eral, the drag-reduction effect itself depends upon the Reynolds number of the flow through the measuring pipe of the apparatus; thus a "calibration curve" such as that shown in Fig. 3 must be drawn for each solvent and, for each test pipe of different dimensions.

The concentration of polysaccharide in the *A. flos-aquae* medium at the time of testing was over 500 ppm, which corresponds well with other similar measurements of concentration (*3*). The only possible fit of the data of Fig. 2 to the chart of Fig. 3, assuming 100-percent culture concentration of *A. flos-aquae* to be 500 ppm polysaccharide, yields a molecular weight of about 100,000.

To investigate by its effect on reduction of friction the nature of bacterial action on the extracellular polysaccharide, we grew two cultures of *Chaetoceros didymus* Ehrenberg simultaneously, one inoculated with syntrophic bacteria, the other bacteria-free (*7*). The culture containing bacteria effected only half as much reduction in friction as the axenic one (*8*). Available data are insufficient to determine whether the effect of bacteria is to lower the molecular weight, reduce the concentration of polysaccharide, or partially block synthesis of polysaccharide. Cultures of *Anabaena flos-aquae* effected considerable reduction in drag even with bacteria present.

Thus it seems entirely possible that algal polysaccharides can influence measurements in tow-tanks and other large-scale hydrodynamic facilities.

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References and Notes

1. R. N. Newton, *Trans. Roy. Inst. Naval Architects* **102**, 435 (1960); K. C. Barnaby and A. L. Dorey, *ibid.* **107** (1965).
2. J. W. Hoyt and A. G. Fabula, in *Proc. ONR 5th Symp. Naval Hyrodynamics, Bergen, Norway, 1964* (Fed. Clearinghouse Sci. Tech. Inform., AD-612 056).
3. B. G. Moore and R. G. Tischer, *Science* **145**, 586 (1964).
4. J. N. Phillips, Jr., and J. Meyers, *Plant Physiol.* **29**, 148 (1954); R. C. Starr, *Am. J. Bot.* **51**, 1013 (1964); G. Soli, *Limnol. Oceanogr.* **9**, 265 (1964).
5. C. T. Bishop, G. A. Adams, E. O. Hughes, *Can. J. Chem.* **32**, 999 (1954).
6. Determined by the manufacturer, Union Carbide Chemicals Co.
7. G. Soli, in *Symposium on Marine Microbiology*, C. H. Oppenheimer, Ed. (Thomas, Springfield, Ill., 1963), chap. 12.
8. Standard sterile techniques were used to grow all algae, even in handling nonaxenic cultures.
9. We thank R. H. Wade, H. I. Scribner, and Carol Ann Flanders for technical assistance, and R. G. Tischer (Mississippi State University) and P. Wold (California Institute of Technology) for gifts of algae.

8 July 1965

Malignant Lymphomas Following Allogenic Disease: Transition from an Immunological to a Neoplastic Disorder

Abstract. *The graft versus host reaction which occurs in F_1 hybrid mice injected with parental spleen cells was used to examine several immunological theories of neoplasia. Long-term survivors of this reaction developed lymphoid neoplasms which resembled Hodgkin's disease and lymphosarcoma. Mice with these tumors were chimeras, but the parental cells present within their spleens had specific immunological tolerance toward host antigens. This, together with the finding that the tumors were transplantable only to isogenic recipients, indicates that the tumors were of host rather than donor origin.*

Chronic lymphocytic leukemia, lymphosarcoma, and Hodgkin's disease are frequently associated with immunological abnormalities. Defects of immunoglobulin synthesis, autoimmunity, and anergy are commonly encountered in patients with these diseases. It is not known whether these aberrations of immunity represent cause or effect of the malignancy. Nevertheless, their elucidation has stimulated considerable interest in immunological theories of neoplasia.

One of these theories (*1*) has as its basis the cellular proliferation that characterizes the response of lymphoid tissue to antigens. It proposes that a continuous or repetitive exposure to antigens could result in a sustained proliferative response which culminates in neoplasia. This concept is supported by Metcalf's (*2*) experiments in which an increased incidence of reticulum cell sarcomas and plasma cell tumors was found in mice which had been given repeated injections of bovine serum albumin or *Salmonella adelaide* vaccine.

Tyler (*3*) has elaborated a theory that neoplasms of lymphoid tissue develop in two steps. In the first step, an immunologically competent cell undergoes a mutation which results in the loss of a histocompatibility antigen. In the second step, normal cells having a full complement of histocompatibility antigens serve as antigenic, and hence proliferative, stimuli for the abnormal, immunologically competent cell. The result is a constant, unrestrained growth leading to what is recognized as a neoplasm of lymphoid tissue.

