AN IMMUNOLOGICAL CONCEPT OF CANCER: A PRELIMINARY REPORT

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This paper is a summary of investigations, begun in 1948, which led to ideas on the nature of the resistance to transplanted tumours induced by chemical carcinogens and related compounds and to an immunological hypothesis of carcinogenesis. Most of the very bulky experimental details are omitted, and will appear in other publications. The present concept arose from an investigation which sought to find non-carcinogenic tumour-inhibiting (T.I.) compounds in tars, particularly coal-tar.

Evidence was obtained that much of the T.I. activity was in fractions of tars which were probably non-carcinogenic. Neither 3:4-benzpyrene (B.P.) nor 1:2:5:6-dibenzanthracene (D.B.A.) could be detected in some active fractions. Moreover, such T.I. fractions did not inhibit somatic growth at all—suggesting, on the basis of the work of Haddow *et al.* (1937), that their T.I. action was not due to the action of a known carcinogen. Their effective dosage was also smaller than that previously found necessary for even pure carcinogens. It had been thought that the T.I. property of carcinogens was due to a general inhibition of somatic growth affecting both normal and neoplastic tissue. However, at an early stage of our work it was apparent that there might be a substance(s) in coal-tar which had specific T.I. action, which did not inhibit somatic growth, and was not a carcinogen. All the following lines of inquiry stemmed from this finding.

The first was to attempt to isolate T.I. compounds; and such work, with the excellent collaboration of Mr. J. W. Westrop, has gone on unceasingly. It is sufficient to state here that from pitch-coking oil six pure compounds having T.I. properties similar to that of the crude oil have been isolated and synthesized; they do not inhibit somatic growth or show any toxicity in doses well above the T.I. level, and have not yet shown evidence of being carcinogenic. All are polycyclic hydrocarbons with five or six condensed rings, and for present purposes it is sufficient to mention the first to be identified—namely, 3:4-benzotetraphene (B.T.)—since this has been most widely used in probing the mechanism of tumour inhibition.

Mechanism of the T.I. Effect

The second line of inquiry was concerned with the mechanism of the T.I. effect. Several relevant facts were early apparent. One was that an active non-carcinogenic hydrocarbon acted on the transplantable tumour (a D.B.A.-induced rat sarcoma) exactly like a T.I. carcinogen. Active T.I. compounds were very insoluble in all organic solvents, and it was shown that this property was related to the T.I. effect (see later). Other early observations were that there was no trace of the active compound in the tumour itself. Active compounds remained mainly at, or close to, the site where injected during the course of tumour inhibition.

None, or very little, was found in other tissues except the local lymph nodes (mesenteric and inguinal after intraperitoneal injection).

Tumour growth in the rat treated from the first to the third day after transplant proceeds as follows: (1) growth at the normal rate, the tumour being lethal at from ten to twenty-five days; (2) growth approximately normal for seven to fifteen days and then regression setting in, which is almost always absolute; (3) growth is slower from the start and the animal survives longer, the tumour proving lethal or regressing at any stage (even when huge).

The T.I. effect was greater the earlier the treatment, and prophylactic treatment was even more effective. Even large transplanted tumours seven to ten days old at the time of treatment occasionally regressed. In striking contrast, not one regression was obtained in over 100 mice and rats with spontaneous or chemically induced tumours even when treatment at the same dosage was started at the first sign of a tumour mass. The transplantable rat tumours—a sarcoma and a Walker carcinoma—responded to these compounds in nearly all of several hundred experiments, the complete regression rate varying from 10 to 100%. This rate varied with the time of treatment, the growth momentum of the tumour, the type of compound used, and, very importantly, with the natural regression rate. In periods when this rate was comparatively high the regression rate in treated rats was almost always very high. The outstanding finding, however, was that usually one or more tumours in a group of 10 grew at a high normal rate, the treatment, powerful in some, not having the slightest effect on others. Such facts reinforced the growing conviction that these T.I. compounds increased the general resistance to tumour growth only when there was some genetic incompatibility between tumour and host.

In transplantable tumours of mice the optimal dose of an active compound was roughly ten times greater than in rats, and complete regression was rare except with the one tumour (S 37), which tended to regress naturally. The other tumours used were the C3H breast carcinoma in C3H pure-line mice, sarcoma 180 in several strains, and a methylcholanthrene sarcoma induced and maintained in the same strain. They (the last excepted) were not quite insensitive, showing in some, but never in all of a group, significant slowing of growth with extended survival times after early treatment with very active compounds.

The most unexpected result was that prolonged treatment of pure-line C3H mice with B.T. delayed the emergence of spontaneous breast carcinoma (by an average of about two and a maximum of four months), thus causing a very significant prolongation of life in this very short-lived strain. This was in striking contrast to their complete lack of effect on the growth of the formed tumour.

