CANCER

Edited by

RONALD W. RAVEN
O.B.E. (Mil.), T.D., F.R.C.S.

Joint Lecturer in Surgery, Westminster Medical School, University of London; Surgeon, Westminster Hospital Teaching Group; Surgeon, The Royal Marsden Hospital; Surgeon, The French Hospital

VOLUME 3

PART III: ADDITIONAL PATHOLOGICAL ASPECTS

PART IV: GEOGRAPHY OF CANCER

PART V: OCCUPATIONAL CANCER

PART VI: CANCER EDUCATION

PART VII: CANCER DETECTION

BUTTERWORTH & CO. (PUBLISHERS) LTD.
LONDON

CONTRIBUTORS TO THIS VOLUME

CHARLES BERMAN, M.D. (RAND), M.R.C.P.
Senior Medical Officer, Consolidated Main Reef Mine Hospital, Maraisburg, Transvaal, South Africa

ETHEL BROWNING, M.D.
H.M. Medical Inspector of Factories

JOHANNES CLEMMESEN, M.D.
Chief Pathologist, Department of Pathology, Finsen Institute and Radium Center, Copenhagen; Director of the Danish Cancer Registry, Copenhagen

EMERSON DAY, M.D.
Chief, Department of Preventive Medicine and Director, Strang Cancer Prevention Clinic, Memorial Center for Cancer and Allied Diseases; Chief, Division of Preventive Medicine, Sloan-Kettering Institute for Cancer Research; Professor, Department of Preventive Medicine, Sloan-Kettering Division, Cornell University Medical College

HAROLD F. DORN, M.D.
Biometrics Branch Division of Research Services, National Institutes of Health, Bethesda, Maryland

NIELS DUNGAL
Professor of Pathology, Director of the Department of Pathology and Bacteriology, University of Iceland

E. W. GAULT, B.SC., M.D. (MELB.), M.S. (MELB.), F.R.A.C.S.
Professor of Pathology, Christian Medical College, Vellore, South India

H. N. GREEN, M.A., M.SC., M.D.
Professor of Experimental Pathology, Leeds University; Director of Cancer Research, Leeds and Sheffield Universities

E. CUYLER HAMMOND, D.SC.
Director of Statistical Research Section, American Cancer Society; Professor of Biometry and Director of Graduate Studies in Statistics, Graduate School, Yale University

J. R. M. INNES, SC.D., PH.D., D.SC., M.R.C.V.S., F.R.S.E.
Chief of Pathology Branch, Medical Laboratories, Army Chemical Center, Edgewood, Maryland

V. R. KHANOLKAR, M.D., F.N.I.
Director, Indian Cancer Research Centre; Director of Laboratories Tata Memorial Hospital, Bombay

ILSE LASNITZKI, M.D. (BASLE), PH.D.
Sir Halley Stewart Fellow

BREWSTER S. MILLER, M.D.
Director of Professional Education, American Cancer Society, New York

R. MURRAY, B.SC., M.B., CH.B., D.P.H., D.I.H.
Formerly H.M. Medical Inspector of Factories, Manchester; now Member, Occupational Safety and Health Division, International Labour Office, Geneva

WALTER E. O'DONNELL, M.D.
Assistant, Division of Preventive Medicine and Assistant, Sloan-Kettering Institute; Assistant Professor of Preventive Medicine, Sloan-Kettering Division of the Cornell University Medical College; Assistant Director and Assistant Attending, Strang Cancer Prevention Clinic, Memorial Center; Clinical Assistant, Department of Medicine, Memorial Center; Clinical Assistant Visiting Physician, James Ewing Hospital, New York

G. R. OSBORN, M.B., B.S. (MELB.)
Pathologist to the Derbyshire Royal Infirmary and the Derbyshire Hospital for Women; Lecturer in Pathology, The University of Sheffield

PAUL E. STEINER, B.A., M.S., M.D., PH.D.
Professor of Pathology, The University of Chicago

PERCY STOCKS, C.M.G., M.A., M.D., F.R.C.P., D.P.H.
Senior Research Fellow, British Empire Cancer Campaign; Late Chief Medical Statistician, General Register Office and Reader in Medical Statistics, University of London

JOHN WAKEFIELD, B.A., F.Z.S., M.R.S.H.
Executive Officer, Cancer Education Project, Manchester Committee on Cancer

J. WATKINS-PITCHFORD, M.D., D.P.H., D.I.H.
Principal Medical Officer, Ministry of Pensions and National Insurance

GEORGE Z. WILLIAMS, M.D.
Chief, Clinical Pathology, Clinical Center, National Institutes of Health; Member, Cancer Control Committee (Cancer Teaching) Field Investigations Branch, National Cancer Institute; formerly Cancer Teaching Co-ordinator and Director, Department of Oncology, Medical College of Virginia, Richmond, Virginia; Past President, Annual Cancer Teaching Conference

