Immunochemotherapy of Cancer with Chlorambucil-carrying Antibody

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Summary

Cell-surface localizing heterologous antibodies against the mouse EL4 lymphoma and a human malignant melanoma could be bound to chlorambucil without causing the loss of the alkylating activity of chlorambucil or interfering with the reactivity of the antibodies with their respective tumour cells. When given to mice pre-inoculated with tumour cells 2, 24, 72, and 120 hours before the beginning of treatment the chlorambucil-bound antibody was a much more effective tumour inhibitor than chlorambucil or the antibody alone. In a patient with disseminated malignant melanoma injection of the chlorambucil-bound anti-melanoma antibody first locally into a few metastatic nodules and then by the intravenous route was followed by the regression of all the metastatic nodules.

Introduction

Though antibodies can be produced against many experimental animal and human tumours there is at present little evidence that they have any significant destructive effect on tumour cells *in vivo* (Southam, 1961; Harris and Sinkovics, 1970; Sophocles and Nadler, 1971). However, it is possible to use surface-localizing antitumour antibodies as carriers for radio-therapeutic agents (Ghose *et al.*, 1967) or a cytotoxic drug such as chlorambucil (Ghose and Nigam, 1972) leading to their selective concentration in tumour tissue. Such an approach to cancer therapy is especially important because most cancer chemotherapeutic agents are not selective; their action is non-specific and directed against all rapidly-dividing cells (Boesen and Davis, 1968). We report here the suppression of the experimental mouse lymphoma EL4 and human malignant melanoma metastases after administration of chlorambucil-bound antitumour antibodies. Chlorambucil was especially selected for these studies because Israels and Linford (1963) showed that chlorambucil could be bound to plasma proteins without losing its alkylating activity, which is the chemical basis of its "anticancer" action (Boesen and Davis, 1968).

Methods

EL4 lymphoma was originally obtained from the Chester Beatty Research Institute, London, and maintained in our laboratory by serial intraperitoneal passage in C57BL mice.

Antitumour Sera.—For the production of anti-EL4 serum adult New Zealand rabbits were immunized first by intra-

muscular injections of a saline suspension of viable EL4 cells mixed with Freund's complete adjuvant and then with tumour cells alone (Ghose *et al.*, 1967). Antihuman melanoma serum was obtained by repeatedly injecting a goat with the homogenate of fresh, surgically excised subcutaneous melanoma nodules from which adipose tissue and skin were carefully removed. The homogenate was mixed with Freund's complete adjuvant for the first two injections. The anti-EL4 serum was successively absorbed with homogenates of liver, kidney, and spleen from normal C57BL mice until the serum reacted, as tested by immunofluorescence, only with EL4 cells. The anti-melanoma serum was absorbed with similar human tissue homogenates and AB red cells after which it reacted only with the patient's melanoma cells and not with the patient's normal skin or with other normal adult human tissues.

Binding of Globulins to Chlorambucil.—The globulin fraction from all sera was obtained after three successive precipitations with 33.3% saturated ammonium sulphate. Globulins were bound with varying amounts of chlorambucil always keeping the ratio of weight of globulin to chlorambucil more than 10:1, a ratio at which about 90% of chlorambucil binds to proteins (Israels and Linford, 1963). After dialysis in cold 0.01 M phosphate-buffered saline (pH 7.1) the antibody-chlorambucil conjugates were found to have retained their antibody activity, as tested by immunofluorescence on smears and suspensions of appropriate tumour cells (Figs 1 and 2). They also retained their alkylating activity, as assessed by the Epstein technique (Epstein *et al.*, 1955).

FIG. 1—Fluorescence photomicrograph of mouse EL4 lymphoma cells treated first with chlorambucil-bound anti-EL4 antibody and then with fluoresceinated goat anti-rabbit globulin. Diffuse cytoplasmic staining of tumour cells shows that the chlorambucil-bound antibody retained its reactivity with EL4 cells. ($\times 260$.)