The effective dose of most of the active non-carcinogenic compounds is 1–2 mg./100 g. rat and 10–20 mg./100 g. mouse (intraperitoneally). Repeated treatment does not appear to enhance the effect greatly. Increased dosage does not enhance T.I. activity, and very high dosage (20–50 mg.) depresses it. In fact, treatment with any toxic substance depresses, or even extinguishes, the T.I. effect. Haddow and Robinson (1937) used doses of from 5 to 50 mg. of carcinogen to achieve tumour inhibition, and such doses depress general growth in the

rat. Our active non-carcinogens did not depress somatic growth even at doses (10–25 mg.) far higher than the T.I. level, whereas at such levels carcinogens, such as D.B.A., were strongly depressing. However, on our rat tumours D.B.A. had strong T.I. activity at the low level (1 mg.) observed with active non-carcinogens, and at this level did not significantly depress somatic growth. The fact that carcinogens could be shown to be tumour-inhibitory at low non-toxic doses indicated that their T.I. action was of the same nature as that of the active non-carcinogens. Another important point is that there was not the slightest evidence of any specific damage to the tumour cell itself, and in regressions induced by treatment the histological changes in the tumour were qualitatively the same as those seen in natural regression (see below).

These early observations now suggested strongly that the T.I. activity of certain polycyclic hydrocarbons was highly specific, and worked by augmenting, often very strongly, the natural resistance to transplanted tumour tissue. It therefore seems possible that they augmented the homologous immunity induced by the iso-antigens in the tumour. The lack of action on spontaneous and induced tumours, and on transplanted tumours which showed genetic compatibility with the host, and the very much feeble effect on tumours derived from and transplanted in pure-line mice, strongly supported this idea. The fact that early treatment was much more effective than that started four or more days later suggested that it augmented the immunity which was developing from the time of transplant. Where there is no iso-antigen in the tumour immunity is, of course, not induced and artificial reinforcement is impossible.

Suppressive Action of Cortisone

We have already seen that the augmented immunity appeared to be induced close to the site of injection of the T.I. compound. It there appeared to act either by some specific stimulant action on the antibody-forming cells or, conceivably, by inducing the formation of an antigenic complex similar to one of the tumour iso-antigens. Several investigations were set up to probe the mechanism involved. Many lines of supplementary treatment which are known, or thought, to enhance antibody production in a non-specific way were tested. The effect of all our T.I. compounds was enhanced by several of these treatments. The decisive proof that these T.I. compounds did increase homologous immunity was obtained by the use of cortisone and related steroids. At the time several investigations on the effect of cortisone on tumour growth and chemical carcinogenesis were in progress (Green, 1951; Green and Savigear, 1951; Green and Ghadially, 1951). Green and Whiteley (1952) showed that cortisone injections *completely* suppressed homologous and partially suppressed heterologous immunity to transplanted tumours. Green (1953) showed that similar treatment invariably *completely* destroyed the augmented immunity induced by D.B.A. and other T.I. compounds. It was noted that here was strong evidence that the T.I. effect of carcinogenic and non-carcinogenic polycyclic hydrocarbons was due to a heightening of resistance to transplanted tumour growth. It can now be stated that the T.I. effect of four compounds (two carcinogens and two non-carcinogens) tested, and of crude tars, pitch-coking oil, and fractions thereof, was abolished by cortisone. If the immune reaction is suppressed during the first three to four days of tumour growth it appears that iso-antibody production then reaches its maximal too late to kill the tumour mass. There seems little doubt from these results that both the T.I. carcinogens and non-carcinogens produce tumour regression by speeding up iso-antibody production in the early days of tumour growth.

“Initiating” and Lack of “Promoting” Action in Non-Carcinogens

It was essential to find the mechanism involved, for this might provide a clue to the nature of carcinogenesis itself. The difficulty confronted us, however, that some of our most active compounds appeared to be non-carcinogens. The hypothesis was therefore developed that the T.I. non-carcinogens possessed the “initiating” but lacked the “promoting” action of the carcinogens. This hypothesis is being tested by direct experiments which up to date favour it, but the precise answer is still awaited. Meanwhile, there is some indirect evidence in its favour. It was shown that the carcinogen D.B.A. increased the mitotic rate of mouse epidermis significantly during the first few days of painting. The chemically related non-carcinogen, B.T., was without effect. Moreover, prolonged painting with any of the T.I. non-carcinogens produced no naked-eye reaction in the skin as compared with the depilation and other evidence of mild, or even severe, inflammation seen with carcinogenic hydrocarbons.

The other relevant fact was that whilst the carcinogens are without doubt tissue-damaging agents, as is shown by the decline in body growth, liver, and kidney damage, etc., produced by high doses (10–25 mg. per rat), the T.I. non-carcinogens are innocuous. This difference is strikingly emphasized after cortisone treatment, which, by suppressing repair, reveals the extensive systemic damage produced by D.B.A. and not by a T.I. non-carcinogen like 3:4-benzotetraphene (Green, 1953, and unpublished results). The inflammatory response to tissue damage produced by carcinogens might well be the “promoting” factor. Both types of active hydrocarbons probably had a similar “initiating” action in the tissues which led directly to an increased iso-antibody production to injected homologous tumour tissue. If so, these immunological changes might be directly concerned in the primary changes of carcinogenesis, but to support this idea further evidence of a relationship between carcinogenic and T.I. potency was required.

One convincing experiment was based on the work of Dickens and Weil-Malherbe (1946), who showed that cholesterol, when intimately mixed with benzpyrene, enhanced the carcinogenic activity of the latter, while, conversely, a lecithin mixture depressed it. Similar treatment, using the above carcinogen and D.B.A. and several T.I. non-carcinogens, showed conclusively that cholesterol strongly enhanced the T.I. capacity of all these compounds and that lecithin weakened it, often to the point of extinction. T.I. potency seemed to be directly related to carcinogenic potency. The fact that the T.I. non-carcinogens were affected in exactly the same manner as the carcinogens is strong support for the suggestion that both produce *initially* the same tissue changes.

