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Effects of cytotoxic antibodies on tumour cells and their possible role in controlling metastases

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A. Introduction

Much of the mortality of malignant neoplasms is a result, direct or indirect, of the dissemination of tumour cells to points at some distance from the primary growth, usually by way of blood vessels and lymph vessels. It seems likely therefore, that if one could learn to limit this mode of spread of carcinomas and sarcomas, then severe suffering and perhaps even death could be postponed or prevented altogether.

Available information indicates that the vascular dissemination of tumours is a complex process [1, 2]. First the viable neoplastic cells invade the lumen of the blood or lymph vessel and next they frequently form obturating masses in the smaller vascular channels, often at some distance from the primary malignant growth. Clotting (thrombosis) of blood elements near the tumour cell emboli appears to favor the intravascular multiplication of these cells [3]. Then the neoplastic cells must grow out through the vessel wall to form discrete new tumour colonies (metastases) which gradually develop their own blood supply.

The purposes of this paper are to survey existing evidence which indicates that:

1. Many more tumour cells circulate than implant and grow.
2. Endogenous cytotoxins (immune and otherwise) exist which have the potentiality of killing neoplastic cells in the circulation.
3. Inhibition of the clotting mechanism will, in all probability, make these cytotoxins more effective.
4. Some malignant neoplasms, particularly cancer of the breast, have a clinical and pathological course which strongly suggests that tumour

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metastases are being suppressed. Clinical-pathological as well as experimental evidence indicates that malignant cells may be dormant for long periods of time and then metastasize after several years of apparent biological (host) control of their dissemination.

Experiments will be described which have helped to establish some of these points and studies will be summarized which are now in progress and which are designed to gain a better understanding of several potential endogenous cancer cell cytotoxic systems.

B. Circulating tumour cells and metastatic implants

Many observations indicate that only a small proportion of the neoplastic cells which enter the blood stream produce progressively growing implants at a distant site [4, 5, 6, 7]. Granting the technical difficulties in identifying viable cancer cells from the blood [8, 9] it is likely that most circulating malignant cells are unable to survive [10, 11]. In fact in some forms of human malignancies it appears that blood borne tumour cells are almost invariably present but, it seems, that their concentration or frequency has little relationship to the prognosis or to the development of distant metastases [12].

There are at least two major factors which probably act to limit the number of viable implants which develop when cancer cells circulate. Either (A) the tumour «virulence» [13] (or growth energy [14]) may not be sufficient to overcome the vicissitudes of embolization and subsequent growth through the vessel wall or (B) the host may be able to utilize one or more cytotoxic reactions to injure or destroy the circulating cancer cells before they can gain a foothold in a fibrin-rich thrombus and start to multiply [3]. In all likelihood both of these factors often influence tumour growth simultaneously [15].

The presence of circulating cytotoxins to circulating cancer cells is not only supported by the evidence suggesting that only a few malignant cells survive dissemination from the primary tumour to a distant site but also by observations suggesting that many of these cells isolated from blood are degenerating [16] and will not grow in tissue culture.

C. Immune serum cytotoxins and cancer cell injury

Cytotoxic antibodies constitute one biological system which must be considered in any analysis of the potential of the host to destroy circulating cancer cells. Although little evidence has been recorded indicating the existence of cytotoxic antibodies to human cancer cells either in

cancer patients or normal patients, this may be due to a lack of available clear cut quantitative methods to demonstrate them.

The mounting evidence that antibodies to a number of organ and tissue cells of the host can be demonstrated in human diseases [17] and the recent demonstration that some of these immune globulins are cytotoxic [18, 19] make it now seem more plausible than it was previously, that antibodies to autologous cancer cells may exist in some cancer patients. Furthermore, the cytopathic potency of circulating antibody in transplantation phenomena has been recently demonstrated [20]. There is also increasing evidence that at least some induced neoplasms in inbred mice are antigenically distinct from host tissues [21, 22], not merely antigenically deleted. These observations strengthen the stimulus to search for circulating autoimmune bodies with some specificity to cancer cells, especially in patients in which the primary tumour has been removed recently.

Study of model systems involving heterologous antibody globulin to transplantable ascites tumour cells [23, 24, 25] have given abundant information about the nature of the immunological cytotoxic action which might be expected to damage or kill circulating cancer cells in the patient.

It is evident that these antibodies are mainly effective in conjunction with serum complement (26, 27, 28), that they produce a rather characteristic metabolic [26, 29, 30] and morphological [26, 27, 30, 31, 33] type of cell damage. Furthermore they are mainly specific for the «membrane» components of the cell [30, 31, 34].

For the most part, it appears that *in vivo* these heterologous tumour antibodies are cytotoxically effective when they come in contact with «free floating» cancer cells as they do in the peritoneal cavity [35, 36] or as they would be expexted to do in the blood stream assuming that the antibody and the tumour cells were present simultaneously.