Immunofluorescence.—Immunofluorescence staining was done by the "sandwich method" using fluorescein isothiocyanate conjugated goat anti-rabbit (for anti-EL4 serum produced in rabbit), rabbit anti-goat (for anti-melanoma serum produced in goat), or rabbit antihuman globulins (for detection of human gammaglobulins) with appropriate controls (Ghose *et al.*, 1968; Ghose and Nigam, 1972). All immunofluorescence tests

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FIG. 2—Fluorescence photomicrograph of snap-frozen and thawed smear of patient's melanoma cells treated first with chlorambucil-bound goat anti-melanoma globulin (absorbed with AB red cells and normal human tissue homogenates) and then with fluoresceinated rabbit anti-goat globulin. Diffuse cytoplasmic staining of the melanoma cells (some of which are binucleated) shows that the anti-melanoma globulin retained its reactivity with melanoma cells after binding with chlorambucil. ($\times 370$)

were performed either on 5- μ m cryostat sections of melanoma and other human or mouse tissues snap-frozen at -176°C or on smears of unfixed snap-frozen and thawed tumour cells. Malignant melanoma cell suspensions were prepared without the use of any enzyme, and the cell suspensions were either irradiated and used immediately for immunization of the patient or frozen at -176°C in 10% dimethyl sulphoxide. Membrane immunofluorescence of melanoma cells was performed by incubating viable melanoma cells in test sera as described by Phillips and Lewis (1970). Malignant melanoma cells from the patient were also cultured in medium 199 supplemented with fetal calf serum.

Cytotoxicity Tests.—These were performed either with the patient's own inactivated serum or the inactivated goat anti-melanoma globulin with or without the addition of fresh human AB blood group serum as the source of complement. Globulins and complement were used in dilutions of 1:4 and

the proportions of trypan blue permeable cells were counted 30 minutes, 2 hours, 6 hours, and 24 hours after exposure of the cells to the antibodies and compared with preparations in which malignant melanoma cells were incubated with normal human globulin with and without added complement or complement only. Lymphocytes were isolated from sterile venous blood with carbonyl iron by means of a mechanical separator (Technicon). Lymphocyte tumour cell interaction was assessed using microtest culture plates (Currie *et al.*, 1971) or by incubating known numbers of tumour cells and lymphocytes for varying periods in medium 199 supplemented with either the patient's serum or normal human AB serum. In the latter preparations note was taken of aggregation of lymphocytes around melanoma cells and their permeability to trypan blue.

Results

MOUSE LYMPHOMA EL4

Intraperitoneal inoculation of EL4 cells in C57BL mice invariably leads to the development of ascites tumour and subsequent death of the tumour-inoculated mice. The survival of the tumour-inoculated mice, however, is dependent on the number of tumour cells inoculated (see Table II).

Five daily intraperitoneal injections of 0.076 mg of chlorambucil bound to 2 mg of anti-EL4 immune rabbit globulin starting two hours after tumour inoculation resulted in the survival of all the injected mice for more than 150 days without developing any detectable tumour (Table I, group 6). Similar injections of 0.076 mg of chlorambucil (group 2), 2 mg of normal rabbit globulin (group 3), 2 mg of immune rabbit globulin (group 4), or 2 mg of normal rabbit globulin bound to 0.076 mg of chlorambucil (group 5) did not cause any significant tumour inhibition. Prolonging the interval between tumour inoculation and the first injection of chlorambucil-bound immune rabbit globulin from 2 hours (group 6) to 24 hours (group 7) did not reduce survival time of the treated mice. However, intervals of 72 hours (group 8) or 120 hours (group 9) between tumour inoculation and the beginning of treatment severely reduced

TABLE I—Survival of Nine Groups of C57BL Mice injected with Chlorambucil-bound Antibody after Intraperitoneal Inoculation of EL4 Cells. Each Group contained Five 12-week-old Female Mice

Group No.	Treatment (Chlorambucil (mg)/Globulin (mg))*	Interval (Hours) between Tumour Inoculation and Beginning of Treatment	Survival (No. of Days $\pm$ S.D.)
1	0/0 (P.B.S.)	2	18.6 $\pm$ 1.2
2	0.076/0	2	22.4 $\pm$ 2.8
3	0/2 N.R.G.	2	17.0 $\pm$ 1.2
4	0/2 I.R.G.	2	20.4 $\pm$ 0.89
5	0.076/2 N.R.G.	2	17.0 $\pm$ 1.22
6	0.076/2 I.R.G.	2	All survived†
7	0.076/2 I.R.G.	24	1 died 64 days after tumour inoculation‡
8	0.076/2 I.R.G.	72	3 survived† and 2 died 28 and 29 days after tumour inoculation
9	0.076/2 I.R.G.	120	1 died 32 days, 3 died 24 days, and 1 died 22 days after tumour inoculation

*Treatment consisted in daily intraperitoneal injections for five days of 1 ml phosphate-buffered saline containing the stated amounts of chlorambucil and globulin.