Since it is known that polycyclic hydrocarbons are able to combine with protein or protein complexes, the possibility was considered that these T.I. compounds modified the antigenic structure of a specific protein complex. If this were so and they were previously bound *in vitro* to protein, not concerned in this immune reaction, the combination should cease to be antigenic to the local antibody-forming cells, and should have no T.I. activity. It was in fact found that mixtures of homogenized rat liver or lung and D.B.A. reduced or abolished the T.I. potency, whilst lymphoid tissue and spleen increased it or left it unchanged. A very convincing result was obtained by conjugating D.B.A. *in vitro* with a pure protein, for when D.B.A. isocyanate was linked to horse-serum albumin the T.I. effect *completely* disappeared. It was shown later that D.B.A. isocyanate itself was quite inactive, suggesting that it had conjugated with non-specific protein at the site of injection, and was thus unable to form the *specific* antigenic combination which induces the homologous immune response. All the evidence now suggested that for T.I. activity the compound must link with a particular protein located at or near the site of injection. Since this protein or protein complex

appeared not to be present in the fixed tissue cells, perhaps it was in some way related to the wandering cells concerned with the phagocytosis of these insoluble compounds.

It appeared that owing to their highly insoluble nature they mainly remained near the site of injection whilst their T.I. effect was being exerted. In fact, relatively more soluble carcinogens, like 9:10-dimethyldibenzanthracene (D.M.B.) and B.P. showed definite T.I. activity only at toxic weight-depressing doses (10-25 mg./100 g. rat), and some of the T.I. effect at such levels could be non-specific. Repeated injections (seven daily doses of 2 mg.), however, induced considerable T.I. activity, which suggested again that an optimal *local* concentration of the compound was necessary if the immune reaction was to reach a sufficient intensity in time to be effective. If so, very soluble forms of active hydrocarbons should be completely ineffective. This proved to be so, for both the endo-succinates of D.B.A. and B.T. showed no T.I. activity.

Role of the Plasma Cell

Histological studies are not complete, but it seems likely that the injected compounds are first engulfed by large mononuclear cells. All methods designed to saturate or "block" these cells abolished T.I. activity. Charcoal injected at the *same* site, but not elsewhere, completely antagonized the T.I. action of the many compounds tested. Following phagocytosis and transference of the compound to neighbouring lymph nodes, the plasma cell seemed likely to be next concerned. Much evidence now suggests its major role in antibody production to transplanted homologous tissue. In the case of homologous tumour tissue we now have evidence that this is so. Most of the observations were made in the rat by Dr. Baruah. The plasma-cell population in certain lymph nodes, the omentum, and the spleen rose greatly during the development of immunity to T.A.B.C. vaccine. The rise exactly paralleled the rise in serum antibody titre. Both rises were much diminished by treatment with cortisone if given for some days before and after the vaccine treatment was begun.

During the growth of the rat sarcoma and Walker carcinoma, certain lymph nodes (right axillary and right para-aortic) and the spleen increased progressively in weight, and showed an increasing proportion of immature and mature plasma cells, with diminishing lymphocytes, throughout the whole course of tumour growth. Early treatment with cortisone strongly diminished this plasma-cell response. Commencing natural regression was accompanied by a massive infiltration of the tumour with plasma cells, which appeared to be solely responsible for the initial stages of tumour regression. It seems very probable that the plasma cells are the source of the antibody(ies) to the iso-antigen(s) in the tumour tissue, and that when they reach a critical level the immune reaction kills the tumour cells.

Are the plasma cells implicated in the primary stages of tumour induction? It seems that they are, for intraperitoneal D.B.A. in small (2 mg.) amounts produced a pronounced rise in the plasma cell (both immature and mature) count in the medullary cords of the mesenteric and para-aortic lymph nodes, and later in the spleen and bone marrow. A similar but less intense change was seen with the non-carcinogenic T.I. compound B.T., but anthracene, a non-carcinogen with no T.I. action, produced no plasma-cell response at all. Both types of T.I. compound appeared to be stimulating the formation of plasma cells from lymphoid reticulum cells, and we have chemical evidence that they do reach these sites. Whether the postulated antigenically modified protein is formed in the transporting cells, the plasma cells, or both is not yet known, but it seems highly likely that the plasma cell is the source of the homologous antibody whose production is so strongly augmented by these compounds.

Evidence for a Specific Antigenic Complex

It now seemed definite that certain carcinogens could elicit or stimulate a similar, or the same, immune reaction

which growing homologous tumour tissue itself elicits. The antibodies so produced circulate, and supplement or complement the natural antibodies induced by the tumour iso-antigens. An unsettled point, however, was whether they induced the formation of an antigenic protein or whether they stimulated the immune reaction concerned. The evidence, particularly the inactivity of compounds attached to "neutral" proteins, favoured the former view. Moreover, all of many methods known to stimulate immune mechanisms non-specifically were relatively ineffective when compared with active hydrocarbons. The circumstantial evidence that a specific antigenic complex was formed by such a compound seemed strong enough to warrant a more direct approach.

