THE IMMUNOLOGIC DIAGNOSIS OF CANCER

MAURICE N. CAUCHI
M.D., M.Sc., Ph.D., D.P.H., F.R.C.P.A.
Monash University, Medical School,
Melbourne,
Victoria, Australia

In view of the explosive nature of the advance in immunology in the last decade, it is inevitable that immunological techniques would be used in an attempt to facilitate the diagnosis of cancer. The purpose of this communication is to put into perspective the various attempts that have been made in this field, and to outline the possible outcome of such research.

The Antigenic Constitution of Human Tumours

Before discussing the possible use of immunological techniques in neoplastic disease it is desirable to outline the ways in which tumour cells differ from normal cells antigenically. As seen in *Table 1*, loss of normal tissue antigens can be an important characteristic of tumours. *Fig. 1* shows a section of human colon stained with an anti-blood group antibody (anti-H) showing that the normal human colonic glands have abundant blood group antigen reactivity while the tumour is depleted of such antigens. Similar loss of histocompatibility, organ specific as well as other normal antigenic constituents have been described. *Fig. 2* shows a section of human colon stained with an anti-colon antiserum. The normal colon stains brightly, while the tumour glands do not.

In contrast to the loss of antigenic constituents by tumour cells described above, it has been shown in recent years that tumours possess antigenic consti-

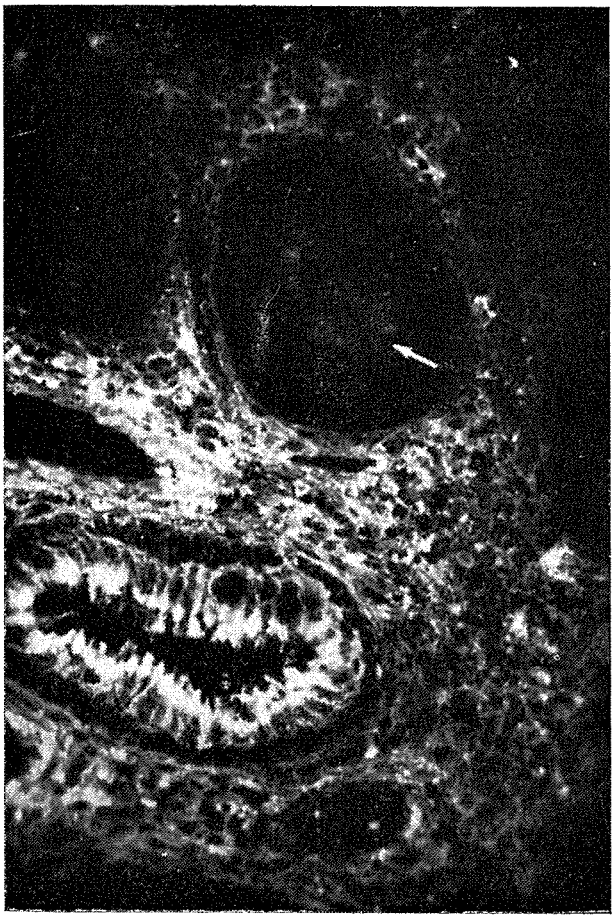

Fig. 1: Section of human colon carcinoma stained with an anti-blood group (anti-H) antiserum. Notice that normal glands stain brilliantly while tumour tissue (arrow) does not. × 210

tuents that are distinct from normal adult antigens. The presence of neoantigens in human tumours has now been adequately established in conditions such as Burkitt's lymphoma (Klein *et al.*, 1967); melanoma (Lewis *et al.*, 1969), neuroblastoma (Hellstrom *et al.*, 1968); osteogenic sarcoma (Morton and Malgren, 1968); bladder tumours (Bubenik *et al.*, 1970); Hodgkin's disease (Order *et al.*, 1971) and leukaemia (Mathe *et al.*, 1969).

In the serum of human foetus one can portant recent results on the identification and practical application of immune responses to the diagnosis of human tumours will be delineated.