The lack of tumour localization of these antibodies and their ineffectiveness in retarding the growth rate of an established solid tumour which has developed its own blood supply [35, 36] suggests that this type of humoral mechanism, if it were applicable to man, would be effective mainly against cancer cells in the circulation and not against an existing vascularized metastasis. That this may be too pessimistic a point of view is suggested by sporadic recent reports [37, 38, 39, 40].

The small lymphocyte assumes importance when one considers the overall possibilities of the host's *in vivo* immune damage to his circulating malignant cells. The role of the circulating and colonizing small lymphocytes which appear to be derived from the pyroninophilic «antibody producing» cells [41, 42] has not yet been clearly established in

relation to immune cell damage. Nevertheless considerable evidence now indicates that the cytotoxic reactions in some kinds of autoimmune disease [18, 19] and in transplantation immunity [20, 43] may involve a combined effect of circulating antibody working with «sensitized or prepared» small lymphocytes. The phenomenon of cytotoxicity produced by «prepared» small lymphocytes is receiving particularly enlightening study *in vitro* by *Rosenau* and *Moon* [44, 45, 46]. Their approaches may ultimately aid in the study of the tumour patient's cellular reaction to his own tumour cells.

It is clear that there is much work to be done if we are to clarify the role of immune cytotoxic reactions to cancer cells in the blood of cancer patients.

Preliminary experiments in our laboratory in Chicago indicate that some patients with carcinoma of the breast appear to have demonstrable quantities of circulating cytotoxic substance when their sera, obtained shortly after removal of the primary tumour, are tested against the D-30 human breast cancer cell line. These observations suggest that there may be a rebound phenomenon when the tumour is removed so that cytotoxic substances previously bound by cancer cells or their products are found to be circulating in the blood. Whether this involves tumour specific antibodies which become more easily demonstrable when a major supply of tumour antigen is removed remains to be established.

At present, at the Kocher Institute, we are concentrating our efforts on approaches which we hope will characterize and quantitate cell damaging substances from the sera of «normal» individuals as well as from cancer patients. Methods which promise to yield quantitative results [47, 48, 49, 50] are being applied to see if cytotoxic activity can be related to identifiable proteolytic components of the serum proteins.

In these investigations certain fractions from the pooled sera of «normal» individuals as well as from cancer patients are receiving special attention. For example, the precipitate which appears on dialysis of either «normal» or cancer patient's sera with P. 005 M PO_4 buffer at pH 7 [51] appears to have unusually high activity as a proteolytic esterase fraction when tested against either TAMe [52] or ATEe [53] as a substrate. Furthermore we have recently isolated a fraction from pooled normal human serum by means of DEAE chromatography of isoelectrically precipitated euglobulin. This fraction which has extraordinary activity as a proteolytic substance has apparently been overlooked by previous investigators since it appears when the ionic strength is raised to 0.5 M NaCl and the pH is raised to 8.0 after the serum con-

taining column has been exposed to a pH gradient from 7.5 to 4.5 as the electrolyte was increased from 0.005 to 0.15 M.

Although evaluation of this fraction is not complete it has a high specific activity for TAMe esterase, seems to show 2 or 3 separate proteins on starch block electrophoresis and immuno electrophoresis and has well localized TAMe activity near the position of the alpha I globulins on the starch block. Ultracentrifugation also shows that it is not a homogeneous fraction but it has no macroglobulin component. All of these analyses indicate a proteolytic enzyme different from those usually separated from Euglobulin.

Both of these active samples are now being evaluated for cytotoxic activity by several methods and it is hoped that this type of analysis can extend on to cancer patients sera before and after surgery.

It is surprizing to find that there has been almost no recorded study of the cytotoxicity of the cancer patient's serum, with or without lymphoid cells (from lymphnodes and blood), against the patient's cancer cells, removed at surgery. It is important to develop methods which will make this possible.

C. Non-specific Cytotoxins of the Serum. Can they injure human cancer cells?

In the previous section of this paper it was indicated that certain protein fractions of cancer patients' sera are also being studied from «normal» control sera in the hope of confirming the fact that the sera from cancer patients do, in fact, develop increasing cytotoxicity to certain malignant cell lines, especially in the early interval following surgical extirpation of their primary tumour.

Although a number of cytotoxins to tissue culture cells have been described in normal human serum [54–61] and especially in serum from schizophrenic patients [62, 63], in only one instance [55] were any of these substances tested against human cancer cell lines and in practically no instance have modern methods of protein and proteinase chemistry been reported to define these in relation to known active proteolytic substances in human serum. Nevertheless, a great deal is known about these systems and certain summarizing statements can be made.

