have an adequate supply of metabolites for the purposes of growth, and if the essential metabolites are not forthcoming then the rate of growth must suffer.

The effect of dietary restriction on the incidence and rate of growth of spontaneous tumours has been the subject of much careful study. Tannenbaum and his colleagues (see review, Tannenbaum, 1947), in a series of brilliant experiments, have shown that dietetic restrict-tion can greatly reduce the incidence of both naturally occurring and carcinogen-induced tumours in experimental animals. Indeed, some 14 different tumours have been shown to be affected in this manner, and the statistical evidence from insurance companies' records collected by L. T. Dublin in 1927 (quoted by Tannenbaum, 1947) shows that the incidence of various neo-

plasms is higher in overweight humans than in under-weight ones. The growth of established tumours is not as dramatically affected by dietetic restriction as is tumour incidence, but nevertheless the rate of growth is dependent upon the nutritional status of the host (Rous, 1914; Tannenbaum, 1947; Ghadially and Wiseman, 1956).

From a consideration of these fundamental studies emerges the concept of a contest between the host and the tumour for the capture of metabolites circulating in the blood and in the extracellular fluid. Although such antagonistic behaviour between tissues in an animal has been suggested for a long time, and superior ability credited to the tumour cell, detailed experimental evidence has only recently become available to indicate an aspect of the nature of the metabolic mechanism which gives the tumour cell an advantage over normal host tissues.

It seems to us that, from a consideration of the work of many investigators, the metabolic superiority of the tumour cell is largely due to its enhanced amino-acid concentrating power. It is the purpose of this paper to evolve a hypothesis to show how infiltration of normal tissues by neoplastic cells, tumour anorexia, tumour cachexia, and the paradoxical growth of tumours in a wasting host can be explained on this simple biochemical basis.

Nature of the Metabolic Competition

Undoubtedly one of the most important factors in the successful synthesis of protein, and hence tissue growth, is the availability of the essential amino-acids for this purpose. Once the extracellular fluid in which the cells live becomes rich in these amino-acids (via the blood stream or autolysis of cells) then the next important step is the uptake of the amino-acids by the cells concerned. In any multicellular tissue we must picture the cells which share a common volume of extracellular fluid as competing with each other for the available metabolites. The cells which are better endowed in regard to the mechanisms concerned for metá-bolite uptake are in a better position for synthesis of new protein.

In 1913 Van Slyke was able to démonstrate that the intact cells of the animal body are able to accumulate amino-acids from the extracellular fluid and that they can concentrate the amino-acids until their content of these substances is about 10 times that of the animal's plasma. Since that time many tissues of the normal animals have been shown to concentrate amino-acids actively, and recently such work has been extended to neoplastic cells. In 1948 Christensen, Streicher, and Elbinger found that glycine, and probably alanine, serine, proline, methionine, and histidine, are actively taken up (that is, against a concentration gradient) by normal liver and muscle in the intact rat. Sassenrath and, Greenberg (1954) and Greenberg and Sassenrath (1955) have published results of experiments in which they estimated, by specific microbiological means, 18 free amino-acids in the livers, muscles, and plasma 'of fed and fasting tumour-bearing rats, and they showed that the amino-acids were all present in higher concentration in the liver and muscle cells than in the plasma. It is a pity that these latter workers did not estimate the concentration of the free amino-acids in the tumours that their animals possessed.

Work with isolated normal diaphragm by Christensen and Streicher (1949) showed that the muscle could concentrate free glycine *in vitro*. In 1953 Wiseman found that the isolated small intestine of the rat was able to concentrate the L-forms (" naturally " occurring) of alanine, phenylalanine, histidine, methionine, and isoleucine. He showed (Wiseman, 1955, 1956) that isolated small intestine of the hamster was able to concentrate 13 different amino-acids and also that they competed with each other for the concentrating mechanism of the cell. While this work with normal tissue

was being done, work with neoplastic cells was being carried out. In 1952 Christensen and Riggs found that the Ehrlich mouse ascites tumour cells actively concentrated amino-acids, and Christensen and Henderson (1952) showed that in the intact animal glycine, and possibly alanine, are concentrated by the Ehrlich ascites cells to a greater extent than by the liver. In 1955 Wiseman and Ghadially showed that suspensions of RD$_3$ sarcoma cells *in vitro* could actively concentrate histidine, proline, ornithine, lysine, and methionine. The results of our experiments indicated a qualitative difference in the capture of amino-acids by the sarcoma cells and normal intestine, in so far as the neo-plastic cells could actively concentrate the diamino-acids, lysine and ornithine, while the intestine could not.