An attempt was made to detect the antibodies thought to be provoked by certain cells in which carcinogens had produced an antigenic change. We first sought to obtain complement fixation and precipitin reactions with sera from D.B.A.-treated rats, using as antigens lipid extracts of various tissues from both D.B.A.-treated and normal rats, and from normal tissues exposed to D.B.A. *in vitro* in various ways. Evidence of a specific antibody was inconclusive. It was then decided to find if cells, removed from an animal and intimately mixed with a carcinogen, would, when reinjected into the *same* animal, produce an immune response. The obvious choice of cell was the red blood cell, and of animal, the rabbit; and red-cell agglutinins were sought for in the rabbit serum, using very many of the accepted techniques.

Of seven rabbits injected with D.B.A.-treated blood two, A and B, showed, after many weeks' treatment, definite alterations in their own blood. The serum of A now strongly agglutinated homologous red cells from all of over 20 rabbits, both controls—that is, injected with their own untreated blood—and normals, whereas none of the sera from these rabbits agglutinated A's red cells. This "pan-agglutinin" in A's serum was active at room temperature and was readily absorbed by all homologous red-cell suspensions. On the other hand, B's serum contained no such agglutinin, but its red cells were agglutinated by sera from *all* rabbits other than its own. This property seemed to be due to a homologous antibody adsorbed on the red cell *in vivo*, for it could be eluted and shown, when mixed with an inactive serum from another rabbit, to behave as a "pan-agglutinin." In both A and B the antibody titre quickly declined after treatment was stopped.

These results, suggesting that an antigenic change had occurred *in vivo* in the red cell after intimate exposure to a carcinogen, were interesting, but confirmation was needed from a different angle. Amos *et al.* (1954), using special techniques, detected circulating agglutinins for homologous blood cells following homologous skin transplantation. This showed that antigens common to skin and blood cells existed in mice. If, therefore, such common antigens existed in the rabbit, it seemed possible that extensive skin painting with a suitable carcinogen might elicit a homologous red-cell agglutinin. Repeated painting of the whole ventral skin of nine rabbits with D.M.B. has now been associated in all with the appearance of homologous red-cell agglutinins in the serum. The room-temperature agglutinin induced was mainly of the A type. In only one instance to date has a definite 37° C. "pan-agglutinin" appeared, and this was readily adsorbed by and eluted from homologous cells, and in two rabbits a change of the B type also occurred.

Many more results still remain to be interpreted, but so far they all accord with the hypothesis which dictated the experiments. This was that carcinogens, and related T.I. non-carcinogens, when in intimate contact *in vivo* with susceptible cells, might induce the production of antibodies to homologous antigens of similar cells.* The protein complex(es) thus made antigenic must be one determining the individual identity of the cell, for the cell becomes "foreign" to its own organism and elicits an antibody to

*Recent findings indicate that the serum antibodies disappear when skin carcinomata appear.

similar, but homologous, cells of the same species. This background necessarily leads to an immunological concept of neoplasia, and that main theme will be developed in the discussion.

Discussion

We have only summarized some aspects of the work which bear directly on our theory of carcinogenesis. It was the successful search for non-carcinogenic tumour-inhibiting (T.I.) compounds in coal-tar which led to the present ideas. The tumour-inhibiting action of many carcinogens (Haddow and Robinson, 1937; Haddow *et al.*, 1937) was thought to be, and probably was in part in the high doses used, incidental to the general depression of somatic growth they produced. If the compounds we isolated had been known to be very active carcinogens we might have accepted such an explanation of their T.I. action. It was fortunate, since it stimulated further investigation, that they were not. It seemed, in fact, that their T.I. action might be specific, for they did not depress somatic growth in T.I. or much higher doses. It thus became necessary to re-explore the mechanism of the T.I. effect of carcinogens, for it seemed most unlikely to be fundamentally different from that of such chemically closely related non-carcinogens. For reasons already elaborated, we became convinced that their T.I. action was due, not to a cytotoxic action in the tumour, but to an enhancement of immunity to homologous tumour tissue.

Was this enhancement due to a direct stimulation, pharmacological in type, of the iso-antibody-forming mechanism? This seemed the most likely explanation, and yet the evidence strongly suggested that formation of antigen and not of antibody was the primary change. The T.I. compounds appeared to act by converting sensitive cells into homologous "foreign" cells, the host reacting, as it does to foreign tissues in general, by attempting to destroy them in the immune reaction so induced. These T.I. compounds affect only transplanted tumours which themselves evoke an immune reaction in the host, the antigens concerned being one or more of the proteins which confer on the cell some degree of the genetic identity of the cells of the individual animal in which they first arose. We shall refer to them as "individual identity protein(s)." When there is no antigenic difference (genetic incompatibility) between tumour and host, as always in spontaneous and chemically induced tumours and sometimes in transplanted tumours, the T.I. compounds are uniformly without effect. Compatibility of host and tumour accounts for the otherwise puzzling finding that usually in any susceptible group of animals a proportion of transplanted tumours show no response to this form of chemotherapy.