†These mice were free of visible tumours for more than 150 days after tumour inoculation.

‡At necropsy this mouse showed bronchopneumonic changes in both lungs. No tumour could be seen in any of the tissues examined.

P.B.S. = Phosphate-buffered saline. N.R.G. = Normal rabbit globulin. I.R.G. = Immune rabbit globulin.

TABLE II—Survival of Five Groups of C57BL Mice injected with 0.076 mg of Chlorambucil bound to 2 mg I.R.G. Daily for Five Days starting Two Hours after Intraperitoneal Inoculation of Varying Numbers of EL4 Cells. Each Group contained Five 12-week-old Female Mice

Group No.	No. of EL4 Cells Inoculated	Survival (No. of Days $\pm$ S.D.)	
		P.B.S.-injected Mice	I.R.G. $\pm$ Chlorambucil-injected Mice
10	10 ³	20.0 $\pm$ 1.0	All survived*
11	10 ⁴	18.4 $\pm$ 0.55	All survived*
12	10 ⁵	15.6 $\pm$ 1.81	All survived*
13	10 ⁶	12.8 $\pm$ 0.83	3 survived and 2 died 22 and 23 days after tumour inoculation
14	10 ⁷	12.4 $\pm$ 0.89	21.8 $\pm$ 3.27

*These mice were free of visible tumour for more than 150 days after tumour inoculation.

P.B.S. = Phosphate-buffered saline. I.R.G. = Immune rabbit globulin.

the survival of the two groups of mice. This was probably due to the increase in the number of tumour cells by the time the treatment began. As can be seen from Table II injections of 2 mg of immune rabbit globulin bound to 0.076 mg of chlorambucil daily for five days, starting two hours after tumour inoculation afforded complete protection against 10^5 EL4 cells per mouse (group 12), but at this dose level protection was only partial with inocula of 10^6 (group 13) or 10^7 (group 14) EL4 cells per mouse. All mice which died of tumour showed widespread tumour deposits in internal organs and large numbers of tumour cells in blood.

The case history of a patient with disseminated malignant melanoma who was treated with chlorambucil-bound anti-melanoma globulin is given below.

CASE REPORT

A 67-year-old white man was admitted to hospital on 12 January 1970 with a nodular bluish ulcerated swelling (1.5 by 2.6 cm) over the nail matrix of the right thumb. The lesion had been present for at least one-and-a-half years, and histologically it was a malignant melanoma (Fig. 3). He also had two intradermal nodules along lymphatic pathways, one in the right forearm and one in the arm and palpable axillary lymph nodes.

FIG. 3—Photomicrograph of section from non-ulcerated part of original lesion in patient's thumb showing sheet-like arrangement of cells and collections of lymphocytes deep under the epidermis. (H. and E. $\times 75$.)

The same day he was given 40 mCi of colloidal radiogold (^{198}Au) into a lymphatic channel cannulated in the right antecubital fossa. On 16 January the right thumb was amputated,

and on the 19th the nodules on the right arm and forearm were widely excised; histologically these were metastatic malignant melanomas (Fig. 4). An exploratory laparotomy on 26 January did not show any involvement of the abdominal organs. This was followed on 2 February by an interscapulothoracic amputation of the right upper extremity.

FIG. 4—Photomicrograph of section from subcutaneous metastatic tumour showing similarity of histological appearance to original lesions. (H. and E. $\times 185$.)

On 10 June a small secondary malignant melanoma nodule was excised from his back. In July additional subcutaneous malignant melanoma nodules appeared and he was given an eight-week course of phenylalanine mustard (2 mg/day).

He continued to develop additional subcutaneous and intradermal tumour nodules.