MICHAEL H. C. WILLIAMS, B.M., M.R.C.P.
Division Medical Officer, Dyestuffs Division, Imperial Chemical Industries Limited

PART III

ADDITIONAL PATHOLOGICAL ASPECTS

CONTENTS

CHAPTER PAGE

Contributors to this Volume vii

PART III: ADDITIONAL PATHOLOGICAL ASPECTS

1. IMMUNOLOGICAL ASPECTS OF CANCER 1
H. N. Green

2. CANCER CELLS IN TISSUE CULTURE 42
Ilse Lasnitzki

3. MALIGNANT DISEASES OF DOMESTICATED ANIMALS . . . 73
J. R. M. Innes

4. STATISTICAL INVESTIGATIONS CONCERNING THE CAUSATION OF VARIOUS FORMS OF HUMAN CANCER 116
Percy Stocks

PART IV: GEOGRAPHY OF CANCER

5. EPIDEMIOLOGY OF CANCER 173
Paul E. Steiner

6. CANCER MORTALITY TRENDS IN ENGLAND AND WALES . . 184
Percy Stocks

7. CANCER MORTALITY TRENDS IN THE UNITED STATES OF AMERICA 208
Harold F. Dorn

8. PRIMARY CANCER OF THE LIVER IN AFRICA 228
Charles Berman

9. ENDEMIOLOGY OF CANCER IN DENMARK 240
Johannes Clemmesen

10. CANCER IN ICELAND 262
Niels Dungal

11. CANCER IN INDIA IN RELATION TO HABITS AND CUSTOMS . 272
V. R. Khanolkar

12. CANCER OF THE UTERUS IN INDIA 281
E. W. Gault

PART V: OCCUPATIONAL CANCER

13. OCCUPATIONAL LEUKAEMIA 287
Ethel Browning

CHAPTER PAGE

14. OCCUPATIONAL CANCER OF THE SKIN 311
R. Murray

15. OCCUPATIONAL TUMOURS OF THE BLADDER 337
Michael H. C. Williams

16. THE MEDICO-LEGAL ASPECTS OF OCCUPATIONAL CANCER . 381
J. Watkins-Pitchford

PART VI: CANCER EDUCATION

17. POSTGRADUATE MEDICAL EDUCATION IN CANCER IN THE UNITED STATES OF AMERICA 387
Brewster S. Miller

18. CANCER EDUCATION IN THE MEDICAL SCHOOL . . . 394
George Z. Williams

19. CANCER EDUCATION FOR THE PUBLIC IN GREAT BRITAIN . . 407
John Wakefield

20. CANCER EDUCATION FOR THE PUBLIC IN THE UNITED STATES OF AMERICA 417
E. Cuyler Hammond

PART VII: CANCER DETECTION

21. CANCER-DETECTION CENTRES 425
Walter E. O'Donnell

22. VALUE OF SIMPLE PROCEDURES IN CANCER EXAMINATIONS . 445
Emerson Day

23. CYTODIAGNOSIS 458
G. R. Osborn

INDEX

CHAPTER 1

IMMUNOLOGICAL ASPECTS OF CANCER

H. N. GREEN

INTRODUCTION

THE RELATIONSHIP of immune reactions to neoplastic growth is of fundamental importance in oncology. It bears closely on the nature, diagnosis and possible treatment of cancer. Yet immunological studies on cancer tended to become relatively fewer as cancer research in general so greatly expanded; there are signs that this is now being reversed.

The explanation for this gradual loss of interest is because of the different biological behaviour of the transplanted and spontaneous tumour. From the time of the first use of transplantation in the late nineteenth century until relatively recently it was assumed that the growth of a tumour in another animal of the same species was essentially of the same nature as growth in the parent host. It could grow, metastasize and kill. It could also grow and regress, which suggested that resistance to tumour growth was a reality, and might be explained in terms of classical immunity studies. This led to experimental work attempting with limited success to detect specific tumour antibodies. These mainly negative results in spite of evidence that resistance to transplanted tumours was often absolute and highly specific, led to another interpretation of the results. It was gradually realized that this resistance was in some way the response of the animal to a "foreign" tissue of the same species and not specifically to tumour tissue at all. Two discoveries in particular were responsible for this revolution in ideas. One was that firm resistance to tumour growth could often be induced by the prior injection of normal tissues (such as red cells or embryonic skin) from the same species; the resistance was as great as if tumour tissue itself were injected. The second arose out of the increasing use of inbred strains of mice for tumour transplantation studies. It then became clear that the closer the genetic relation of the tumour donor and the recipient animal, the greater was the chance of successful transplantation. This relationship was so precise that, between members of the completely pure-line strains, successful and lethal transplantation was always attained. On the other hand the tumour might never become established, let alone show progressive growth, in mice of another genetic strain. In other words once the experimental conditions corresponded to spontaneous growth (that is, growth in tissue of the same genetic type) all evidence of tumour immunity vanished.