We were also able to show that apart from this qualitative difference there were two quantitative differences. Under similar conditions the sarcoma cells not only concentrated the amino-acids to a greater extent but the effect of methionine as an inhibitor of amino-acid uptake by cells was less marked. In the case of isolated normal intestine it was found that when methionine was present with an accompanying amino-acid in equimolecular amounts the methionine completely prevented the accompanying amino-acid from being concentrated. The effect of methionine on the isolated sarcoma cells, however, was only to depress, but not to abolish, the concentrative uptake of the second amino-acid.

Various earlier workers (Pilsum and Berg, 1950; Graham, Hier, Waitkoff, Saper, Bibler, and Pentz, 1950; Wretlind and Rose, 1950) have noted that excess dietary methionine retards the growth of young rats, and the results obtained by Wiseman (1955) show that this growth retardation is probably due to the powerful inhibitory effect of excess methionine on the uptake of other amino-acids by normal tissues. A consideration of the differences of the action of methionine on isolated intestine and sarcoma cells (Wise-man and Ghadially, 1955) led to the conclusion that excess dietary methionine should increase the rate of growth of a tumour in a tumour-bearing animal while at the same time causing the host tissues to waste. This, in fact, was shown to be the case (Ghadially and Wiseman, 1956) when 4% DL-methionine was added to the diet of rats bearing RD$_3$ sarcoma. The results of those experiments strongly suggested that the excess methionine decreased the ability of the normal tissues to take up other amino-acids and thereby provided a readier source of amino-acids for the growing tumour, whose amino-acid uptake was not so greatly affected by the excess methionine. Thus there was confirmation *in vivo* of the earlier *in vitro* findings.

Metabolic Superiority of the Neoplastic Cell

Numerous other experiments have also demonstrated this metabolic superiority of the neoplastic cell over its normal counterpart. Zamecnik, Frantz, Loftfield, and Stephenson (1948) found that slices of rat hepatomata (induced by the use of 4-dimethylaminoazobenzene) incorporated more radioactivity from labelled alanine and glycine than did slices of normal rat liver. Rutman, Cantarow, and Paschkis (1954) have shown that mitochondria from preneoplastic livers and from hepatomata of acetoaminofluorine-treated mice showed an enhanced ability (20 to 50% increase) in incorporating alanine-1-^{14}C into peptide linkages. Studies by Burke and Miller (1956) revealed that in the preneoplastic phase during 3'-methyl-4-dimethylaminoazobenzene carcinogenesis the livers of rats incorporated into proteins between two and three times the normal amounts of L-lysine. This was associated with a depression of katabolism of the amino-acid as evidenced by a yield of $^{14}CO_2$ from the labelled lysine lower than expected, and a decreased urea production. Tumours other than hepatomata show an increased uptake of radioactive amino-acids.

Zamecnik *et al.* (1948) and Winnick (1950) found that the rate of uptake of ^{14}C-labelled glycine and alanine into mouse mammary tumour homogenate was several times as great as that of normal mouse liver homogenate. LePage (1953)

has been able to demonstrate that the Gardner lymphosarcoma cells and the Ehrlich carcinoma cells can incorporate up to 50 times as much glycine-2-^{14}C into protein as can normal mouse liver cells. Mouse mammary tumour mitochondria show essentially the same phenomenon (Winnick, 1950). Roberts and Tishkoff (1949) have found that the preneoplastic epidermis of mice receiving applications of methylcholanthrene can concentrate free amino-acids to a greater extent than normal mouse epidermis. It would seem, therefore, that the ability to outdo normal tissues in the capture of amino-acids is an early acquisition of the neoplastic cell, and this we would expect if the neoplastic cell is to compete rapidly and successfully for these essential metabolites.

The rapid rate of incorporation of amino-acids into cellular protein in the neoplastic and preneoplastic cell, as compared with its normal counterpart, would keep the level of free intracellular amino-acid lower than it might otherwise be and thereby help the movement of more amino-acid into the cell from the extracellular fluid. Thus not only is the rate of production of neoplastic tissue kept high, but also the field is set for the continued deprivation of the local normal cells of adequate amounts of the precursors of their protein. An added advantage occurs in the event of the neoplastic cell being able to concentrate an essential amino-acid actively while the local normal cells are unable to do so. Under these conditions the local normal cells would be largely deprived of one or more essential amino-acids, and they would then virtually cease to produce their own intracellular protein, as all the precursors of a protein molecule must be present at the same time for protein synthesis. In the absence of one or more essential amino-acids, cells are unable to retain for any length of time those amino-acids which are available, and a negative nitrogen balance occurs (Cannon, 1948).

Activity of in vitro Preparations

We must point out at this stage that the enhanced activity of many of the in vitro preparations to incorporate labelled amino-acids into protein has not always been demonstrable in vivo (Griffin, Bloom, Cunningham, Teresi, and Luck, 1950; Kit and Greenberg, 1951; Tyner, Heidelberger, and LePage, 1952). It seems likely, however (Zamecnik, Loftfield, Stephenson, and Steele, 1951), that this is a result of inherent technical limitations, for it was observed that, although the avascular portions of the tumours were relatively inactive, the regions with a good blood supply showed high activity. From these observations we may conclude that the rate of uptake found in the in vivo experiments indicates only the average activity of the whole tumour and not the true activity of its most rapidly growing periphery. A similar view is put forward by Caspersson and Santesson (1942), and by Rutman et al. (1954).