No cytotoxic lymphocytes or membrane-localizing antibody against autologous melanoma cells could be detected in the peripheral blood in September. He reacted mildly to tuberculin. One metastatic nodule (about 1 cm in diameter) was injected with 0.1 ml of B.C.G. (Connaught Laboratory, Toronto) on 16 September. Two more nodules were similarly injected the next day. There was a severe inflammatory reaction around the injected nodules about 24 hours after the B.C.G. injection. After subsidence of the inflammation the injected tumour nodules were seen diminishing in size, and by December all the three nodules were undetectable. However, new metastatic tumour nodules continued to appear, and on 10 December 12 more metastatic nodules were locally injected with 0.2 ml of B.C.G. per nodule. Again there was inflammation in the injected nodules, after which they diminished in size. On 16 December histological examination of one of the nodules which had been injected with B.C.G. on 17 September and excised on 16 December showed a granulomatous reaction but no identifiable tumour cells (Fig. 5). Immunofluorescence showed a few IgG and IgM containing cells in the granulomatous area.

TABLE III—Measurements (in cm) of Patient's Seven Metastatic Nodules showing Gradual Decrease in Size after Treatment with Chlorambucil-bound Anti-melanoma Antibody. The Two Largest Diameters of the Nodules approximately at Right-angles to one another were recorded

Date measurement made:	12 Oct. 1971	25 Oct. 1971	8 Nov. 1971	22 Nov. 1971	13 Dec. 1971	7 Jan. 1972 and 14 Mar. 1972
Forehead recurrence in scar (in skin)	1.2 by 1.0	1.0 by 0.8	0.6 by 0.6	Vague induration	Nil	Nil
Small satellite above and medial to forehead recurrence (in skin)	0.2 by 0.2	0.1 by 0.1	Nil	Nil	Nil	Nil
Right side of neck (in skin)	1.8 by 1.2	1.5 by 1.1	1.2 by 1.0	0.8 by 0.8	0.5 by 0.5	Nil
Left side of neck near angle of jaw (in skin)	—	1.5 by 1.0	1.2 by 1.0	0.9 by 0.9	0.6 by 0.5	Vague induration 0.3 by 0.3
Back of left shoulder scar (subcutaneous)* (2.0 by 2.0†)	2.0 by 1.6	1.5 by 1.2	Vague induration 0.8 by 0.5	Nil	Nil	Nil
Medial end of right clavicle (subcutaneous)* (1.5 by 1.5†)	2.0 by 2.0	1.9 by 1.5	1.5 by 1.2	0.8 by 0.5	0.5 by 0.5	Nil
Left buttock (subcutaneous)* (1.0 by 1.0†)	0.7 by 0.6	0.6 by 0.6	Nil	Nil	Nil	Nil

* These three tumour nodules were injected with goat anti-melanoma globulin bound to chlorambucil.

† Measurements just before direct injection of tumour nodules.

— = Not recorded.

FIG. 5—Photomicrograph of section of subcutaneous malignant melanoma metastasis three months after local injection of 0.1 ml B.C.G. Note formation of granulomas containing epithelioid and giant cells and dense collections of lymphocytes. No melanoma cells could be identified in any of the sections examined. (H. and E. $\times 60$.)

On 3 February 1971 5×10^8 melanoma cells were irradiated (10,000 rads), mixed with B.C.G., and then injected subcutaneously into several sites. Serum collected seven days after injection of irradiated melanoma cells showed membrane immunofluorescence in a titre of 1:4 but not in a titre of 1:16. The patient's peripheral blood lymphocytes did not aggregate around his own tumour cells, no anti-melanoma antibody could be detected in his serum 16 days after immunization. A fresh crop of metastatic nodules then began to appear. On 11 February 11 more metastatic nodules were locally injected with B.C.G. Within a few hours he had chill, fever (39.5°C), nausea, and shivering. These nodules did not decrease in size. Subsequent to this a total of 50 mg of Concanavallin A (Sigma, St. Louis) was injected into 13 metastatic nodules and about 1 mg of a lyophilized phenol extract of *Candida albicans* was injected into each of 12 other nodules. These injections did not cause regression of any of the tumour nodules.

FIG. 6—Fluorescence photomicrograph of suspension of patient's melanoma cells first exposed to his own serum (collected after regression of his tumour nodules) and then to fluoresceinated goat antihuman globulin. Dead cells show diffuse cytoplasmic and nucleolar staining whereas live cells (arrowed) show discrete granular staining of cell surface. Fluorescent dots scattered throughout field are due to granular surface staining of cells floating at different levels of focus. ($\times 315$.)