Table 1

Antigenic Changes in Tumours

1. *Loss of Normal Antigens*, e.g.
   a. blood group antigen
   b. organ specific antigen
   c. histocompatibility antigen.
2. *Reversion to Foetal Type Antigenic Constitution*, e.g.
   a. Carcinoembryonic antigen
   b. α-foetoprotein production
   c. metaplasia-associated antigen.
3. *Gain of Tumour Specific Antigens*
   a. individual specific antigens, e.g. carcinogen induced.
   b. group antigen (cross-reacting) e.g. viral induced tumours, Burkitt's lymphoma, melanomas, carcinoma of the colon, etc.

α-Foetoprotein and Hepatoma

In the following pages the more imdetect by gel diffusion and immunoelectrophoresis techniques a globulin which migrates in the α_1 region *(Fig. 3)*. Such a globulin is not detected in normal adult sera. In 1964 Tatarinov reported the presence of this foetoprotein in the sera of patients with primary hepatoma. *Fig. 4* shows the gel diffusion pattern of foetal serum and serum from patients with hepatoma. This is a simple test for hepatoma, being positive in 50-80% of patients who have it. This test is being used at the moment in a WHO survey of the incidence of hepatoma in Africa (Muir, C.S., personal communication). The recent demonstration of minute amounts of foetoprotein by radioimmunoassay techniques in normal adult sera (Rouslahti and Seppala, 1971) does not detract from the practical usefulness of this test in clinical practice.

The significance of a foetoprotein reemerging in the adult circulation is a very intriguing one. Using tissue culture techniques Gitlin and Boesman (1967) have shown that the protein can be produced by foetal liver cells. We have localised

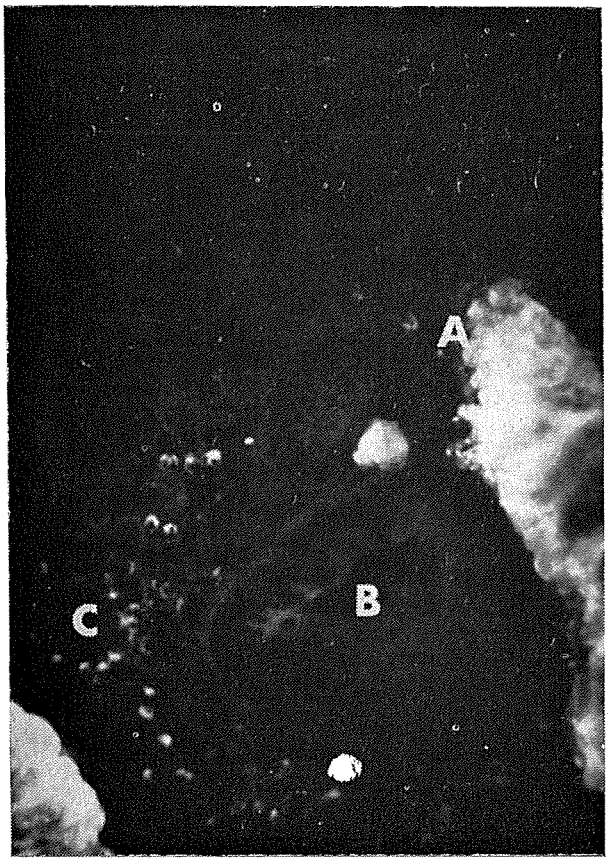

Fig. 2: Section of human colon carcinoma stained with a specific anti-colon antiserum to show absence of staining in the tumour glands (B) compared to normal (A), indicating loss of organ-specific antigens. × 210

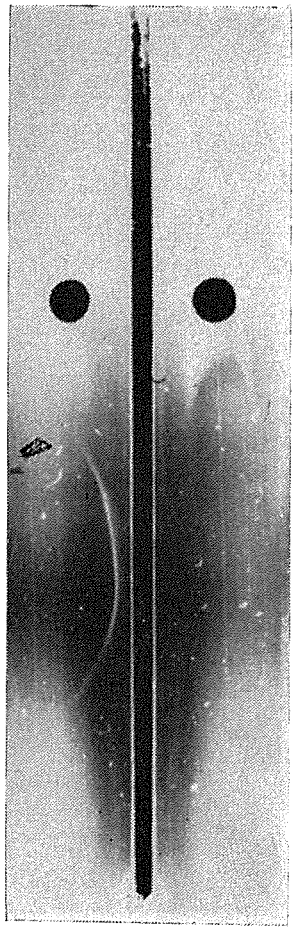

Fig. 3: Immunoelectrophoresis of human foetal serum reacted with an antifoetoprotein antiserum: α-foetoprotein migrates in the α_1 globulin region