1. More than one substance is probably identifiable. Bjorklund's substance [55] is a prealbumin which does not require complement for its cytotoxic action but Bolande's [60], Willheim's [56] and Ginsburg's [61] substances require only one component of complement. On the

other hand Landy's [57] as well as Federoff's [63] material require all components of complement.

2. It is noteworthy that Federoff's cytotoxin to strain L cells apparently requires human complement whereas most immune sera, even so-called natural or homologous antibody, can usually be activated by complement from any of several species [63].

3. In many respects, however, as Federoff has recently reported, from a functional stand point his heat stable cytotoxic material closely resembles an antibody [63].

It is obvious that much more work is necessary in order to characterize chemically these natural or «normal» cytotoxins, to define their inhibitor systems, if any, to ascertain whether they are active on any or many *human* cancer cell lines and to find out if they increase or decrease in cancer patients sera in response to surgery, in relation to rate of cancer cell dissemination, etc.

D. Other Factors Controlling Cancer Cell Spread

Obviously many other factors have to be considered when one tries to understand why cancer cells spread rapidly in one patient and slowly or not at all in the next patient who seems to have a comparable primary malignant growth.

Some of these factors are being systematically studied by the Fisher brothers at the University of Pittsburgh [64–69], by J. Sumner Wood jr. at Johns Hopkins [2, 3, 70, 71], by Cole and McDonald [7, 13, 15, 71] at the University of Illinois. These are 3 of the currently active American teams who are trying to understand the mechanisms controlling cancer cell dissemination in its broadest context.

Without attempting a comprehensive review of this rapidly changing field of investigation the following tentative generalizations can be listed as guidelines for future work:

1. All three of the groups listed above have done much of their work using non inbred strains of rats and rabbits and long established tumour lines (Walker 256, and V-2 carcinoma) which are probably not as compatible with their hosts as the usual «spontaneous» malignancy of man, although in all probability the «virulence» or growth energy of these experimental tumours may be considerably greater than the average human cancer. Only a few recent studies of the mechanisms of metastases have been performed in inbred animals using an «induced» or «spontaneous» transplantable malignant tumour which arose in that same inbred line. For this reason some caution must be used in applying

the results of these experimental studies to the spread of a primary tumour to a distant secondary site.

2. Embolization [1, 2], tumour cell sticking to endothelium [1, 2], and thrombosis about the embolized cells [2, 3] seem to be very important components of the sucessful spread of the tumour.

3. Cortisone treatment [70] and several other types of manipulation of the experimental animal [64–68, 71] often increase the number of «metastases» which occur.

4. The number of tumour cells injected intravenously [15] or the size of the subcutaneously growing tumour [11] are, within certain limits, directly related to the number of metastases forming.

5. The phenomenon of the «dormant» tumour cell [72, 73] is demonstrable in the experimental animal but its explanation is not apparent.

It is not known whether the results of all of these studies would be expected if the host were reacting to its own tumour but it is evident that there are many factors to consider when one tries to analyze why some cancer cells in the circulation develop into metastases.

E. Coagulation and cancer cell dissemination

One of the most consistent clinical-pathological [74, 75] and experimental [1, 2, 10] observations in relation to metastases is that there is formation of a true thrombus adjacent to the tumour cell emboli which potentially form an implant after blood stream invasion or intravenous injection. Furthermore there is a great quantity of evidence indicating that therapy with anticoagulants or with fibrinolysins will rather remarkably decrease sucessful metastases [63, 77, 78, 79, 80]. Recent evidence obtained using tissue culture methods [81] suggests that all of the metastasis-preventing effect of heparin and much of the dicumarol and fibrinolysin effect is due to action on the clotting mechanism and not to direct toxic action on the tumour cells.

These observations indicate that cytotoxic substances, including antibodies to specific cancer cell antigens, would be much more effective if given with an anticoagulant. Unfortunately, to my knowledge, no work has been reported with this experimental approach.

It is noteworthy that this is just the opposite effect one strives for in trying to localize iodine 131 labelled anti-fibrinogen in tumour bearing animals [82, 83]. In this therapy epsilon amino caproic acid (EACA) has been used to inhibit the «plasmin» mechanism and thereby prevent fibrinolysis in or near the tumour. It is evident that this approach could

turn out to be a two edged sword with new metastases forming during the period of EACA therapy while some of the older implants were being successfully treated.

F. Cancer of the breast and the intravascular cytotoxic concept of limiting metastases

Recently published evidence [84] suggests that among the malignant neoplasms carcinoma of the breast is one of few cancers which permits the patient a long favorable course following initial surgery and before metastases become manifest. This characteristic of this carcinoma has often impressed us also and the University of Chicago Cancer Registry statistics illustrate the frequent long course. Furthermore, we have studied a few cases which showed this clinical course of many years after *both* breasts were removed, thus helping to eliminate the possibility of a second breast primary which one has to rule out in this kind of case. A large number of cases survive for more than one decade, often with virtually no evidence of the dormant tumour, before widespread metastases become manifest.