There is, of course, good evidence from the work of Miller and Miller (1952) and others that the carcinogenic hydrocarbons combine with proteins *in vivo*. Our own work suggests that they may modify a protein complex which now becomes "foreign" to the organism and excites an immune response. Whether the linkage is with lipid, polysaccharide, or nucleic acid, or directly with protein does not concern us here. The protein modification may not of course occur in this direct way, but by linkage to, and modification of, the enzymes concerned in its synthesis. The word "foreign" is used here to embrace the most delicate degree of antigenic difference—that is, that conferring individual and even perhaps tissue identity on the cell. These "foreign" cells excite the production of homologous antibody which must be similar to that elicited by the transplanted tumour itself, for it augments the reaction often to the point where the tumour is destroyed.

If our hypothesis is correct, the antigenic change induced by the carcinogen must be related in some direct or indirect way to the carcinogenic process itself. Might not this even be the essential primary change in susceptible cells exposed to a carcinogen? If so, are the affected cells usually destroyed by the immune reaction so elicited? Probably this is so, for numerous examples in studies on tumour

development support the idea that many or all pre-cancerous (or in carcinogenic phraseology, "initiated") cells may disappear at any stage before a malignant tumour emerges, while some may remain dormant for long periods of time. Only repeated and continuous exposure to a powerful hyperplastic agent ultimately results in the outgrowth of a malignant neoplasm.

"Identity Proteins"

What, then, is the cause of the early proliferation of susceptible cells usually seen after exposure to a carcinogen? It is suggested that it is in part the result of an inflammatory process which the products of immune reactions are known to induce. Concomitant proliferation both of the neighbouring normal and of the still surviving modified cells occurs, but the immune reaction may be completely effective and destroy all the modified cells. Some may escape either because they are not yet sufficiently modified to elicit a lethal immune reaction or because continuing inflammatory hyperplasia more than offsets the rate of destruction. Here the finding that certain (and there are probably many) polycyclic hydrocarbons also induce an immune change without exciting the further change of carcinogenesis is very relevant. They augment immunity to homologous tumour tissue at least as much as related carcinogens. They must therefore, *ex hypothesi*, induce the formation of "foreign identity proteins." They differ from the T.I. carcinogens, however, in one striking way, for they are very innocuous to the body tissues generally, whereas chemical carcinogens are tissue-damaging agents.

It is postulated that the relatively mild hyperplasia produced by the specific immune reaction is, in the case of the carcinogen, greatly augmented by a non-specific inflammatory hyperplasia. Continuous hyperplasia may be alone sufficient to develop a tumour, after only one adequate exposure to a carcinogen. Continuous exposure to a carcinogen is more effective than one exposure followed by a non-specific hyperplastic agent, because not only has the carcinogen a similar hyperplastic action but it is also able to modify more and more of the normal cells affected by the hyperplastic stimulus. The immune reaction will heighten as the population of "foreign" cells increases. Compounds like 3:4-benzotetraphene, with strong T.I. and no carcinogenic action, induce the antigenic change, but do not stimulate the degree of non-specific hyperplasia necessary if some cells are to survive the immune reaction and proceed to neoplasia. It is postulated, therefore, that a chemical carcinogen has the dual property of inducing a highly specific antigenic change and a non-specific hyperplasia of the affected cells.

How, then, does this lead to the neoplastic change? There are sound reasons for thinking that the type of highly malignant cell which is capable of growing without restraint in any homologous host completely lacks individual "identity proteins." This unique property is not easily explained in any other way in the light of all the firm evidence for the immuno-genetic basis of tissue transplantability. If this is so then lesser degrees of malignancy will be associated with a correspondingly smaller loss of what we have called "identity proteins." It is argued, moreover, that this loss constitutes *in itself* the neoplastic change, and that the gradations and progressions in neoplasia are completely accounted for by the extent of the loss.* Variations from slight to great loss, first in specific tissue identity and then in individual identity, gradually or relatively suddenly developing according to the potency of the carcinogen, with the almost infinite gradations possible in degrees of immunological change, could account for the whole apparently perplexing range of neoplasia.

*While this article was in proof the work of Weiler and Hörlein (*Z. Naturforsch.*, 1952, 7, 324) was noted, which clearly showed that microsomes and mitochondria from butter-yellow induced liver tumour cells lacked specific liver antigen(s). Its relevance here needs no stressing.

How could this loss of "identity protein" take place? As in all unicellular reactions to a harmful stimulus, by adaptation and selection, a cell emerging which has extruded or lost the antigenically modified proteins or the enzyme system(s) responsible for their synthesis. Adaptations to cells of simpler structure in the presence of a strong immune reaction are so familiar in bacteria that no microbiologist would deny its possibility in animal cells. At some point, if one or more of the continually proliferating modified cells are to survive the ever-increasing antibody production during carcinogenesis a biologically effective way is to dispense with one or more modified "tissue identity" proteins. The cell is then in some degree free from immunological restraint, and free to invade and wander to the extent defined by its new antigenic state.

The transplanted neoplastic cell differs from its normal prototype in the greater growth momentum it can achieve in a homologous animal, or, in the final analysis, in the "foreign" tissues of the animal in which it developed or of a genetically similar one. Fine gradations of antigenic loss must be envisaged. First there is the benign tumour which survives only when in normal anatomical contact with its progenitor's tissues and has no more capacity for growth in another animal than its normal ancestor. Following this, every grade of transplantability is seen with progressively increasing loss of "identity proteins" until the extreme grade is reached, usually only after a long transplantation history, when the tumour grows on transplantation to any homologous host, and even for a while in a different species. In fact, all our experimental criteria of malignancy are really based on the degree of immunological compatibility. The more malignant a cell the more it lacks the substances which give it "identity," first as a tissue, then as an individual, and last, maybe to a slight extent, as a species.