α-foetoprotein in human foetal liver cells and hepatoma using indirect immunofluorescent techniques (Cauchi and Nairn, 1972a). Briefly, fresh tissues were snap frozen in a liquid nitrogen-isopentane mixture and 6μ thick sections were cut, stained with a specific rabbit anti-α-foetoprotein, washed and a fluorescein-conjugated goat-anti-rabbit globulin was added. Using this technique, the presence of cells which are capable of binding with an anti-α-foetoprotein anti--serum, (and hence presumably containing α-foetoprotein) could be demonstrated in both foetal liver *(Fig. 5)* as well as in sections from a hepatoma, but not in normal tissues.

Testing for α-foetoprotein has now become a routine test in a large number of hospital laboratories and has proven to be the most simple specific test for human hepatoma. The test is indicated in patients with:

a. a hepatic mass of unknown aetiology,

b. cirrhosis or chronic liver disease of any type,

c. presumed "metastatic" cancer where a primary tumour cannot be detected (Stillman and Zamcheck, 1970).

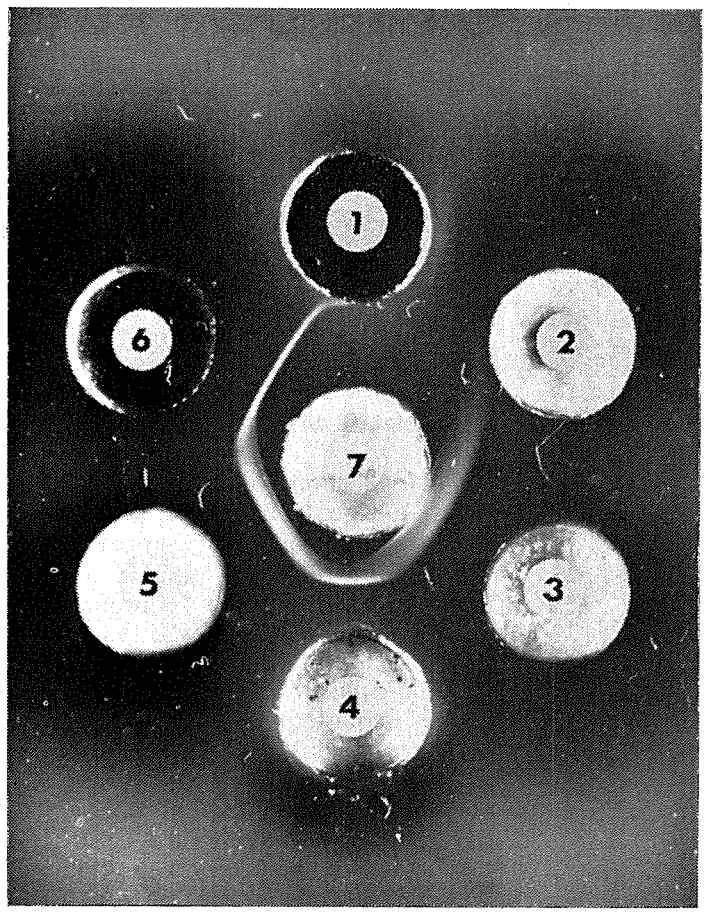

Fig. 4: Gel diffusion showing immunological reaction between a specific anti-foetal serum (centre well) and human foetal serum (wells 3 and 6), amniotic fluid (well 4), and serum from a patient with hepatoma (well 5). There is no reaction with normal adult serum (well 2).

Carcinoembryonic Antigen (CEA) and Gastrointestinal Cancer

In 1965, Gold and Freedman obtained an extract from carcinoma of the colon and succeeded in immunizing rabbits to produce an antibody which reacted specifically with colonic cancer but not with normal tissue. Later on this antigen was also found in other tumours of entodermal origin, e.g. oesophagus, stomach, pancreas, as well as in metastases of such tumours in the liver. As this antigen was shown also to be present in the normal gastrointestinal tract of human foetuses of 2-6 months gestation, this antigen was called carcinoembryonic antigen (CEA).