Knowledge of the historical development of this subject is vital to understanding the revolution in regard to cancer immunity it evoked. Spontaneous tumours were now regarded as being genetically of exactly the same nature as the host tissues from which they derived. Both spontaneous and induced tumours were thus antigenically neutral to their host and behaved immunologically on transplantation

exactly like the normal tissues of the same animal. After decades of search for specific tumour immunity the answer appeared to be that it had only revealed in one more direction the normality of the cancer cell. In the author's opinion this disappointment tended to blind workers to the otherwise obvious fact that the transplanted tumour, though it did behave immunologically like normal tissues, did so only in a qualitative sense. That it can ever show progressive growth in genetically different animals is, however, evidence that the malignant tumour may override transplantation iso-antigenic immunity. It was apparently assumed that this was due to its greater growth momentum, which was the inherent and causally unknown fundamental property of the malignant cell.

Studies on normal tissue far exceed those on tumour tissue transplantation though the latter paved the way for the former. It is likely that they will both now run parallel until a vital clue is obtained in one or the other which may provide a practical approach to the successful surgical homografting of all tissues, and at the same time the nature of cancer. It is emphasized that these studies are vital to the understanding of the immunology of cancer. Knowledge of the chemical nature and the biological actions of the antigens in cell components, including the nucleus, cytoplasmic particles like the mitochondria and microsomes, and the cell membrane is essential. Similar knowledge is required about the antigenic mixtures which determine organ or tissue (tissue antigen), individual (iso-antigen) and species (species antigen) specificity and the interrelationships between them.

The subject of tissue transplantation is introduced because it embraces the most important aspect of cancer immunology. All the studies on tumour transplantation may be seen in a different light, as the work of the past few years has established without doubt that the reaction of the host to the foreign graft, and the graft to the host, is an antigen-antibody reaction following at least some of the known immunological laws. If, *as is likely*, this tissue immunity proves to have special features, separating it from the classical reaction to foreign proteins from another species, *it is now certain* that it is an immunological process. For work on normal tissues two recent symposia should be consulted (Ciba Foundation, 1954; Moore, 1956).

In earlier studies on tumour grafting the main object was to relate them to tumour therapy ultimately in man. Tumour immunity might be explained in terms of bacterial immunity and a similar approach achieve the prophylactic and therapeutic successes of bacteriology. As it gradually emerged that immunity to the transplanted tumour gave no immunity to the spontaneous and chemically induced tumour and that even one transplanted tumour might give no immunity against another, these hopes faded; the work was reviewed by Woglom (1929). Later, the subject of immuno-genetics was developed and reviewed by Snell (1952). It is not possible here to cover the whole of the work; reference is made to a few excellent reviews which cover the previous 10–20 years; important modern developments are indicated together with past work which may now assume a new relevance. Of the reviews, those of Tyzzer (1916), Woglom (1929), Spencer (1942), Stern and Willheim (1943), Hauschka (1952a) and Stern (1953) should be consulted.

It is proposed to use the author's own concept as a basis for discussion of the facts bearing on the antigenic structure of the cancer cell. No attempt is made to

restrict the facts to those favouring any one theory. Some unifying hypothesis is, whether right or wrong, essential for clear thought in this involved field.

ANTIGENIC CHANGE IN THE CANCER CELL

If the malignant change is immunological in nature the point to be solved is whether loss, gain or qualitative alteration in the cellular antigens is involved. The search for a "mutant" tumour antigen has been almost in vain (Kidd, 1940, 1944, 1946a and b; Kidd and Friedewalde, 1942; Cheever, 1941; Jacobs and Houghton, 1941; MacKenzie and Kidd, 1945).

No specific tumour antibody has been detected, but there is evidence for the presence during transplanted tumour growth of iso-antibodies to the normal iso-antigens in the tumour (Lumsden, 1931; Gorer, 1942, 1947, 1948, 1950; Amos and his colleagues, 1954). The significance of this may be wider than is usually thought, for it is one pointer to the possibility that progressive growth of a spontaneous tumour, when transplanted, might be due to iso-antigenic lack. If so, these iso-antigens might assume greater importance in the cancer problem, as bearing on a possibly related antigenic lack as a factor in the growth of the spontaneous tumour.