"Marker Proteins"

The concept that the cells of each tissue contain tissue "identity proteins" is difficult to explain briefly. It is probably true that a "benign" neoplastic tissue has a better chance of growing as an autograft—that is, outside its normal environment—than the corresponding normal tissue. Certain normal cells, particularly those with a high growth momentum, will survive and even grow for *limited* periods as autografts in favourable sites. Progressive growth is, however, not usual in translocated tissues, except when removed to a comparable site (epidermis to epidermal bed). Certainly some embryonic tissues will grow as "autografts" in the maternal tissues, and so will tissues displaced during embryonic life—that is, heterotopic tissues. But such tissues are more likely to have in the former, and will have in the latter, a "neutral" antigenic status. Here we are indebted to Burnet and Fenner (1949) for the "self-marker" hypothesis.

To account for the absence of specific antibodies in the individual for the antigens determining the red-cell type it is postulated that during embryonic life the "scavenger" cells, by specific contact with "marker proteins," develop "recognition units," units complementary to the "marker protein," as with enzyme and substrate. Late in foetal development antibody production is elicited only by proteins differing from a body "self-marker," but nevertheless sufficiently alike to be capable of partial reaction with a "recognition unit." This reaction leads to a modification of the enzymic "recognition units," which multiply, and after transference to the antibody-forming cells provide a permanent source of self-reproducible antibody. So runs this concept, and in our view it could account for the toleration of heterotopic tissues by adjacent normal tissues. Such a toleration usually does not long exist if displacement occurs in post-embryonic life, with the significant exception of the neoplastic cell.

This concept can be extended into the realm of neoplasia. Having long thought in terms of "identity protein" in developing this immunological concept it is easier to continue using it, though it may prove to be synonymous with

"marker protein." It is likely that, as with the red blood cell, all cells of each defined tissue possess tissue "identity proteins." There is increasing evidence that specific tissue antibodies can be induced by injection of many types of tissue into a foreign species. This is a strong pointer to tissue specificity *within* the individual. A cell lacking one or more tissue "identity proteins" will only partially, or not at all, be "recognized" outside its normal boundaries by the phagocytes concerned with the removal and disposal of displaced or effete normal cells of a similar kind. It has no "identity proteins" to react with "antibody" of foetal origin and therefore does not attract cells normally concerned in its disposal. Such cells are therefore in some degree "neutral" cells, lacking identity, free to wander without other than purely physical hindrances, and free to seek adequate nutrition wherever available. The degree of freedom will depend on the number of tissue "identity proteins" lost; for several, or even many, are likely to determine completely identity in the normal cell. Loss of all should produce a wildly malignant tumour capable of growing in many tissues, whilst a limited loss would produce a cell more restricted in its range.

Support for the Immunological Concept

This whole concept was evolved during six years' experimental work, and is founded on some practical approach at each step. Latterly we have been encouraged by the findings of a few other workers which seem to lend support to the hypothesis. Miller and Miller's (1952) work in particular is most striking. Following chemical analyses of the proteins in skin and liver after repeated exposure to chemical carcinogens, it was suggested that the tumour cell might be the result of protein deletion. Another finding which exactly accords with our hypothesis has been recorded by Pollard and Russel (1954). It had been shown (Green, 1953) that chemical carcinogens were able to increase the general resistance to transplanted tumours. The above workers found that when such carcinogens were injected into the mouse the spleen acquired the property of inhibiting the growth in tissue culture of tumour tissue, of similarly induced type, from another mouse. When a tumour emerged in the treated mouse the spleen lost its tumour-inhibiting properties.* Our interpretation of this is that antibodies to modified "identity proteins" induced at the site of injection by the carcinogen were produced in the spleen. The antibodies would react with iso-antigens in the homologous tumour cells and thus destroy them. When the tumour emerged the antigenic stimulus would no longer be present, as tumour cells are such because they lack the "identity proteins" concerned in the immune reaction. The facts seem to fit our hypothesis neatly.

Clayton and Spain (1954) found that partial reticulo-endothelial blockage with ferric oxide strongly decreased the incidence of azo-dye-induced liver tumours. No explanation was found, but one is provided on our hypothesis that without an adequate local immune response *chemical* carcinogenesis is impossible. If the reticulo-endothelial cells are blocked and therefore incapable of transferring carcinogens to antibody-forming sites no such response can occur. In our experiments local blockage of these cells destroyed the tumour-inhibiting property of the carcinogen. Another recent observation of quite a different kind may also, if confirmed, be of significance. It was observed by Hall *et al.* (1953) that one of the partners in pairs of rats in parabiosis tended to develop lymphosarcoma at a relatively early age. The rat thus affected was always the one which had previously suffered from "parabiotic intoxication," presumably as the result of an excess of iso-antibody induced by its own "identity proteins" in the immunologically stronger partner. Here is an instance where the individual "identity proteins" are solely concerned in the immune reaction, and they are submitted to severe attack from the high antibody concentration induced by the powerful iso-antigenic stimulus which must occur

*See footnote p. 1377.

in parabiosis. Adaptation might be the only chance of survival, and such an adaptation could *only*, on the present concept, be neoplastic in nature.