The real impetus to the clinical application of this knowledge came with the discovery that CEA is present in detectable concentrations in the sera of patients with cancer of the gastrointestinal tract. Using a sensitive radioimmunoassay technique capable of detecting 2.5ng of CEA/ml (1 ng = 10^{-9} gm) Gold and his associates (Thompson *et al.*, 1969) have shown that the level of CEA can be higher than 300 ng/ml in some patients with carcinoma of the colon. 97% of patients with carcinoma of the colon had significantly higher than normal levels of CEA in the serum.

Since these original discoveries a number of tumour immunologists have been engaged in the production of a simple and reliable immunoassay test for the detection of carcinoma of the colon. At the moment we are in the process of providing a simple radiometric test that can be

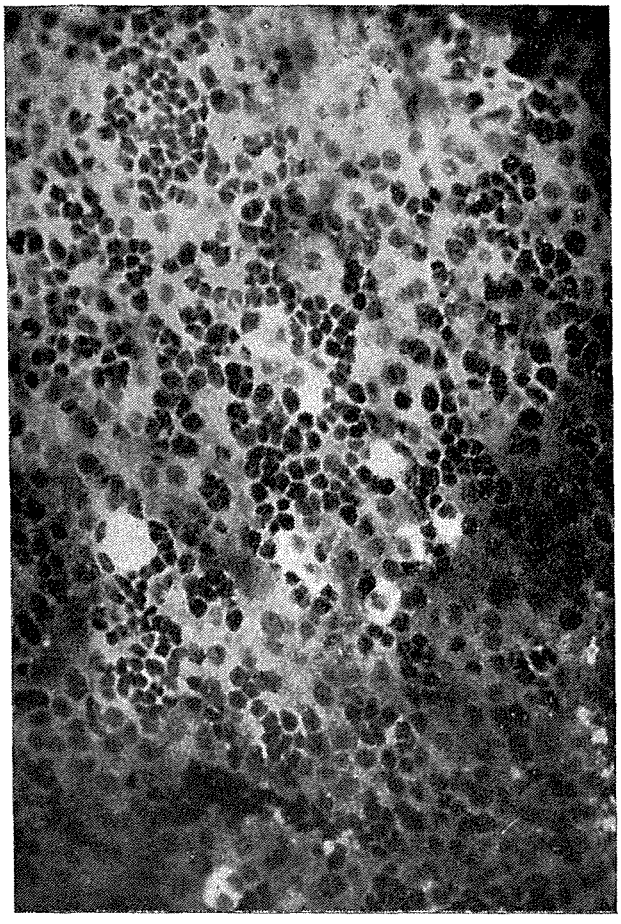

Fig. 5: Frozen section of foetal human liver stained with anti-foetal serum showing fluorescence of hepatic cells × 210

applied to the detection of CEA (Reid and Cauchi, 1972).

The presence of *antibody* specific to CEA in the serum of some patients with carcinoma of the colon has been described (Gold, 1967) but data on this topic are conflicting (Lo Gerfo *et al.*, 1972). It is conceivable that a large tumour mass can act as an antigenic "sink" absorbing all serum antibody activity. It is more likely, however, that the host is in some way incapable of responding to the large paralysing dose of circulating antigen, giving rise to a situation analogous to "immunological paralysis" (Mitchison, 1964). It is unlikely that the host is inherently incapable of responding to foetal-type antigen if these were presented in a suitable manner, since we have shown that a host can produce specific anti-foetal antibody activity when immunized with foetal tissues (Cauchi and Nairn, 1972b). *Fig. 6* shows a foetal intestinal cell stained by a specific isologous antifoetal intestine antiserum, showing the typical membrane staining. As illustrated in this figure, CEA is largely a surface bound antigen.

Other "Embryonic" type Antigens Associated with Neoplastic Disease

Table 2 summarises the data on the various antigenic systems described in human tumours relating to embryonic type antigens. The placental alkaline phosphatase (Regan isoenzyme) is found in the sera of 4-5% of patients with various malignancies. Stohlbach *et al.* (1969) found an alkaline phosphatase isoenzyme in the serum of a patient (Regan by name) but not in normal adult tissues. It is chemically and immunologically indistinguishable from placental alkaline phosphatase. It appears to be produced by the tumour itself. The heterophile foetal antigen described by Edynak *et al.* (1970) is non-species-specific and seems to be present in a large variety of tumours, benign or malignant. To date these tests have not found widespread clinical application, but might be useful eventually as non-specific tests for the detection of cancer.