There is more in favour of the possibility of antigenic gain than for qualitative antigenic change. Theoretically it could account for the growth of a transplanted tumour, for a cell which was able to fix more antibody and thus diminish its effective concentration below the lethal point would grow under conditions where its prototype with a normal iso-antigenic content would die. The relationship of heteroploidy to transplantability which Hauschka (1952b), Hauschka and Levan (1951, 1953) discovered bears strongly on this point. Increasing chromosome numbers are correlated with an increasing range of transplantability and even strain immunity is broken down, tetraploid neoplasms surviving in several unrelated strains of mice. According to Hauschka (1952a) transplanted tumours are not genetically homogeneous, but mosaics, wherein mitotic aberrations provide ample opportunity not only for physical loss of histocompatibility genes but for additive ploidy changes bringing about antigenic modifications. With continuous selection those cells with the most balanced chromosome sets are better fitted to survive than unbalanced competitors. Hauschka (1952b) found that mouse ascites tumours retained diploid and tetraploid forms for over 50 generations. It seems that the frequent occurrence of antigenic change during serial tumour transplantation is associated with and perhaps due to the presence of the polyploidal cells. Whether these apparently often multiple losses (Little, 1951; Strong, 1926) of histocompatibility genes reflect antigenic simplification (Gorer, 1948) or not, the behaviour of the polyploidal cell is best explained thus. The alternative concept of antigenic gain with antibody saturation would ascribe the significant tumour iso-antigens mainly to the nucleus whereas, as will be seen, there is evidence against this. Polyploidy is, moreover, seen not only in tumours but in all normal tissues from the embryo onwards (Hauschka and Levan, 1951; Koller, 1947; Timonen, 1950; Beatty, 1951). It is thus difficult, though not impossible, to conceive of its direct causal relationship to the malignant change. It seems more likely that in the tumour cell it reflects iso-antigenic loss and it may be an expression of high reproductive activity and reduced function promoted by antigenic simpli-

city. This is an important cytological change and knowledge of its nature would advance considerably the knowledge of progression towards greater malignancy.

Quantitative antigenic gain thus remains a possibility in the transplanted tumour, but there is some agreement that progression (that is, increasing range of transplantability) must be due to antigenic simplification. The term antigenic loss is perhaps avoided to allow for the re-arrangement of genic equilibrium, one antigen crowding out others, bringing about masking, or functional elimination, of weaker histocompatibility factors (Gorer, 1948). These inherent immuno-genetic complications have obstructed the simple conception of direct antigenic loss in the face of sustained antibody attack. Even antigenic simplification is regarded as a pure transplantation phenomenon. So much was it accepted that transplanted and spontaneous tumours are, in immunological terms, distinct, that the idea that antigenic simplification might be a feature of all cancer cells and the factor responsible for homologous transplantation when that is possible, was not seriously considered.

Green (1954) first suggested on the basis of experimental work, that antigenic loss was not only a feature of the malignant cell but its *raison d'être*. Whether this is correct or not the concept accords with so many facts that its consideration in some detail will illustrate many aspects of cancer immunology.

The immunological concept arose from a synthesis of the results of two distinct experimental approaches; both at their inception were unconcerned with immunological ideas. The first was a search amongst coal-tar products for possible anti-cancer agents. There were several theoretical reasons for this, but the practical one was that coal tar itself was powerfully inhibitory to many transplanted tumours. Nor was this effect thought to be directly related to that described by Haddow and Robinson (1937) and Haddow, Scott and Scott (1937) who found that carcinogenic hydrocarbons in very large doses restrained transplanted, and in much less degree, spontaneous tumour growth (Haddow, 1938; Haddow and Robinson, 1939). The coal-tar effect described by Green was different. It was not due to a known carcinogen, for active amounts of tar contain only 0·3 milligram benzpyrene (this should be compared with the doses of 10–15 milligrams carcinogen used by Haddow). Moreover, strong activity was found in some non-carcinogenic fractions corresponding to those described by Berenblum and Schoental (1947). These fractions were also non-toxic and did not restrain, even in high dosage, somatic growth. It was the fact that Haddow's non-specific growth effect was not involved that led to the work being continued. A specific anti-tumoral effect had been sought and, it seemed possible, had been found.

On this basis chemical fractionation with therapeutic tests at each stage continued for two years until fractions active in doses as small as 4 milligrams per 100 gramme rat were obtained and a preliminary report (Green, 1949) was made. Green and Westrop (1957) continued their chemical studies using pitch coking oil as the crude source. Fractional adsorption on alumina columns and spectrophotometric observations ultimately provided clues to the nature of the active compound(s) as being polycyclic hydrocarbon(s) with five or more rings. Finally, a pure active compound, naphtho-2′ : 1′-1 : 2-anthracene (formerly termed 3 : 4-benztetraphene) was isolated and synthesized. This had all the biological properties sought. It produces regression of roughly half the rat transplanted tumours treated in small dosage (2 milligrams), is non-toxic at much higher dosage and has no

general growth inhibitory effect. Moreover, it is either non-carcinogenic in the mouse or nearly so. Meanwhile many inactive and also four active additional 5-ring and 6-ring hydrocarbons were isolated; of the latter, 11 : 12-benzfluoranthrene, a highly potent compound, has been used in many biological experiments. It is non-toxic and non-carcinogenic. Some important biological tests have been previously referred to (Green, 1954 and 1955).