Dr. Fred Stewart, the great pathologist of the Memorial Hospital and the Sloan Kettering Institute in New York has emphasized this behaviour of cancer of the breast [85]. He also described the microscopic feature of a particular variety of breast cancer, medullary cancer with lymphoid stroma for which prognosis after surgery was much better than with other types of infiltrating mammary carcinoma [86].

That a broad spectrum of tumour-host balance may be present in carcinoma of the breast is strengthened by the observations of Berg [87] who studied the histological pattern of 58 patients with large mammary cancers who were clinically free of neoplastic disease for an average of 10 years after radical mastectomy. «Plasma cell» infiltrates at the edge of the tumour were correlated with the nonlethal anaplastic infiltrating carcinomas but not with the short term survivors.

One may add to this the observations that Black [88, 89] has reported concerning the lymph nodes in patients with carcinoma of the breast which often appear to be highly reactive, again with some prognostic value.

All of these observed phenomena make it seem probable that carcinoma of the breast would be a likely primary for further study in order to learn more about the correlation of circulating cytotoxic substances with prognosis of malignancy, the frequency of metastases, etc.

Summary

Evidence, both clinical-pathological and experimental, has been reviewed which indicates that many more cancer cells circulate than are able to establish implants. Immune cytotoxins, both those already studied in relation to transplantable tumours and those which seem to be important in autoimmune disease in man and in transplantation immunity are considered as model systems to increase our understanding of tumour cytotoxins. The potentional role of the «sensitized» lymphocyte is also discussed.

Certain recently isolated proteolytic substances from human serum are described and the importance of studying these in the sera of cancer patients before and after surgery is emphasized.

Some of the knowledge of human serum cytotoxic substances to established transplantable tumours and tissue culture lines is summarized and the ways in which this knowledge might be applied to the problem of cancer cell dissemination is indicated.

Recent experimental work from a number of laboratories is briefly reviewed in relation to the development of metastases. In particular the role of the clotting mechanism in favoring metastases is given special consideration.

The phenomenon of the «dormant» tumour implant and delayed metastases in carcinoma of the breast is described along with certain other characteristics of some primary cancers of the mammary gland which indicate that it is a particularly important malignancy to study in regard to factors limiting the dissemination of neoplastic disease.

Zusammenfassung

Klinisch-pathologisches und experimentelles Beweismaterial zeigt an, daß viel mehr Krebszellen zirkulieren als Metastasen gebildet werden. Immunocytotoxine, die schon im Zusammenhang mit transplantabeln Tumoren geprüft wurden und solche, die für autoimmune Krankheiten beim Menschen und für die Transplantationsimmunität von Bedeutung sind, werden als Modellsysteme erachtet, die uns ein besseres Verständnis für Tumorcytotoxine ermöglichen. Der Autor diskutiert auch die potenzielle Rolle der sensibilisierten Lymphocyten.

Er beschreibt außerdem gewisse, neuerlich aus dem menschlichen Serum isolierte proteolytische Substanzen und betont die Bedeutung der Untersuchung dieser Stoffe im Serum Krebskranker vor und nach operativen Eingriffen.

Er gibt auch einen Überblick über unsere Kenntnisse der gegenüber etablierten transplantabeln Tumoren und Gewebekulturstämmen cytotoxischen Substanzen des menschlichen Serums; er gibt an, in welcher Weise diese Kenntnisse auf das Problem der Krebszelldissemination angewendet werden können.

Neuere experimentelle Arbeiten einiger Laboratorien über die Entwicklung der Metastasen werden kurz referiert. Die Rolle des Gerinnungsmechanismus bei der Begünstigung der Metastasenbildung wird ganz besonders hervorgehoben.

Die Phänomene der latenten Tumormetastase und der verzögerten Metastasenbildung des Brustkrebses werden mit anderen Eigentümlichkeiten einiger Primärtumoren der Brustdrüse zusammen beschrieben. Der Brustkrebs stellt hinsichtlich des Studiums der Faktoren, welche die Dissemination der Neoplasie zu limitieren vermögen, eine besonders wichtige Art von maligner Geschwulstbildung dar.

Résumé

La pathologie aussi bien clinique qu'expérimentale a montré qu'il y a beaucoup plus de cellules cancéreuses circulant dans le sang qu'il n'y a de métastases. Les immuno-cytotoxines, aussi bien celles étudiées en relation avec les tumeurs inoculables que celles qui semblent avoir une importance dans les affections d'auto-immunité et dans l'immunité par inoculation, peuvent être considérées comme des exemples typiques, qui nous permettent de mieux comprendre les cytotoxines tumorales. L'auteur discute aussi le rôle possible des lymphocytes «sensibilisés».