Metaplasia-associated Antigen

It has been shown that intestinal metaplasia which occurs in gastric tumours in man (Ming *et al.*, 1967) and animals (Feit *et al.*, 1967) may be associated with the loss of adult-specific antigens and the re-emergence of an intestinal-type antigen (de Boer *et al.*, 1969; de Boer and Cauchi, 1971). This can be shown quite readily in the experimental animal following such carcinogenic agents as X-irradiation. Such a distribution of intestinal antigen in the stomach is incidentally the normal finding

in the foetus (de Boer *et al.*, 1969), indicating that this antigenic change can be considered as a reversion to a foetal antigenic distribution. *Fig.* 7 shows a frozen section of human carcinoma stained with an antiserum to show intestinal-type antigen.

The search for antigenc constituents in gastrointestinal secretions which are specific for cancer appears attractive. Hakkinen and Viikari (1969) reported a new test for the diagnosis of gastric cancer based on the detection of a sulphoglycoprotein in the gastric juice of patients with gastric cancer. This test was positive in 96% of patients with gastric cancer compared to 9.4% of "control" patients. The relatively high incidence of false positive tests in conditions like benign peptic ulcer (14%) limits the usefulness of such a test until it can be demonstrated that the test can be made more specific for gastric neoplasm.

Tumour Antigens in Lymphoma and Leukaemia

In 1964, Epstein *et al.*, found intracellular DNA virus, now known as the Epstein Barr Virus (EBV) in cultured cells from a patient with Burkitt's lymphoma. Antibodies to EBV were found in a large proportion of normal people tested, and their distribution is world wide. However, high levels of anti-EBV antibody titres (more than 1:160) have been found only in three conditions viz: infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma (Henle and Henle, 1969). Klein *et al.*, (1966) using indirect immunofluorescent techniques demonstrated 7S immunoglobulins in the sera of patients with Burkitt's lymphoma that reacted with surface antigens of lymphoma cells, but not of normal cells. Other antibodies reacting with cytoplasmic constituents of

Table 2

Embryonic-Type Antigenic Constituents in Human Tumours

Antigen	*Cancer*	*Localisation*	*Ref.*
α-foetoprotein	Hepatoma, teratoma	serum, tumour	Abelev *et al.* 1967
Carcinoembryonic (CEA)	GIT cancer	cell membrane (glycocalix) serum	Gold and Freedman 1965
Foetal Sulphoglycoprotein	Gastric cancer	gastric juice	Hakkinen 1966
Tumour glycolipids	GIT cancer	tumour	Rapport *et al.* 1959
T-globulin	Various cancers	serum	Tal *et al.* 1970
Regan isoenzyme	Digestive tract and various malignant tumours	tumour cells	Stolbach **et al.** 1969
α_2-ferroprotein	Malignant tumours of childhood (nephroblastoma, neuroblastoma, teratoma, etc.)	serum	Buffe *et al.* 1970
Heterophile foetal Antigen	Various tumours	serum	Edynak *et al.* 1970