Increasing numbers of metastatic nodules continued to appear all over his body. On 23 September 25 mg of anti-melanoma globulin combined with 0.38 mg of chlorambucil was injected into one metastatic nodule. The next day two more nodules were injected with a total of 25 mg of anti-melanoma globulin combined with 0.38 mg of chlorambucil. On 30 September 25.5 mg of the anti-melanoma globulin combined with 2.5 mg of chlorambucil was injected intravenously. Similar intravenous injections were given on 2 October (58.5 mg of anti-melanoma globulin and 3 mg of chlorambucil) and 3 October (40 mg of anti-melanoma globulin and 4 mg of chlorambucil). The injections of chlorambucil-bound anti-goat globulin did not cause any adverse reaction or complications of renal, pulmonary, and haematological systems. At the end of the first week of October 1971 the patient reported that some of his tumour nodules were becoming smaller. This was confirmed by measurements of seven of the tumour nodules (Table III). All metastatic nodules regressed irrespective of whether these were locally injected with chlorambucil-bound antibody or not. At the time of writing the patient was leading an active life and gaining weight.

Samples of serum collected on 13 December 1971 and 6 February 1972 showed the presence of both cytoplasmic and cell surface localizing antibody up to a titre of 1:32 (Fig. 6). Lymphocytes collected from his peripheral blood also aggregated around his own melanoma cells after about 12 hours of incubation (Fig. 7). No antibody against normal goat globulin or chlorambucil-bound goat globulins could be detected in the serum by immunodiffusion.

FIG. 7—Photomicrograph showing aggregation of patient's peripheral blood lymphocytes on his own melanoma cells grown as a monolayer after liquid nitrogen storage. Lymphocytes were collected after regression of patient's melanoma nodules. ($\times 500$.)

Discussion

EL4 is a chemically-induced lymphoma in which the presence of a tumour-specific antigen was established by Gorer and Amos (1955). Whether the heterologous antibody produced by us was directed against the particular antigen described by Gorer and Amos is uncertain. Immunofluorescence with living EL4 cells showed the availability of antigen on tumour cell surface. The results presented here clearly show that chlorambucil when bound to this antibody was a more effective tumour inhibitor than chlorambucil by itself, the antibody alone, or chlorambucil bound to a non-specific protein carrier such as normal rabbit globulin.

Malignant melanomas and their metastases are known to undergo occasional spontaneous regression (Sumner and Foraker,

1960; Everson and Cole, 1966; Smithers, 1967; Bodenham, 1968). However, an examination of the case histories of patients with reported regression of malignant melanoma (Boyd, 1966; Everson and Cole, 1966; Moore and Gerner, 1971) reveals that only a small fraction of these cases showed spontaneous complete regression of all metastatic nodules, and it appears that "the incidence of these occurrences has been magnified" (Moore and Gerner, 1971).

The part that injections of B.C.G. and chlorambucil might have played in the regression of the metastases of the patient reported on here must also be considered. Similar to our observation is the report of Morton *et al.* (1970) that local infiltration of B.C.G. into tumour nodules resulted in the regression of injected nodules in five out of eight patients with disseminated malignant melanomas. Krementz *et al.* (1971) reported on one patient whose non-injected nodules regressed about one month after local injection of B.C.G. into some of the metastatic melanoma nodules. In the present patient during a period of about a year—that is, 17 September 1970 to 23 September 1971—no tumour nodules regressed apart from those injected direct with B.C.G. in September 1970. On the contrary, fresh crops of progressively enlarging melanoma nodules continued to appear during this period. Also at no time before immunochemotherapy did any other tumour nodules show spontaneous regression. It is unlikely that the total dose of 10.26 mg of chlorambucil given over a period of about 10 days could have caused tumour regression by itself.

Johnson and Jacobs (1971) reported the lack of any objective tumour regression in patients with disseminated malignant melanoma after administration of 0.2 mg/kg/day of chlorambucil continuously for six weeks. Of the several alkylating agents tried in these patients only phenylalanine mustard caused tumour regression in one patient. The present patient did not respond to phenylalanine mustard before immunochemotherapy. The goat anti-melanoma globulin was not cytotoxic to his melanoma cells and it is unlikely that this antibody by itself caused tumour regression. Passive transfer of serum or plasma from cured patients, in contrast to sensitized allogeneic leucocytes (Sumner and Foraker, 1960; Nadler and Moore, 1970; McPeak, 1971), has failed to cause regression of disseminated melanoma (Everson and Cole, 1966). The observations by Ikonopisov *et al.* (1970) and Currie *et al.* (1971) also suggest that the appearance of anti-melanoma antibody and cytotoxic lymphocytes in this patient's blood subsequent to immunochemotherapy was probably the result of the diminution of his tumour tissue. The absence of detectable antibody to goat globulin in his serum after injections of chlorambucil-bound anti-melanoma goat globulin is not surprising, as all anti-melanoma globulin preparations were freed of aggregates by passage through Millipore filters and centrifugation (Wiegler, 1971).