It might be argued that the increased rate of incorporation of amino-acids into cellular and mitochondrial protein does not indicate that new and extra protein is being produced and that the results obtained by many of the above experiments might be the result of exchange of an amino-acid for another molecule of the same amino-acid without new protein synthesis occurring. However, the ability of the neoplastic cells to capture and concentrate free amino-acids at a rate higher than that of normal cells has been clearly demonstrated, and when that fact is added to the well-known observation that tumour tissues grow at a rate far greater than the host tissues it seems that the most likely explanation is that the tumour cells do in fact incorporate amino-acids into protein more rapidly than their normal counterparts.

An enhanced power to capture amino-acids is, of course, seen in fast-growing tissues other than malignant tumours—for example, placental tissue (Christensen and Streicher, 1948). The malignant tumour cannot, therefore, be looked upon as being unique in this respect; furthermore, it must be emphasized that an increased ability to capture amino-acids cannot be regarded as the hall-mark of malignancy

or the essential neoplastic change. However, once this fundamental metabolic superiority is possessed by a tissue it has a potentiality to grow at the expense of its neighbours and will in fact do so whenever other growth-restraining influences fail to act. The understanding of this metabolic superiority enables many of the perplexing paradoxes of tumour–host relationships to be explained. It is, of course, possible that other important biochemical factors are involved at various stages in the neoplastic disease. Among such possible factors are competition for metabolites other than amino-acids, the production of toxic materials by established tumours, and changes in energy requirements.

Infiltration

The capacity of malignant cells to infiltrate into and destroy normal tissues is well known. One of the most recent and intriguing accounts attempting to explain the essential malignant change and the ability of neoplastic cells to wander is Green's (1954) antigenic theory of cancer. According to this hypothesis the ability of the malignant cell to wander and infiltrate is due to the failure of the antigenic mechanism of the body to " recognize " the cell once it has lost certain marker antigens. The growth of cells in unusual places and their extension beyond normal planes will therefore go unnoticed by the controlling mechanisms of the body. Loss of a series of such marker antigens, one by one, will confer an increasing independence upon a cell, and eventually such a cell will have the potential to infiltrate local tissues and spread beyond the normal physiological boundaries without arousing an adequate defence reaction on the part of a host. The development by a cell of the attribute to wander, however, seems to be in itself insufficient to account for its continuous growth unless such potentiality were aided by some metabolic superiority. Exploitation of its newly acquired growth independence, however acquired, must ultimately depend on its ability to outdo the local cells in a contest for available metabolites.

Infiltration by neoplastic cells does not only imply their growing between normal cells or along paths of least physical resistance, but it is essentially and ultimately a cell-by-cell destruction of the normal at the interface of the tumour and the normal tissue. In cases of low malignancy —that is, without marked metabolic superiority—there may be merely compression of neighbouring tissues and little real infiltration, but with highly malignant growths with overwhelming metabolic superiority it is not long before the normal cells are replaced by tumour tissue. Over a fairly broad zone surrounding a tumour one can as a rule detect islands of cells in various stages of disintegration, atrophy, and necrosis. Pressure exerted by the increasing tumour mass is usually the explanation given to account for the death of the normal cells at the tumour's advancing edge, but cannot in fact be the explanation (as already pointed out by Ghadially and Wiseman, 1957). The growing edge of the tumour derives its blood supply from vessels at the tumour/normal tissue interface (Algire and Chalkley, 1945), and any appreciable rise in pressure at this region would destroy the tumour cells as well as the normal cells.

It seems to us that the phenomenon of infiltration by a cell which has lost its marker antigens can best be explained on the basis of a metabolic competition of the type described in the early part of this paper. The advancing columns of tumour cells are bathed in the same extracellular fluid and share the same local environment as the adjacent normal cells. Thus it is inevitable that a local competition for available amino-acids will arise. The more efficient power of concentration possessed by the tumour will have a two-fold action. Firstly, it provides the tumour with material necessary for protein synthesis and growth, and, secondly, it deprives the adjacent normal cells of their basic metabolic requirements and thereby induces atrophy, degenerative changes, and finally necrosis. It is for this reason, in our opinion, that the normal cells at the periphery of a growing tumour suffer most. The disintegrating normal cell no doubt provides further metabolites for the insatiable

demands of the flourishing tumour. Thus our hypothesis explains not only why malignant cells flourish and infiltrate but also why the normal cells are destroyed.