Can the immunological concept be extended to include tumours induced by other than chemical agents, and spontaneous tumours of unknown cause? To consider in this connexion all the known exciting causes of neoplasia would require a volume, and only a few examples can be selected here.

The "Virus" Hypothesis

It has been shown that prolonged prophylactic treatment with one active T.I. compound (3:4-benzotetraphene) very significantly delayed the outgrowth of a mouse spontaneous breast tumour, though of course it had no effect on the formed tumour. The effect was not due to calorie restriction, for the gain in body weight was normal. The primary neoplastic change, preceding the appearance of a spontaneous tumour, appeared to be held in check. If the proposed mode of action of these T.I. compounds on *transplanted* tumours is correct then it would seem that in the *spontaneous* development of the C3H mouse breast carcinoma the "pre-cancerous" cell was eliciting an immune reaction, which restrained its outgrowth for some time. If so, how, in this case, could the antigenic modification occur? One possibility in this particular tumour is through the agency of a foreign protein, the milk factor "virus." Viruses are capable of becoming an integral part of the nucleoprotein of the infected cell, and if they thus happen to modify the synthesis of "identity protein" so as to make it "foreign" to the organism, the same sequence of events could occur as postulated with the chemical carcinogens, the "promoting" hyperplastic agent being in this case hormonal, and usually oestrogenic.

Can the hypothesis explain the pure virus tumours—for instance, the Shope papilloma of the rabbit skin? The "benign" forms of this and other virus tumours are probably the result of excessive hyperplasia induced by the immune reaction to the infecting virus. In the naturally infected wild rabbit the antibody response to modified "identity protein" may be not strong enough to elicit the drastic adaptation to a malignant cell. In the more "foreign" domestic rabbit the greater antibody response may eventually induce the malignant transformation. If this is so, the virus protein must in some degree replace "identity protein" and be deleted with it, for once the malignant cell appears the virus disappears. This explanation cannot hold for the Rous fowl sarcoma, in which the virus often persists in large amounts. This tumour is unique in that the tumour cell is formed almost at once by virus invasion. To comply with our theory virus protein must quickly replace "identity proteins" or compete, rapidly and successfully, for the enzyme systems responsible for their synthesis.

Ionizing radiations are so ubiquitously carcinogenic that the hypothesis would be still more in doubt if it could not explain this action. Their interference with chromosomal structure and genetic constitution, often leading to mutant strains of cells, and their disintegrating effect on nucleic acids, are well known. Some modification of "protein complexes," perhaps involving the nucleic acids, is almost to be expected. If so, "identity proteins" would be affected sometimes, and perhaps always at certain energy levels. We have indirect evidence of such a change in experiments designed to raise resistance to tumour transplants by general irradiation, based on the methods used with T.I. compounds. Further confirmatory work is, however, necessary.

Neoplasms Resulting from Long-continued Hyperplasia

What of the important group of neoplasms which appear to be solely the result of long-continued hyperplasia, without any intervention of a known carcinogen? Long-continued excessive hyperplasia leads to premature senescence of the overworked cells. Involution follows, and is accompanied by extensive degenerative changes in the stroma and blood vessels. The senile cells are still being

stimulated to excess in a region where malnutrition is continually increasing. Under such conditions a cell might well fail to synthesize one or more of the highly specialized "identity proteins," which in any case have no survival value. Such a failure would result in the emergence, sometimes sudden, of a neoplastic cell. This is not too speculative an idea, for the finding of Earle *et al.* (1943), that very prolonged tissue culture of a pure cell race in a heterologous medium could frequently, without exposure to a carcinogen, induce a malignant change, might be regarded as an experimental vindication of it.

On our theory the change would be due to the imperfect nutritional conditions of the tissue culture. These finally result in the emergence of a new cell which has adapted itself to its environment by failing to synthesize certain highly specialized protein complexes. Since the individual "identity proteins" in tissue culture are redundant to the cell, they might well be one of the first involved, and such deficient cells would grow as a malignant tumour when injected in a homologous host. These experiments were originally designed to induce malignant change by prolonged carcinogen treatment of fibroblasts in tissue culture, but such a change was no more frequent than in the controls. The results may therefore be an additional demonstration of our original postulate that some reaction in the *intact* animal is essential for *chemical* carcinogenesis. Later (Sanford *et al.*, 1950) it was suggested that the malignant transformation might be due to selection of latent sarcoma cells, or that the completely heterologous culture medium used had induced some change, possibly enzymic, in the cell. We have indicated our conception of the nature of this change and think that these important findings may prove to be one of the clearest demonstrations that lack of "identity protein" complexes is the state which determines *all* the attributes of the neoplastic cell.