It was now established that there are in coal tar, tumour-inhibiting (TI) hydrocarbons which are probably non-carcinogenic and not growth inhibitory, but which, in small doses, either parenterally or by mouth, inhibit the growth of transplanted tumours. This was a specific effect limited to tumour growth and not a general effect on tissues, both normal and neoplastic alike, described by Haddow. This work involved a fundamentally different method of approach and led directly to an immunological concept of cancer. As active compounds are not carcinogenic the carcinogens are implicated as follows. As the non-carcinogenic TI compounds are chemically closely related to the carcinogenic polycyclic hydrocarbons and Haddow had shown the latter, albeit in very high doses, to be tumour-inhibitory, it seemed unlikely that two different effects were involved. It was soon shown that they were not. Although carcinogens, like methylcholanthrene, 3 : 4-benzpyrene and 9 : 10-dimethylbenzanthracene had, in similar dosage, feeble TI action, 1 : 2 : 5 : 6-dibenzanthracene proved to be as potent as the TI non-carcinogens. All these compounds are feebly soluble in water but the latter is, for all practical purposes, insoluble, and, in most organic solvents, much less soluble. It was possible that the rate of absorption from the injected tissue was a factor. Repeated small doses, or one big dose of the "inactive" carcinogens, revealed activity. It now seemed likely that both groups of compounds shared a specific TI effect, but that an optimal local concentration was essential for activity. They were, however, all active on intravenous injection possibly because they reached the reticulo-endothelial (R.E.) system intact by this route. These TI* compounds enhanced resistance to transplanted tumours, which is of an immunological nature. It was concluded that the TI compounds decisively altered the immune state of the animal. Where the tumour-host relationship involved little or no iso-antigenic difference the compounds were ineffective or relatively much weaker. This was observed in spontaneous breast tumours, chemically induced tumours of rats and mice, and transplanted tumours in inbred strains of mice. It is true that Haddow (1938) found some TI effect on spontaneous breast tumours, but the carcinogen doses were at the toxic level and the effect may have been non-specific. It is significant also that a mouse tumour (sarcoma 180) which grows well in most strains and has thus undergone "antigenic simplification" was, next to the autologous tumours, the least susceptible to the TI compounds.

The plasma cell, cortisone and tumour growth

There is considerable evidence that carcinogens do not enhance antibody formation in general, but tend to depress it (Malmgren and Bennison, 1952; Malmgren, Bennison and McKinley, 1952). Baruah (1956) found no increase in the titre of T.A.B. induced antitoxin after the injection of dibenzanthracene in rats. There is evidence that carcinogens may cause R.E. organs to hypertrophy in species

* From henceforth TI compounds cover both carcinogenic and non-carcinogenic compounds unless otherwise stated.

susceptible to cancer induction. Dibenzanthracene (and other TI, but not non-TI compounds) produced enlargement of the lymph nodes, particularly those draining the area injected, and of the spleen in the susceptible rat, but atrophy of these tissues in the non-susceptible rabbit (Green and Baruah, 1956). It seemed likely (Green, 1954) that non-specific stimulation of the R.E. system was not the cause of the increased resistance. Nevertheless this system was stimulated by the TI compounds, and the unexpected idea of an increased formation of antigenic substances resembling tumour iso-antigens had to be considered. This could not arise by some local change in the tumour because it was shown that TI compounds do not reach this site. They were found in only detectable amounts at the local site of injection, in the draining lymph nodes and occasionally in the spleen. It therefore appeared to induce the postulated antigen locally, or in the R.E. system, or during transport there. During transplanted tumour growth in homologous rats and mice a similar enlargement of the lymph nodes and spleen is now familiar (*see* Parsons, 1936). It is the result of a very active immune response.