Anorexia and Cachexia

As previously mentioned, three phases have been identified in the growth history of the tumour and the tumour-bearing animal. In the first stage the volume of tumour tissue is small and the needs of both the normal and the neoplastic cells are easily met from the general metabolic pool. By the time that the second stage is reached, however, the volume of tumour tissue has increased, and it is at this stage that the severe competition for essential amino-acids must arise. The tumour, by virtue of its more powerful amino-acid concentrating mechanism, deviates large amounts of the available amino-acids for its own protein synthesis, and the normal tissues begin to suffer as the metabolic pool is heavily depleted. The katabolic processes in normal tissues continue (perhaps at an increased rate), but the amino-acids for the anabolic process are no longer freely available.

One of the tissues to suffer early on in this metabolic competition is the intestine, and as a result food intake is reduced. The intestine of a tumour-bearing host is found to suffer a severe weight loss in common with most other normal tissues (Bloor and Haven, 1955), and in rats bearing a Walker carcinoma 256 the intestinal weight may be reduced to about three-quarters of that in control animals. Herein probably lies a good deal of the explanation of the anorexia seen both in patients and in experimental animals suffering from neoplastic diseases. The rate of turnover of cells in the intestinal mucosa has been demonstrated to be very rapid indeed, and the entire mucosa is replaced every 1½ days in the rat (Leblond and Stevens, 1948) and every 2½ days in the cat (McMinn, 1954). This rate of tissue production entails much protein synthesis and calls for a constant supply of amino-acids from the general metabolic pool. Such a demand on the metabolic pool will tend to become more onerous in the later stages of neoplastic disease, and when scarcity of essential metabolites begins to make itself felt the tumour will continue to grow at the expense of the intestine as well as of other normal tissues.

Increasing anorexia and cachexia then herald the third and terminal stage of the disease, for not only is the metabolic pool being rapidly drained of amino-acids by the tumour, but in addition the intestine is no longer able to replenish that pool from the diet efficiently.

As well as obtaining amino-acids in large amounts from the extracellular fluids of the body, there is an actual translocation of amino-acids from normal to neoplastic cells. The work of White (1945) showed that mouse mammary carcinoma continued to grow even when the diet contained almost no nitrogenous substances. Much of the amino-acids necessary for the growth of the mammary tumour could have come from no other source except the normal tissues.

In contrast to other normal tissues, the effect of the growing tumour on the liver is more complex. Instead of the wasting as seen in most other normal tissues there is often an actual gain in liver weight during the earlier stages of the disease (Medigreceanu, 1910 ; Cramer and Lochhead, 1913 ; McEwan and Haven, 1941 ; Yeakel, 1948), and Abels, Rekers, Binkley, Pack, and Rhoads (1942) found that there was liver hypertrophy in 42% of patients suffering from gastro-intestinal cancer. The increase in weight of the liver is due to an increase in liver nitrogen (Yeakel, 1948 ; Sherman, Morton, and Mider, 1950) as well as an increase in liver water content (McEwen and Haven, 1941). It has been suggested that some growth-promoting factor is produced by the growing tumour and acts specifically on the liver (Paschkis, Cantarow, Stasney, and Hobbs, 1955).

The liver hyperplasia may, on the other hand, be due to an increased liver activity concomitant with the increasing metabolism of the growing tumour, and may be similar to the liver enlargement seen in pregnancy (Cramer and Lochhead, 1913 ; Walter and Addis, 1939 ; Poo, Lew, Lee, and Addis, 1940). The stimulating effect on the liver of a growing transplanted tumour has also been shown by Annau, Manginelli, and Roth (1951) and by Malmgren (1956). The latter was able to demonstrate an increase in the number of mitoses in the liver, and similar changes in liver mitosis were observed after the administration of saline extracts of tumours (Malmgren, 1956). But eventually, in the third stage of the disease, when most of the normal tissues are rapidly losing weight and even the rate of growth of the tumour is reduced, the liver suddenly shows a marked weight loss (McEwen and Haven, 1941). It appears that when the last usable sources of nitrogen from the normal tissues of the body have been utilized by the growing tumour mass the liver suddenly surrenders its own nitrogenous materials to keep the malignant cells supplied with metabolites. At this stage of the disease the derangement of the host's nitrogen metabolism has proceeded so far that death rapidly brings the whole process to an end.

Conclusion

The success of the neoplastic cell once it has appeared in the host seems to us to be in the superior ability of such cell to capture from the internal environment, and to concentrate intracellularly, the free amino-acids essential for its protein synthesis. In this activity the neoplastic cell is superior to the normal cell, and the degree of such superiority decides the eventual history of the tumour. Those tumours which are only slightly more active than the normal in their ability to concentrate amino-acids will grow slowly and produce only minor metabolic disturbances, while the more active ones will infiltrate faster, cause anorexia, and produce rapid cachexia and eventual death.

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