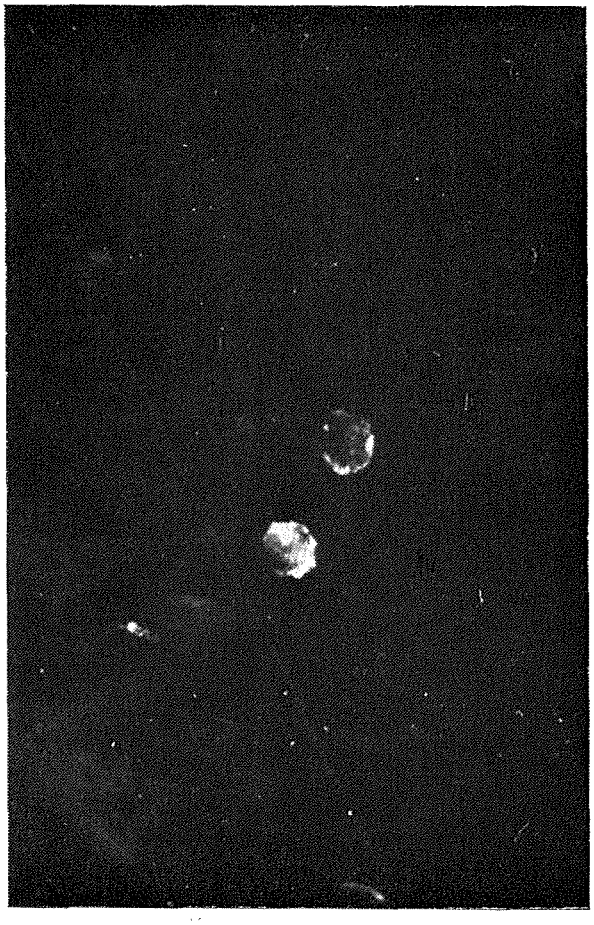

Fig. 6: A foetal intestinal cell stained by an anti-foetal serum to illustrate cell membrane immunofluorescence × 540

lymphoma cells have been described by Henle *et al.*, (1969). The following antigens have now been described in Burkitt's lymphoma cells (Clifford, 1972).

1. *Early antigen* (EA): a soluble antigen which appears intracellularly soon after infection with EBV.
2. *Membrane antigen complex* (MA): on the surface of Burkitt's lymphoma cells, probably representing viral envelope constituents.
3. *Viral capsid antigen* (VCA): a "late" viral product present in the cytoplasma of Burkitt's lymphoma cells.

Although such antigenic systems have not been used for the diagnosis of Burkitt's lymphoma, further studies might indicate that changes in the levels of antibody might be related to prognosis following surgery, chemotherapy etc. For example, the highest levels of anti-VCA titres were found in patients with large tumours, while low levels were found in patients with long term remission. On the other hand, high anti-MA titres correlated better with a small tumour mass, indicating absorption of the antibody by the tumour mass (Henle *et al.*, 1969). A rise in anti-MA antibody titres usually occurs in patients who respond well to chemotherapy or local irradiation (Einhorn *et al.*, 1970).

During the last few years we have been working on the antigenic constitution of leukaemic cells and their ability to stimulate an immune response in the patient. There is little doubt now that leukaemia-specific antigens do exist (Viza *et al.*, 1969) and that the patient can respond to such antigens by the production of antibodies or a cell-mediated immune response (Powles *et al.*, 1971). More work is, however, required before these data can be of prognostic value. In particular with the more widespread use of immunotherapeutic techniques, the accurate measurement of the immune status will be an essential parameter in detecting host response. That such a response is necessarily complex, time — as well as dose — related has been shown extensively in experimental systems (Cauchi, 1972).

Immunologic Detection of Tumour Secretions

Certain tumours secrete substances or hormones which can be detected by sensitive immunological techniques. For example, radioimmunoassay techniques have been used to measure chorionic gonadotrophin from chorionepitheliomas, calcitonin from thyroid carcinoma, gastrin from pancreatic tumours producing the Zollinger Ellison syndrome, insulin from pancreatic tumours, and ACTH from Pituitary and lung tumours (WHO, 1972). These methods may eventually become useful in

Fig. 7: Section of irradiated stomach stained with an anti-colon antiserum to show the presence of intestinal-type antigens × 340

the diagnosis of hormone-secreting tumours.

Macrophage Slowing Factor (MSF) in Cancer

It has been shown recently that blood lymphocytes from patients with neoplastic disease were sensitized to an antigen which could be extracted from normal brain (Field and Caspary, 1970), or from a variety of malignant tumours (Caspary and Field, 1971). Following interaction of lymphocytes with the antigen, a factor is released which slows the migration of macrophages by about 14-20% of normal. This is a relatively simple test which might eventually be useful as a non-specific test for cancer.