Puis, il passe en revue certaines substances protéolytiques isolées dans le sérum humain et souligne l'importance qu'il y a de les étudier dans le sérum des malades du cancer, avant et après l'intervention chirurgicale.

L'auteur résume ensuite nos connaissances sur les substances du sérum humain à effet cytotoxique sur des tumeurs inoculables établies et sur des séries de cultures tissulaires, et indique comment ces connaissances peuvent être mises à profit dans l'étude de la dissémination des cellules cancéreuses.

Après une revue rapide des recherches expérimentales récentes de plusieurs laboratoires sur le problème du développement des métastases, l'auteur souligne tout particulièrement le rôle de la coagulation sanguine dans la fixation des métastases.

Enfin, l'on décrit le phénomène de la métastase tumorale «latente» et des métastases tardives du cancer du sein, ainsi que certains caractères de quelques tumeurs primaires de la glande mammaire. Le cancer du sein

représente, du point de vue de l'étude des facteurs capables de limiter la dissémination de la néoplasie, une tumeur maligne d'importance particulière.

Riassunto

La patologia clinica ed esperimentale ha dimostrato che ci sono molte più cellule cancerose circolanti nel sangue che metastasi proprie. Le immuno-citotossine, sia quelle studiate in relazione a tumori inoculabili che quelle che sembrano avere una certa importanza nelle affezioni di auto-immunità e nell'immunità per inoculazione, possono essere considerate come tipici esempi che ci permettono di comprendere meglio le citotossine cancerose. L'autore discute pure l'importanza potenziale dei linfociti sensibilizzati.

Descrive poi certe sostanze proteolitiche isolate nel siero umano e sottolinea l'importanza della loro identificazione nel siero di ammalati di cancro, prima e dopo l'intervento chirurgico.

L'autore riassume indi le nostre conoscenze su quelle sostanze del siero umano che possiedono un effetto citotossico contro tumori inoculabili fissati e serie di colture di tessuti, indicandone in che modo queste conoscenze possano essere applicate al problema della disseminazione di cellule cancerose.

Dopo un rapido esame di recenti ricerche esperimentali di diversi laboratori sul problema dello sviluppo di metastasi, l'autore sottolinea particolarmente l'importanza della coagolazione sanguigna nel favorimento della formazione di metastasi.

Infine sono descritti il fenomeno della «metastasi dormente», della formazione tardiva di metastasi nel cancro al seno e qualche carattere particolare di certi tumori primari della mammella, fenomeni che sembrano dimostrare che si è in presenza di un tumore di una malignità tutta particolare che deve essere esaminata in rapporto ai fattori limitanti la disseminazione di neoplasmi.

1. *Baserga R.* and *Saffiotti U.:* Experimental studies on histogenesis of bloodborne metastases. A.M.A. Arch. Path. **59,** 26 (1955).
2. *Wood S.* jr.: Pathogenesis of metastasis formation observed in vivo in the rabbit ear chamber. A.M.A. Arch. Path. **66,** 550 (1958).
3. *Wood S.* jr., *Yardley J. H.*, and *Holyoke E. D.:* The relationship between intravascular coagulation and the formation of pulmonary metastases in mice injected intravenously with tumor suspension. Proc. Amer. Ass. Cancer Res. **2,** 260 (1957).
4. *Weil R.:* Intravascular implantation of rat tumours J. med. Res. **28,** 497 (1913).
5. *Takahashi M.:* An experimental study of metastasis. J. Path. Bact. **20,** 1 (1915).
6. *Engell H. C.:* Cancer cells in the circulating blood. Acta chir. scand. (Suppl.) **201,** 1 (1955).
7. *Cole W. H.*, *Roberts S.*, *Watne A.*, *McDonald G.*, *McGrew E.:* The dissemination of cancer cells. N.Y. Acad. med. Bull. **34,** 163 (1958).