That homologous, and even heterologous, immunity can be abrogated by cortisone was first shown by Green and Whiteley (1952), and substantiated by later workers. This treatment combined with x-irradiation has been used as a method of "culturing" human tumours in animals (Toolan, 1953, 1954a, b). It is, therefore, revealing to find (Green, 1953a, 1954, 1955) that cortisone treatment completely abolishes the otherwise greatly augmented immunity induced by dibenzanthracene and many other TI compounds. With the right technique (that is, treatment in the initial stages of tumour growth) the effect is always obtained. Cortisone treatment combined with a fairly large dose of a carcinogen precipitated a rapid fall in the body weight of the rat. This was a true potentiation of effects and in suitable dosage early death occurred. The lesions included honeycomb vacuolation of the liver and patchy necrosis of the lung and myocardium with tremendous bacterial invasion and no sign of repair. The inhibition of reparative processes by cortisone had "unmasked" the potential tissue-damaging action of the carcinogen, but, in spite of this, the tumour grew to a lethal size. This cortisone effect gave a further indication of the specific nature of the TI action of carcinogens and a possible clue to its mechanism.

The effect of cortisone and related steroids in preventing tissue repair and in lowering immunity to infection is well known; its mechanism is still disputed. Every aspect of the defence process from arrest of phagocytosis to destruction of lymphoid tissues has been incriminated (*see* Dougherty and White, 1945, 1946; Chase, White and Dougherty, 1946). Nevertheless it seems likely that the suggestion (Green, 1954) that the depressing effect on immune reactions is due to an antimitotic action on R.E. antibody-forming cells may prove correct. Fuller knowledge of the nature of this effect may bear closely on the nature of carcinogenesis, for cortisone has other striking effects on tumour induction and growth. The lymphocyte was regarded by many, particularly Dougherty and White (1945), as the cell mainly affected by cortisone, for lymphoid tissue almost melts away with immunologically effective doses of cortisone, and it was thought that pre-formed antibody was released and thus, for a time, the immune response enhanced. This is not now generally accepted and the main problem is whether the dissolution of lymphoid tissue is itself responsible for the decreased immunity. The realization that the plasma cell is an antibody-producing cell, and that this might even be

its sole function, led the author to instigate intensive studies on the plasma cell and its reaction to cortical steroids, carcinogens and tumour growth.

The main conclusions of Baruah (1956) regarding the plasma cell, its immunological role, and its significance in tumour growth, are as follows. The rise in the serum antibody titre of the rat to the heterologous antigens, typhoid vaccine, sheep red cells and rabbit serum, runs parallel with increasing plasma-cell formation mainly in the splenic red pulp and the medulla of the lymph nodes. If cortisone is given some days before and during the period of immunization little antibody is produced and new plasma cells are not formed. If cortisone is given at later stages of immunization antibody production proceeds unchecked. Cortisone inhibits the formation of immature plasma cells from primitive reticulum cells. Direct agglutination of bacteria on the surface of splenic plasma cells of immunized rats, as observed by Moeschlin and Demiral (1952), occurs, whereas the lymphocyte does not show this property. All the evidence pointed to the plasma cell, and not the lymphocyte, as the source of heterologous antibody which is synthesized while the cell is maturing to its adult form.

Tumour iso-antigens evoke a large plasma-cell reaction in the draining lymph nodes and spleen. Around the growing tumour there is little reaction, but when regression, natural or induced by a TI compound, is imminent, not only is the general plasma-cell response much augmented, but plasma cells accumulate in large numbers around and inside the periphery of the tumour. Both homografts and heterografts of living normal tissues evoke a qualitatively similar response, whereas heat-killed tumour tissue produces no plasma-cell reaction.

The carcinogens dibenzanthracene and methylcholanthrene and two non-carcinogenic TI compounds induce considerable plasma-cell formation in the local lymph nodes and spleen, whereas the non-carcinogens anthracene and phenanthrene are inactive in this respect. The mixing of tumour cells with excess of splenic or lymph-node pulp from transplanted tumour-bearing rats prevents *in vivo* growth of the cells, and splenic pulp from carcinogen (dibenzanthracene) treated rats behaves similarly, whilst in both instances the blood serum is inactive. Adequate early treatment with cortisone inhibits the formation of immature plasma cells otherwise induced by any of these methods.

These are the relevant findings which provide support for the thesis that the plasma cell is the major site of antibody formation in response both to normal and tumour iso-antigens. They demonstrate that transplantation immunity conforms, at least in part, with the immune state induced by foreign proteins generally. They underline the fact that carcinogenic hydrocarbons, and a few closely related compounds, alter the immunological state of the animal. The bearing of this on the mechanism of carcinogenesis will be discussed later, but the fact in itself indicates that the role of immunology in cancer studies extends far beyond the immunology of tissue transplantation.

It is clear that the increased tumour immunity induced by carcinogens is linked up with plasma-cell proliferation. The carcinogens tend to depress antibody production in general, which suggests that the plasma-cell response is to a specific antigen(s). Perhaps because the immune mechanism is already engaged the response to other antigens is weakened. The antigen(s) induced by TI compounds must be closely related to the tumour iso-antigens, for the antibody it elicits not only augments the action of tumour iso-antibody, but the spleen of the normal

animal treated with a carcinogen contains a factor having a lethal action on homologous tumour cells.