Autoantibodies in Neoplastic Disease

It is very difficult to interpret the significance of autoantibodies to normal tissue constituents in patients with neoplastic disease. Firstly some forms of neoplastic disease have a greater tendency to occur in patients with specific autoimmune diseases. For example carcinoma of the colon is about 20 times more common in patients with ulcerative colitis than in the general population. Secondly, tumours and autoimmune disease process might be associated in some ill defined way, e.g. the presence of thymoma and myasthenia gravis. Thirdly, there is the well known association between autoimmune haemolytic anaemia and conditions like Hodgkin's disease, lymphatic leukaemia and more recently other tumours, e.g. ovarian dermoid cyst (Baker *et al.*, 1968). Fourthly, and perhaps more specifically, tumours might stimulate the production of antibodies that cross react with normal constituents. The finding of a smooth muscle antibody in 67.5% of patients with malignant disease (Whitehouse and Holborow, 1971) falls in this category. This work needs confirmation. We have not been able to confirm such findings (Tannenberg *et al.*, 1972).

The destruction of a large mass of tissue by irradiation, chemotherapy etc., might itself produce sufficient stimulus for the production of autoantibodies. Antibody formation against skin (Quismorio, *et al.*, 1971), leucocytes (Price *et al.*, 1969), nuclei (Weir, 1966), etc. have been shown to occur after tissue necrosis following heat, radiotherapy, or infarction.

Although interesting biologically, such autoimmune phenomena are unlikely to be of practical value in the diagnosis of cancer.

Conclusions

One can envisage a number of ways in which immunologic tests for cancer can be useful in future clinical practice.

a. In the diagnosis of tumours prior to surgical biopsy. So far this is a routine test only in the case of hepatocellular carcinoma.

b. In the detection of recurrence after surgical removal of a primary carcinoma. This has obvious application in gastrointestinal tract cancer, hepatoma, etc.

c. In screening programmes for the detection of cancer where there is an endemically high incidence, e.g. hepatoma, Burkitt's lymphoma in Africa.

d. In determining the prognosis of tumour patients. It has been shown that antibody titres vary according to tumour mass, the presence of metastasis etc. This may be correlated with ultimate prognosis.

e. Using radioactively labelled highly specific antitumour antibodies, the localisation of tumour metastasis, involved lymph nodes, etc. would provide a rapid objective assessment of tumour spread.

f. As an objective test in the control of tumours by immunotherapy.

In painting an optimistic future for the role of tumour immunology in clinical practice one must not forget that a great deal of fundamental work is still required to delineate the exact biological significance of tumour-specific antigens. Such work at the moment is being carried out in major tumour immunology centres throughout the world, and one would hope that the next ten years will witness the same rate of growth in the application of immunological techniques to the cancer problem that has been characteristic of basic immunological research in the last decade.

References

ABELEV, G.I., ASSECRITOVA, I.V., KRAVESKY, N.A. et al. (1967). Int. J. Cancer, **2**, 551.

BAKER, L.R.I., BRAIN, M.C., AZZOPARDI, J.G., WORLLEDGE, S.M. (1968). J. Clin. Path., **21**, 626.

DE BOER, W.G.R.M., FORSYTH, A., NAIRN, R.C. (1969). Brit. Med. J., **3**, 93.

DE BOER, W.G.R.M., CAUCHI, M.N. (1971). Pathology, **3**, 291.

BUBENIK, J., PEARLMAN, P., HELMSTRON, K., MOBERGER, G. (1970). Int. J. Cancer, **5**, 39.

BUFFE, D., RIMBAUT, C., LEMERLE, I, SCHWIRSGUTH, O., BURTIN, P. (1970). Int. J. Cancer, **5**, 85.

CASPARY, E.A. and FIELD, E.J. (1971). Brit. Med. J., ii. 613.

CAUCHI, M.N. (1972) in preparation.

CAUCHI, M.N. and NAIRN, R.C. (1972a). Pathology, in press.

CAUCHI, M.N. and NAIRN, R.C. (1972b). J. Immun. Conm. in press.

CLIFFORD, P. (1972) in Recent advances in Cancer and Radio therapeutics: Clinical Oncology. ed. K.E. Halnan, (Churchill, Livingstone), p. 113.

EDYNAK, E.M., OLD, L.J., VRANA, M., LARDIS, M. (1970). Proc. Amer. Assoc. Cancer Res., **11**, 22

EINHORN, L., KLEIN, G. and CLIFFORD, P. (1970). Cancer **26**, 1013.

EPSTEIN, M.A., ACHONG, B.G. and BARR, Y.M. (1964). Lancet, i, 702.