8. *Sandberg A. A., Moore G. E.:* Examination of blood for tumour cells. J. nat. Cancer Inst. **19,** 1 (1957).
9. *Roberts S., Watne A., McGrew E., Cole W. H.:* Technic and results of isolation of cancer cells from the circulating blood. A.M.A. Arch. Surg. **76,** 334 (1958).
10. *Iwasaki T.:* Histological and experimental observations of the destruction of tumour cells in the blood vessels. J. Path. Bact. **20,** 85 (1915).
11. *Wood S.* jr., *Holyoke D. E., Closm W. P. C., Sommers S. C., Warren S.:* An experimental study of the relationship between tumour size and number of lung metastases. Cancer **7,** 437 (1954).
12. *Candar Z., Ritchie A. C., Hopkirk J. F., Long R. C.:* The prognostic value of circulating tumour cells in patients with breast cancer. Surg. Gynec. Obstet. **115,** 291 (1962).
13. *Chan P.Y.M., Hodden D. H., McDonald G. O., Cole W. H.:* The use of «aging» in reducing the development of tumours after inoculation of carcinosarcoma 256 Walker cells. Cancer **14,** 1057 (1961).
14. *Loeb L.:* On some conditions determining variations in energy of tumour growth. Amer. Med. (Philad.) **10,** 265 (1905).
15. *Overstreet R. J., McDonald G. O.:* The role of cellular dosage on «takes» following inoculation of Walker 256 tumour cells in the rat. Surg. Forum **8,** 161 (1957).
16. *Pruit J.:* Personal communication.
17. *Wissler R. W.:* Effects of specific antibodies on tissue cells. Ann. Rev. Microbiol. **16,** 265 (1962).
18. *Irvine W. J.:* The cytotoxic factor in thyroid disease. Scot. med. J. **5,** 511 (1960).
19. *Harwin S. M., Paterson P. Y., Didakow N. C.:* Antibodies against autologous brain in rats with allergic encephalomyelitis. Nature (Lond.) **189,** 322 (1961).
20. *Stetson C. A., Jensen E.:* Humoral aspects of the immune response to homografts. N.Y. Acad. Sci. **87,** 249 (1960). (Art. 1: 4th Tissue Homotransplantation Conference).
21. *Prehn R. T.:* Tumor-specific immunity to transplanted Dibenz (a, h)-antracene-induced sarcomas. Cancer Res. **20,** 1614 (1960).
22. *Klein G., Sjögren H. O., Klein E., Hellström K. E.:* Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res. **20,** 1561 (1960).
23. *Wissler R. W., Barker P. A., Flax M. A., LaVia M. F., Talmadge D. W.:* A study of the preparation, localization and effects of antitumor antibodies labeled with I^{131}. Cancer Res. **16,** 761 (1956).
24. *Ellem K. A. O.:* Studies on the mechanism of the cytotoxic action of antiserum. Aug. J. exp. Biol. med. **20,** 116 (1957).
25. *Ellem K. A. O.:* Some aspects of the ascites tumour cell response to a heterologous antiserum. Cancer Res. **18,** 1179 (1958).
26. *Flax M. H.:* The action of anti-Ehrlich Ascites tumour antibody. Cancer Res. **16,** 774 (1956).
27. *Wissler R. W., Flax M. H.:* Cytotoxic effects of antitumour serum. Ann. N.Y. Acad. Sci. **69,** 773 (1957).
28. *Horn E. C.:* Ascites tumour development. II. Cytotoxicity of various antisera prepared against Ehrlich Ascites tumour cells components. Cancer Res. **16,** 595 (1956).
29. *Green H., Fleisher R. A., Barrow P., Goldberg B.:* The cytotoxic action of immune globulin and complement on Krebs-Ascites tumour cells. II. Chemical studies J. exp. Med. **109,** 511 (1959).
30. *Lindner A.:* Mechanism of immune lysis of Ehrlich Ascites tumour cells. Amer. J. clin. Path. **34,** 426 (1960).
31. *Fitch F. W.:* Immunohistochemical study of Ehrlich Ascites tumour. A.M.A. Arch. Path. **73,** 144 (1962).