PROTEIN BINDING BY CARCINOGENS

One way in which these chemical carcinogens and the TI compounds in general could produce a new antigen is by haptenic binding with tissue proteins, a possibility already revealed by Landsteiner (1945), in linking aromatic amines to proteins by diazotization. Conjugates were formed with a new type of antigenic activity evoking an antibody which reacted with the conjugate, and either the protein or haptene alone. Moreover, it was shown by Landsteiner and Paroles (1935) that allergic sensitization of animals was possible with simple organic compounds and that the formation of a new antigen could be induced by benzene compounds *in vivo*. The knowledge that allergic reactions in skin and mucous membranes induced by many compounds and elements is due to the formation of such conjugates (allergens) is now established.

As will be seen, the *in vivo* binding of carcinogens is now also established, but many earlier facts suggested the possibility. Mayer (1930), Hueper (1936), Hueper, Briggs and Wolde (1938) and Reuterwell (1931) suggested that tumour formation might be the result of an allergic reaction to chemical carcinogens. When it is recollected that substances commonly producing clinical allergy are aromatic amines (such as aniline, benzidine and naphthylamine), tar products and even inorganic substances like nickel, arsenic and chromates, and that many compounds in these groups (including all the inorganic elements) are potentially carcinogenic, the view is not surprising. Moreover some allergic tissue reactions simulate tumour formation (for example, nasal polyps). Hueper, Briggs and Wolde (1938) failed, however, to demonstrate an immune response in rabbits, rats and dogs injected with 2-naphthylamine, or in men with bladder tumours. They used precipitin and complement fixation, and active and passive skin sensitization tests. Boyland and Warren (1937) also failed to obtain anaphylaxis in guinea-pigs injected with serum suspensions of dibenzanthracene, 3 : 4-benzpyrene, methylcholanthrene and 3 : 4 : 5 : 6-dibenzcarbazole. A normal dose response curve was obtained with methylcholanthrene-induced skin tumours and a fairly large percentage obtained with a single dose, so that the authors considered that sensitization is not involved for if so there should be a relatively greater number with two doses than with one. In spite of these negative results Hueper (1942) suggested that allergic mutation is the most acceptable theory of chemical carcinogenesis. The view is based on the great mass of evidence that many compounds related to the carcinogens, particularly the aromatic amines and azo dyes, are not only easily conjugated with proteins *in vitro* but produce sensitization and a state of local allergy *in vivo*. Strong morphological resemblances were also found in the development of the carcinogen-induced bladder polyps and in the allergic rhinitis leading to the nasal polyp. Moreover, Reuterwell (1935) produced tumour-like masses in the rabbit ear by repeated injections of ovo-albumin. Hueper regarded the tumour itself as remaining in a sensitized state, the reaction of the transplanted tumour (Schwartzman, 1935) to bacterial toxins being an expression of this.

Viewed retrospectively it appears that whilst these views established the probability of protein-binding by carcinogens the evidence for allergic mutation was

purely by analogy and experimental evidence was lacking. On the evidence it appears more likely that the carcinogens are, unlike some closely related compounds, not capable of allergic sensitization. However, they are able to form antigenic complexes.

The experiments cited above suggest that they are not able to form these active complexes *in vivo*, but there are possible flaws in this interpretation. If they are not carcinogenic to the species under test this might imply that specific protein binding did not occur. Boyland and Warren (1937) used guinea-pigs, a refractory species. Hueper, Briggs and Wolde (1938) did use one susceptible animal (the dog and 2-naphthylamine), but the humans with bladder tumours are obviously uncertain material. There are, however, several difficulties. One is that the *in vivo* carcinogen is now known to be a metabolite (2-amino-1-naphthol—Bonser and her colleagues, 1952) and therefore the appropriate substance was not used in the tests. Another is that the tissue sensitized, if any, would be the bladder mucosa, and not the skin. Ideally the sensitization tests would have to be made with the active metabolite on bladder mucosa. Moreover, antibodies, if produced by antigenic change in a small mass of tissue, would be unlikely to appear in detectable amounts in the blood stream and would have to be sought for locally (for example in lymph nodes). Again, antibodies of the sensitizing type even induced by such active agents as the tubercle bacillus (*see* Burnet and Fenner, 1949) are often difficult to detect.