FEIT, J., SVEJDA, J., SOCHOROVA, M. (1967). Neoplasma, **14**, 285.

FIELD, E.J. and CASPARY, E.A. (1970). Lancet, ii, 1337.

GITLIN, D. and BOESMAN, M. (1967). J. Clin. Investig., **46**, 1010.

GOLD, P. (1967). Cancer, **20**, 1663.

GOLD, P. and FREEDMAN, S. (1965). J. Exp. Med., **121**, 439.

HAKKINEN, I.P.T. (1966). Scand. J. Gastroenterol, **1**, 28.

HAKKINEN, I. and VIIKARI, S. (1969). Ann. Surg., **169**, 277.

HELLSTROM, I., HELLSTROM, K.E., PIERCE, G.E., BILL A.H. (1968). Proc. Nat. Acad. Sci., **60**, 1231.

HENLE, W. & HENLE, G. (1969). E. Afr. Med. J., **46**, 402.

HENLE, G., HENLE, W., CLIFFORD, P. et al, (1969). J. Natn. Cancer Inst., **43**, 1147.

KLEIN, G., CLIFFORD, P., KLEIN, E., STJERNSWARD (1966). Proc. Nat. Acad. Sci., **55**, 1628.

KLEIN, G., CLIFFORD, P., KLEIN, E., et al (1967). Nat. Cancer Inst., **39**, 1027.

LEWIS, M.G., IKONOPISOV, R.L., NAIRN, R.C. et al (1969). Brit. Med. J., **3**, 547.

LA GERFO, P., HERTLER, F.P., BENNETT, S.J. (1972). Inst. J. Cancer, **9**, 344.

MATHE, G., AMIEL, J.L., SCHWARZENBURG, L. et al (1969). Lancet. **2**, 697.

MING, S.L., GOLDMAN, H. & FREIMAN, D. (1967). Cancer **20**, 1418.

MITCHINSON, N.A. (1964). Proc. Roy. Soc. B., **161**, 275.

MORTON, D.L., MALGREN, R.A. (1968) Science, **162**, 1278.

ORDER, S.E., PORTER, M., HELLMAN, S. (1971). New Eng. J. Med., **285**, 471.

POWLES, R.L., BALCHIN, L.A., HAMILTON FAIRLEY, G., ALEXANDER, F. (1971). Brit. Med. J., i, 486.

PRICE, W.R., WOOD, M., CHILDERS, D. (1969). Amer. J. Surg., **118**, 871.

QUISMORIO, F.P., BLAND, S.L., FRIOU, G.J. (1971). Clin. Exp. Immunol., **8**, 701.

RAPPORT, M., GRAF, L., SKIPSKI, V. et al. (1959). Cancer, **12**, 438.

REED, R. & CAUCHI, M.N. (1972). Paper to be read at the Australian Society for Immunology Meeting, Sydney.

ROUSLAHTI, E., SEPPALA, M. (1971). Int. J. Cancer, **8**, 374.

STILLMAN, A. & ZAMCHECK, N. (1970). Amer. J. Dig. Dis., **15**, 1003.

STOHLBACH, L.L., KRANT, M.J., FICHMAN, W.H. (1969). N. Eng. J. Med., **281**, 757.

TAL, C. & HALPERIN, M. (1970). Is. J. Med. Sci., 6, 708.
TANNENBERG, A.E.G., MULLER, H.K., CAUCHI, M.N. & NAIRN, R.C. (1972) in preparation.
TATARINOV, Y. (1964). Vop. Med. Khim., 10, 90.
THOMPSON, F., KRUPEY, J., FREEDMAN, S. et al (1969). Proc. Nat. Acad. Sci., 64, 161.
VIZA, D.C., BERNARD-DEGANI, O., BERNARD, A. (1969). Lancet, Aug. 30, 1969.
WEIR, D.M. (1966). Immunological reactions after tissue injuries ("Research in Burns" ed. A.B. Waller & A.N. Wilkinson, pp 389. E. & S. Livingstone, Edinburgh & London.
WHITEHOUSE, J.M.A. & HOLBOROW, E.J. (1971). Brit. Med. J. 4, 511.
W.H.O. Technical Report Series No 496: Clinical Immunology. Geneva, 1972