Tyler further proposed that the reaction between graft and host produced in hybrid mice by the injection of parental lymphoid cells into F_1 hybrids represents a potentially useful model for studying the etiology of cancer. Since the transplanted parental lymphoid cells lack one or more histocompatibility antigens present in the F_1 hybrid host, they are analogous to the hypothetical antigen-deleted, immunologically competent cell of lymphoid neoplasms. Kaplan and Smithers (*4*) and Green *et al.* (*5*) have also advanced the idea that the graft versus host reaction is analogous to malignant lymphomas, but on somewhat different grounds.

In our experiments the graft versus host reaction (runt disease, allogenic disease, homologous disease) (*6*) was studied with particular reference to the development of neoplasms. An important technical drawback to the use of this reaction for testing immunological theories of neoplasia is that ordinarily it is rapidly fatal. This objection was overcome in two ways: (i) The recipients were 4- to 6-week-old F_1 hybrids rather than infant mice, which are customarily used in studies of the graft versus host reaction. The mortality due to the graft versus host reaction was therefore reduced from 100 to 70 percent. (ii) The F_1 hybrid recipients of parental spleen cells were treated with amethopterin, which reduces the mortality due to the graft versus host reaction to 30 percent (*7*). It was possible by these methods to accumulate adequate numbers of mice for a long-term study.

The graft versus host reaction was induced in 6-week-old male (C57Bl/$6 \times$ DBA/2)F_1 mice (hereafter referred to as BDF_1) by four weekly injections of approximately 80×10^6 male C57Bl/6 spleen cells. The cells were prepared by mincing whole spleens with fine scissors and gently pressing the fragments through tanta-

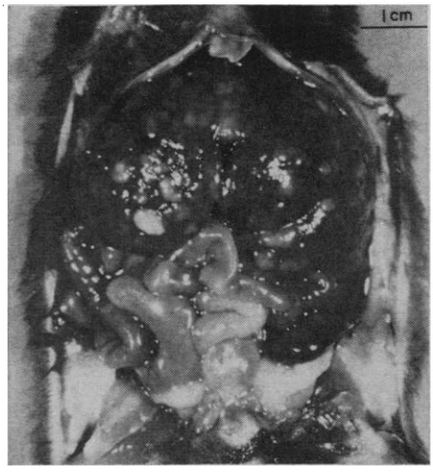

Fig. 1. This BDF_1 mouse developed transient allogenic disease, only to succumb to a widespread malignant lymphoma 1 year after the administration of C57Bl/6 spleen cells. The liver is studded with tumor nodules and the enlarged spleen extended into the pelvis.

lum gauze. The resulting suspension was diluted to the desired concentration of cells with Ringer solution and administered intraperitoneally. Two groups of BDF_1 recipients were used: (i) 65 mice that received 320×10^6 C57Bl/6 spleen cells and (ii) 50 mice that received the same dose of spleen cells followed by five intraperitoneal injections of amethopterin (3 mg per kilogram of body weight). The drug was given every other day beginning the day after the last injection of parental spleen cells was administered. These two groups of mice and six control groups are listed in Table 1. In our system, all deaths due to allogenic disease occurred during the first 150 experimental days. The surviving animals were then maintained under standard laboratory conditions for from 3 to 12 months. The tissues of all groups of mice were examined grossly and microscopically at the times indicated in Table 1.

Table 1. Incidence of malignant lymphomas in experimental and control mice.

Parental cells administered	Amethopterin	Mean age (mo)	Malignant lymphomas*
	Strain BDF_1		
Spleen	0	8	7/18
	Strain BDF_1		
Spleen	+	12	14/29
	Strain BDF_1		
None	0	10	0/36
	Strain BDF_1		
None	+	10	0/12
	Strain BDF_1		
Frozen-thawed spleen	0	10	0/12
	Strain BDF_1		
Liver	0	12	0/12
	Strain C57Bl/6		
	0	10	0/24

* Number of mice with tumors/total number of mice in group.

Approximately 70 percent of the mice of group (i) and 40 percent of the mice of group (ii) died of overt allogenic disease. One-half of the surviving mice developed transient, mild-to-severe runting, usually during the first 50 to 60 days of the experiment. Thereafter, their appearance was normal except for those mice that developed large tumor masses.