Further Facts Concerning Neoplasia

We have only space left to consider, in the light of this concept, a few more of the very many facts relating to neoplasia. This will be done more generally in a later publication, when it is hoped to make clear that the concept can be applied as a unifying hypothesis. At this stage the reader should himself make the attempt in his own special field. For instance, the true hereditary tumours can be regarded as springing from cells in which the genetic mechanism controlling the enzymes responsible for synthesis of "identity proteins" is unstable. The malignant tumours of early life might be thought of as springing from heterotopic cells, which, displaced during late foetal life, evoke no "recognition units," and thus become antigenic when the immune mechanism develops after birth. The same line of reasoning would apply to neoplastic changes occurring in malformed or congenitally displaced tissues, provided the displacement occurred in later foetal life. The theory covers all stages of progression of tumours (see Foulds, 1954), and can also be used to explain the important "enhancing" transplantation techniques which all involve a heightening of iso-antibody production. It can also be used to explain metastatic dormancy, for the malignant cell has, of course, not usually lost all its tissue "identity protein," and, transported to other tissues, the normal, though weaker, disposal mechanism may, if it does not destroy them, hold some in check indefinitely until a hyperplastic stimulus (usually inflammatory or hormonal) leads, in the way we have described, to further adaptation. Why carcinogenic stimuli at any period of life result in frank neoplasia many years later can also be similarly explained. The capacity of the tumour for metastatic spread is the basic expression of the malignant state. An attempt to explain the nature of that state has been our object throughout the discussion.

Finally, it must be emphasized that the basic concept could still be reasonably maintained solely on the known immunogenetic facts of homologous transplantation. Altogether it appears that the immunological approach to the cancer problem is likely to become dominant and it may well lead eventually to a final solution.

The concept arose step by step directly from our initial observations on the non-carcinogenic tumour-inhibiting substances in coal-tar. It was developed as a basis for planned experimental work and as such has proved most useful. I am much indebted to Burnet's stimulating idea of "self-markers," which, extended into the cancer field, provided at a later stage an essential link in the hypothesis.

Summary

Non-carcinogenic polycyclic hydrocarbons which strongly inhibited the growth of some transplanted tumours were isolated from coal-tar oil.

These compounds, as compared with related *carcinogenic* hydrocarbons, were non-toxic and did not inhibit somatic growth. Nevertheless their tumour-inhibiting effect was shown to be of the same specific nature as that induced by the carcinogens. Neither had any direct cytotoxic effect on the tumour.

Both types failed to inhibit spontaneous or chemically induced tumours, or transplanted tumours lacking antigenicity to the host.

It was conclusively shown, partly with the aid of cortisone, that the tumour-inhibiting property was due to a stimulation of the immune reaction to homologous tumour tissue.

Evidence is presented which favours the view that these compounds have the specific property of altering certain protein complexes so that they behave as iso-antigens and excite the production (mainly locally) of homologous antibody. Their mode of action is discussed and the important role of the plasma cell in the immune response demonstrated.

Some evidence was obtained that chemical carcinogens when in intimate contact with red cells and epidermal cells induce the production of homologous antibodies to iso-antigens in similar cells.

Evidence is presented suggesting that tumour-inhibiting and carcinogenic capacity are biologically closely related. It is therefore postulated that the immune reaction induced by a carcinogen is the essential primary stage in chemical carcinogenesis.

It is suggested that all the tumour-inhibiting polycyclic hydrocarbons are able to induce the primary "initiation" stage, but that only the carcinogens are able to "promote" the cell to the stage of neoplasia by their additional non-specific hyperplastic action.

It is postulated that the "initiation" stage of chemical carcinogenesis is that at which an antigenic change in the "identity protein(s)" to an iso-antigen occurs. The antibody response thus elicited either destroys the pre-cancerous cell at some stage, or continuous hyperplasia leads to an ever-increasing immune reaction which may finally induce an adaptation in the cell. The adaptation involves the loss of "identity-protein" complexes, and the neoplastic cell emerges.

Evidence was obtained that a tumour-inhibiting polycyclic hydrocarbon delayed the *outgrowth* of a mouse spontaneous tumour. For this and many other reasons it is suggested that the initial stage in the development of every malignant tumour may involve the loss, or antigenic change followed by loss, of "identity proteins."

Suggestions are made concerning how this loss could occur with such different cancer-inducing agents as viruses, ionizing radiations, and long-continued hyperplasia alone.

The basic concept is that all neoplastic cells lack one or more substances (probably protein complexes or their related enzyme systems) which confer tissue and individual identity on the cell. The cell thus becomes more antigenically "neutral" and does not attract in the same degree the normal immunologically regulated disposal mechanism. It is argued that this lack of "identity proteins" constitutes in itself the neoplastic state, and that the attributes, gradations, and progressions of this state are defined by the nature and extent of the loss.

Individual acknowledgments for all the willing help we had will be expressed in later detailed papers, for the work described is linked up with other investigations that did not concern us here.

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A NEW THERAPY FOR PREVENTION OF POST-OPERATIVE RECURRENCES IN GENITAL AND BREAST CANCER

A SIX-YEARS STUDY OF PROPHYLACTIC THYROID TREATMENT*

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All who have treated operable cancer of the breast or female genitals have tried to eradicate the cancer by radical operation, by radium or deep x-ray treatment, or by a combination of these methods. But after this initial treatment we tend to sit back and wait. Wait—let me be frank—for a recurrence of the cancer. The mistake made in cancer therapy is that we begin the fight most vigorously, but halt the battle too early without having tried post-operative prophylactic treatment against the killer's recurrence.

In 1938 I mentioned for the first time the beneficial effect of male hormone in two cases of inoperable breast cancer, after having proved the effect of testosterone on the breast and female genitals (Loeser, 1938a, 1938b). I thought at that time I had found the solution in prophylactic treatment against cancer recurrence. This hormonal treatment—which was published long before Huggins's contrasexual therapy in cancer of the prostate

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