In our animal experiments chlorambucil or ^{131}I -bound antibodies consistently caused much more tumour inhibition than antibody, chlorambucil, or ^{131}I alone. This might be the result either of (a) sensitization of tumour cells to ionizing radiations and cytotoxic drugs brought about by surface-localizing antibodies (Ghose and Cerini, 1969) or (b) antibody-mediated selective concentration of cell-damaging agents in tumour tissue (Ghose and Nigam, 1972) or of both. At present we are treating other disseminated malignant melanoma patients with chlorambucil-bound anti-melanoma antibody.

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References

- Bodenham, D. C. (1968). *Annals of the Royal College of Surgeons of England*, 43, 218.
- Boesen, E., and Davis, W. (1968). *Cytotoxic Drugs in the Treatment of Cancer*. London, Arnold.
- Boyd, W. (1966). *Spontaneous Regression of Cancer*. Springfield, Illinois, Thomas.
- Currie, G. A., Lejeune, F., and Fairly, G. H. (1971). *British Medical Journal*, 2, 305.
- Epstein, J., Rosenthal, R. W., and Ess, R. J. (1955). *Analytical Chemistry*, 27, 1435.
- Everson, T. C., and Cole, W. H. (1966). *Spontaneous Regression of Cancer*. Philadelphia, Saunders.
- Ghose, T., and Cerini, M. (1969). *Nature*, 222, 993.
- Ghose, T., Cerini, M., Carter, M., and Nairn, R. C. (1967). *British Medical Journal*, 1, 90.
- Ghose, T., Nairn, R. C., and Cerini, M. (1968). *Experimental Cell Research*, 49, 513.
- Ghose, T., and Nigam, S. (1972). *Cancer (Philadelphia)*, 29, 1398.
- Gorer, P. A., and Amos, D. B. (1955). *Cancer Research*, 15, 338.
- Harris, J. E., and Sinkovics, J. G. (1970). *Immunology of Malignant Disease*. St. Louis, Mosby.
- Ikonopisov, R. L., *et al.* (1970). *British Medical Journal*, 2, 752.
- Israels, L. G., and Linford, J. H. (1963). In *Proceedings of the Fifth Canadian Cancer Research Conference*, p. 399. New York Academic Press.
- Johnson, F. D., and Jacobs, E. M. (1971). *Cancer (Philadelphia)*, 27, 1306.
- Krementz, E. T., Samuels, M. S., Wallace, J. H., and Benes, E. N. (1971). *Surgery, Gynecology and Obstetrics with International Abstracts of Surgery*, 133, 209.
- McPeak, C. J. (1971). *Cancer (Philadelphia)*, 28, 1126.
- Moore, G. E., and Gerner, R. E. (1971). *Surgery, Gynecology and Obstetrics with International Abstracts of Surgery*, 132, 427.
- Morton, D. L., *et al.* (1970). *Annals of Surgery*, 172, 740.
- Nadler, S. H., and Moore, G. E. (1970). *Archives of Surgery*, 100, 244.
- Phillips, T. M., and Lewis, M. (1970). *Revue Européenne d'études Cliniques et Biologiques*, 15, 1016.
- Smithers, D. W. (1967). *Annals of the Royal College of Surgeons of England*, 41, 160.
- Sophocles, A. M., and Nadler, S. G. (1971). *Surgery, Gynecology and Obstetrics with International Abstracts of Surgery*, 133, 321.
- Southam, C. M. (1961). *Cancer Research*, 21, 302.
- Sumner, W. C., and Foraker, A. G. (1960). *Cancer (Philadelphia)*, 13, 79.
- Weigle, W. O. (1971). *Clinical and Experimental Immunology*, 9, 437.