Green (1954) thought he had obtained indirect evidence of auto-antibody production following massive exposure of the rabbit skin to a potent carcinogen, or by injecting large quantities of red cells mixed with the carcinogen. The idea of the former experiment was that the rabbit skin might share a common antigen with the red cell as it does in the mouse (Amos and his colleagues, 1954). In a few rabbits a 37° C saline panagglutinin for homologous rabbit red cells appeared in the serum, often erratically. Frequently a non-saline panagglutinin, active at room temperature and probably of the nature of a "cold" agglutinin, appeared. Active agglutinins could be eluted from the red cells in some cases. Their presence appeared to make the cells more sensitive to agglutination by other normal sera so that they became panagglutinable. This apparent production of a wide-ranging homologous antibody recalls the activity of protein conjugate antibodies, which often react with a wide range of substances related chemically or biologically to the haptene (*see* Hueper, 1942). The results were interpreted as being due to the formation of an iso-antigen in the carcinogen-treated skin. Unfortunately it has not proved easy to repeat these results (Green, 1956a) working in another laboratory and with rabbits of different origin. Other collateral evidence obtained on these rabbits suggests that complement fixing and precipitating antibodies for lipid antigens may appear in the serum. No firm conclusions can be drawn, but it seems likely that positive serological tests will eventually be consistently found.

Whilst firm proof of an immune reaction in the animal treated with a pure carcinogen is lacking, there is abundant proof that its protein conjugates are capable of antigenicity. This was known from Landsteiner's work and developed by Fieser and Creech (1929) and Creech (1952, 1955), Creech, Havas and Andre (1953). Creech worked in the hope of ultimately obtaining protection against carcinogenesis with appropriate antisera. Isocyanates of the carcinogens were coupled through the carbamide–NHCONH–linkage to bovine or horse serum

albumin. The linkage occurs to the E-amino groups of the lysine side-chain. The purified conjugates dissolve readily in water. A great variety of conjugates were made, the haptenes including 1 : 2-benzanthracene, 1 : 2 : 5 : 6-dibenzanthracene, 9 : 10-dimethyl-1 : 2-benzanthracene, 2-acetylaminofluorene, 4-dimethylaminostilbene and 2′-methyl-4-dimethylaminostilbene. It was shown by fractionation with ammonium sulphate and by electrophoretic analysis that the conjugates were homogenous, and not only had they different mobilities from the serum albumin, but with a mixture of similar conjugates containing different numbers of determinant groups, resolution was possible. The conjugates are highly fluorescent and the method can be used for labelling antibody globulins, tracing them *in vivo*. The precipitation reaction products were estimated by their nitrogen content and the amount of antigen in them by spectrophotometry. It was possible to show clearly that specific antisera were formed. There were some cross-reactions, such as between 1 : 2-benzanthracene and 3 : 4-benzpyrene, which were not unexpected as the only factors of importance in this immune reaction are the size and shape of the non-polar determinant group. The number of attached groups affected the antigenicity and there were indications of a minimal, maximal and optimal number. Specific reactions with the carcinogen were shown by conjugating it with an amino acid or peptide and using the inhibition technique (usually no direct precipitate is found) when sometimes all the serum antibody was removed. Creech does not appear to have attempted immunization with rabbit serum albumin conjugates. This is surprising as this would much more closely mimic the conditions that might obtain during normal carcinogenesis.

The chemical and immunological difficulties were successfully overcome, but purely biological difficulties were met in attempting to obtain passive immunity by treating rabbits with antisera. It was difficult to find an appropriate submaximal dose of 9 : 10-dimethylbenzanthracene to give a constant yield of tumours. Effective antisera could not be obtained from mice. The attempts to immunize mice with protein conjugates had one interesting result, for not one of these ever produced tumours under a wide variety of conditions. Nor did the isocyanates alone (except dibenzanthracene) or amino-acid conjugates. No reason for this is advanced, but it suggests that the carcinogen-determining centres were effectively blocked and could not, therefore, react with autologous tissue proteins. The results might indicate that specific protein binding can occur *in vivo* and that it is an essential stage in carcinogenesis, for if binding with the specific protein(s) is not possible tumour formation does not occur. An alternative explanation is that the heterologous protein conjugates are neutralized by the immune reactions they elicit. It would be interesting to know their metabolic fate and rate of excretion.

These results have a parallel with those of Green (1954). Here the TI activity of dibenzanthracene was completely abolished when it was given as the isocyanate itself, or as isocyanate coupled with horse serum albumin. It was suggested that the isocyanate bound with non-specific protein at the site of injection, thus preventing specific binding in the appropriate tissue. Further evidence was that dibenzanthracene mixed with homogenized rat lung or liver had much less TI activity, whilst mixed with spleen or lymph nodes had sometimes higher activity. These findings and those of Creech support the view that specific protein binding is responsible both for tumour inhibition and for carcinogenicity. The writer thinks that the protein conjugate behaves like an iso-antigen. He found (Green,