32. *Latta H.:* A cellular reaction to antibody in tissue culture studies with E. M. J. biophys. biochem. Cytol. **5,** 405 (1959).
33. *Goldberg B., Green H.:* The cytotoxic action of immune gamma globulin and complement on Krebs Ascites tumour cells. I. Ultrastructural studies. J. exp. Med. **109,** 505 (1959).
34. *Aston J. E., Goldberg B., Green H.:* Immune cytolysis: electron microscopic localization of cellular antigens with Ferritin-antibody conjugates. J. exp. Med. **115,** 275 (1962).
35. *Wissler R. W.:* Combined effects of antitumor serum and other therapy on the growth and appearance of tumors. Cancro **14,** 433 (1961).
36. *Stone M., Dzoga K., Wissler R. W.:* Combined inhibitory effect of antitumor antibody and an oncolytic virus on the solid Ehrlich tumor. Lab. Invest. **11,** 300 (1962).
37. *Graham J. B., Graham R. M.:* Antibodies elicited by cancer in patients. Cancer (Philad.) **8,** 409 (1955).
38. *Finney J. W., Byers E. H., Wilson R. H.:* Studies in tumor autoimmunity. Cancer Res. **20,** 351 (1961).
39. *Sumner W. C., Foraker A. G.:* Spontaneous regression of human melanoma: clinical and experimental studies. Cancer (Philad.) **13,** 79 (1960).
40. *De Cavalho S.:* Preliminary experimentation with specific immunotherapy of neoplastic disease in man. I. Immediate effect of hyperimmune equine gamma globulins. Cancer (Philad.) **16,** 306 (1963).
41. *Wissler R. W., Fitch F. W., LaVia M. F.:* Reticuloendothelial system in antibody formation. Ann. N.Y. Acad. Sci. **88,** 134 (1960).
42. *Gunderson C. H., Juras D., LaVia M., Wissler R. W.:* Tissue and cellular changes associated with antibody formation in the rat spleen. J. Amer. med. Ass. **180,** 1038 (1962).
43. *Batchelor J. R., Boyse E. A., Gorer P. A.:* Synergic action between isoantibody and immune cells in graft rejection. Plast. reconstr. Surg. **26,** 449 (1960).
44. *Rosenau W., Moon H. D.:* Lysis of homologous cells by sensitized lymphocytes in tissue culture. J. nat. Cancer Inst. **27,** 471 (1961).
45. *Rosenau W., Moon H. D.:* Lysis of homologous cells by sensitized lymphocytes in tissue culture. J. nat. Cancer Inst. **27,** 471 (1961).
46. *Rosenau W., Moon H. D.:* The inhibitory effect of hydrocortisone on lysis of homologous cells by lymphocytes in vitro. J. Immunol. **9,** 422 (1962).
47. *Renis H. E., Johnson H. C., Bhuyan B. K.:* Collagen plate assay for cytotoxic agents. Cancer Res. **22,** 1126 (1962).
48. *Born G. V. R.:* Adenosine triphosphate in blood platelets during clotting. Proc. physiol. Soc. **133,** 61 (1955).
49. *Born G. V. R.:* Changes in distribution of phosphorus in platelet rich plasma during clotting. Biochem. J. **68,** 695 (1958).
50. *Bettex-Galland M., Lüscher E. F.:* Studies on the metabolism of human blood platelets in relation to clot retraction. Thromb. Diath. Haem. **4,** 178 (1960).
51. *Burstein M., Samaille J.:* Nouvelle méthode de séparation et de dosage des lipoprotéines de faible densité. Ann. Biol. clin. **1,** 1 (1959).
52. *Sherry S., Troll W.:* The action of thrombin on synthetic substrates. J. biol. Chem. **208,** 95 (1954).
53. *Levy L. R., Lepow I. H.:* Assay and properties of serum inhibitor of C_1-esterase. Proc. Soc. exp. Biol. (N.Y.) **101,** 608 (1959).
54. *Lumsden T., Kohn-Speyer A. C.:* Tumour immunity: Natural cytotoxins (heterotoxins) protection of cells against homologous antibodies. J. Path. Bact. **32,** 185 (1929).
55. *Björklund B.:* A serum cytolytic factor active against HeLa and other established cell strains. Proc. Soc. exp. Biol. (N.Y) **103,** 1 (1960).

56. *Wilheim R., Ivy A. C., Janacek H. M.:* Cytolysis of Ehrlich Ascites carcinoma cells by normal human blood sera. Exper. Med. Surg. **15,** 300 (1957).
57. *Landy M., Michael J. G., Trapani R. J., Achinstein B., Woods M. W., Shear M. J.:* An antibody-complement system in normal serum lethal to mouse tumour cells. Cancer Res. **20,** 1279 (1960).
58. *Bolande R. P., Todd E. W.:* The cytotoxic action of normal human serum on certain human cells propagated in vitro. Arch. Path. (Chicago) **66,** 720 (1958).
59. *Bolande R. P.:* Cytotoxic action of human serum on atypical mammalian cell lines. Lab. Invest. **9,** 475 (1960).
60. *Bolande R. P., McLain J. P.:* Cytotoxic action of normal human serum on Ehrlich Ascites and sarcoma 180 cells. Proc. Soc. exp. Biol. (N.Y.) **103,** 345 (1960).
61. *Ginsburg I., Dishon T., Bloch M., Grossm J.:* A thermostable cytotoxic factor in normal human serum active against Landschutz Ascites tumor cells. Proc. Soc. exp. Biol. (N.Y.) **107,** 235 (1961).
62. *Fedoroff S.:* Toxicity of schizophrenics' blood serum in tissue culture. J. Lab. clin. Med. **48,** 55 (1956).
63. *Fedoroff S., Doerr J.:* Effect of human blood serum on tissue cultures. III. A natural cytotoxic system in human blood serum. J. nat. Cancer Inst. **29,** 331 (1962).
64. *Fisher E. R., Fisher B.:* Experimental studies of factors influencing hepatic metastases. I. The effect of number of tumour cells injected and time of growth. Cancer **12,** 296 (1959).
65. *Fisher B., Fisher E. R.:* Experimental studies of factors influencing hepatic metastases. II. Effect of partial hepatectomy. Cancer **12,** 929 (1959).
66. *Fisher E. R., Fisher B.:* Effect of reticuloendothelial interference on experimental metastases. Surg. Forum **11,** 55 (1960).
67. *Fisher E. R., Fisher B.:* Experimental studies of factors influencing hepatic metastases. IV. Effect of cirrhosis. Cancer **13,** 860 (1960).
68. *Fisher B., Fisher E. R., Lee S. H.:* The effect of alteration of liver blood flow upon experimental hepatic metastases. Surg. Gynec Obstet. **112,** 11 (1961).
69. *Wood S.* jr., *Holyoke E., Sommers S. C., Warren S.:* Influence of pituitary growth hormone on growth and metastasis formation of a transplantable mouse sarcoma. Bull. Johns Hopk. Hosp. **96,** 93 (1955).
70. *Wood S.* jr., *Holyoke E. D., Yardley J. H.:* An experimental study of the influence of adrenal steroids, growth hormone and anticoagulants on pulmonary metastasis formation in mice. Proc. Amer. Ass. Cancer Res. **2,** 149 (1956).
71. *Chan P., McDonald G. O., Cole W. H.:* The role of hepatic damage on development of Walker 256 carcinosarcoma. Surg. Forum **11,** 55 (1960).
72. *Hadfield G.:* The dormant cancer cell. Brit. med. J. **2,** 607 (1954).
73. *Fisher B., Fisher E. R.:* Experimental evidence in support of the dormant tumour cell. Science **130,** 918 (1959).
74. *Schmidt M. B.:* Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome in den leukämischen Neubildungen. Gustav Fischer Verlag, Jena, 1903.
75. *Saphir O.:* The fate of carcinoma emboli in the lung. Amer. J. Path. **23,** 249 (1947).
76. *Terranova T., Chissone F.:* Il fattore coagulazione nell'attaccamento delle cellule neoplastiche immesse in circolo. Bull. Soc. Ital. Biol. Sper. **28,** 1224 (1952).
77. *Cliffton E. E., Grossi C. E.:* Effect of human plasmin on the toxic effects and growth of blood-borne metastasis of the Brown-Pearce carcinoma and the V2 carcinoma of rabbit. Cancer **9,** 1147 (1956).
78. *Cliffton E. E., Agostino D.:* Factors affecting the development of metastatic cancer. Cancer **15,** 276 (1962).
79. *Grossi C. E., Agostino D., Cliffton E. E.:* The effect of human fibrinolysin on pulmonary metastasis of Walker 256 cacinosarcoma. Cancer Res. **20,** 505 (1960).

80. *Lacour F., Oberling C., Guerin:* Influence de l'éthyldicoumarol sur l'évolution des métastases de l'épithelioma T8 chez le rat: nouvelles recherches. Bull. Assoc. Franc. étude Cancer **43/44,** 88 (1956–57).
81. *Lismell A., Mellgren J.:* Effect of heparin, protamine, dicoumarol, streptokinase and epsilon-amino-n-caproid acid on the growth of human cells in vitro. Acta path. microbiol. scand. **57,** 145 (1963).
82. *Day E. D., Planisek J. A., Pressman D.:* Localization in vivo of radioiodinated antirat-fibrin antibodies and radioiodinated fibrinogen in the Murphy rat lymphosarcoma and in other transplantable rat tumours. J. nat. Cancer Inst. **22,** 413 (1959).
83. *Bale W. F., Spar I. L., Goodland R. L.:* Experimental radiation therapy of tumours with I^{131}-carrying antibodies to fibrin. Cancer Res. **20,** 1488 (1960).
84. *Henneford J., Baserga R., Wartman W. B.:* The time of appearance of metastases after surgical removal of the primary tumour. Brit. J. Cancer **16** (4), 599 (1962).
85. *Stewart F. W.:* Experiences in spontaneous regression of neoplastic disease in man. Tex. Rep. Biol. Med. **10,** 239 (1952).
86. *Stewart F. W.:* Tumours of the breast. Fascicle 34, Altas of Tumour Pathology. Washington, D.C., A.F.I.P., 1950.
87. *Berg J. W.:* Inflammation and Prognosis in breast cancer. Cancer **12,** 714 (1959).
88. *Black M. M., Stase K., Speer F. D.:* Lymph node structure in patients with cancer of the breast. Amer. J. Path. **29,** 505 (1953).
89. *Black M. M., Speer F. D.:* Lymph node reactivity in cancer patients. Surg. Gynec. Obstet. **110,** 977 (1960).