The incidence of tumors for all groups of mice is shown in Table 1. Only those mice which received living, parental spleen cells developed neoplasms. The difference in incidence of tumors between the amethopterin-treated and untreated mice may be due to the longer period of observation in the former group. All tumors originated in lymphoid tissue and involved retroperitoneal and peripheral lymph nodes, spleen, and small bowel. The thymus was never involved and histologically was either normal or slightly atrophied. Liver metastases were common, and invasion of the kidney by tumor was occasionally seen. The gross features of the tumor are shown in Fig. 1. Histologically the tumor consisted of reticulum cells, histiocytes, lymphocytes, and plasma cells (Fig. 2); multinucleated cells with a close resemblance to Reed-Sternberg cells were occasionally seen. Eosinophils were abundant in some tumors, absent in others. The overall histological picture was that of a granulomatous, neoplastic proliferation resembling Hodgkin's disease in some areas and reticulum cell sarcoma in others.

In order to determine whether parental cells persisted in the host animals, the discriminant spleen-cell assay (*8*) was carried out in 12 tumor-bearing mice. Cell suspensions prepared from the spleens of these animals were inoculated intraperitoneally into 8-day-old BDF_1 and $(C57Bl/6 \times A/Jax)F_1$ mice (referred to as BAF_1). In each litter selected for assay two mice were not injected, and these served as controls. Eight days later the mice were killed and their spleen and body weights were determined. The discriminant spleen-cell assay depends upon the development of splenic enlargement in recipients of the cell suspension. This indicates the presence of immunologically competent cells in the inoculum. The size of the spleen is evaluated by a "spleen index," the ratio of spleen weight in an injected mouse to the spleens of the control mice. Enlargement of the spleen is considered present if this index is 1.4 or higher. Splenomegaly in both BDF_1 and BAF_1 recipients indicated the presence of C57Bl/6 cells in the test inoculum; splenomegaly in BAF_1 recipients only indicated the presence of C57Bl/6 cells which were specifically tolerant of BDF_1 antigens; the absence of splenomegaly in both strains indicated failure to detect C57Bl/6 cells. Eleven of the 12 tumor-bearing mice tested in this manner were chimeras; that is, their spleens contained cells derived from the original inoculum of parental tissue (Table 2). Of these 11 chimeric mice, 10 harbored parental cells which had acquired specific immunological tolerance for host antigens.

The lymphoid tumors were transplanted into C57Bl/6, BDF_1, and BAF_1 recipients. Implants of tumor fragments were made subcutaneously with a trochar or by the intraperitoneal administration of suspensions of tumor cells. Newborn, suckling, and adult recipients were used, and these recipients have been observed for 6 to 10 months. Thus far, the tumor has been transplantable only to adult or suckling BDF_1 mice. Transplants have been successful only in those mice that had received suspensions of tumor cells intraperitoneally. In contrast to the original neoplasm, which never occurred in the thymus, the transplanted tumor characteristically involved the thymus. The gross features of a typical second-generation tumor are shown in Fig. 3. The histological features of this neoplasm were similar to those of the original tumor.

In these experiments normal adult tissues were transferred to normal mice with the result that neoplasia developed. Since mice of the donor strain did not develop tumors spontaneously, it is unlikely that the foregoing results represent merely the transfer of "premalignant" tissue from one animal to another. The special circumstances of the experiment were that lymphoid

neoplasms arose in F_1 hybrid mice injected with parental spleen cells. Since the first effect of this procedure is induction of the graft versus host reaction, a possible relation between the immunological disorder and neoplasia must be considered.

The analogies beween the graft versus host reaction and malignant lymphomas mentioned previously tend to be supported by these experiments. However, the extent to which this comparison can be carried appears to be limited by the experimental data. For example, if the tumors had developed from parental cells which were constantly stimulated by hybrid antigens, they should have been histocompatible with parental strain mice and hence transplantable to that line. This was not the case, and, contrary to the expectations of this hypothesis, the tumor was transplantable only to isogenic F_1 hybrid recipients. Thus the neoplasm behaved, at least insofar as its transplantation characteristics were concerned, like tissue of host, rather than of donor, origin. Tyler (*3*) predicted this result on the supposition that parental lines would lack the antigenic stimulus necessary for the proliferation of neoplastic cells. However, the finding that those parental, immunologically competent cells which were detectable in the tumor-bearing animals had specific immunological tolerance of host antigens is argument against this explanation. Since immunological tolerance of host antigens is acquired by foreign lymphoid grafts before any evidence of neoplasia (*7*, *9*), chronic antigenic stimulation leading to a neoplastic transformation of grafted cells does not seem likely. Nevertheless, since the mechanism of immunologic tolerance is poorly understood, this possibility cannot be entirely dismissed.

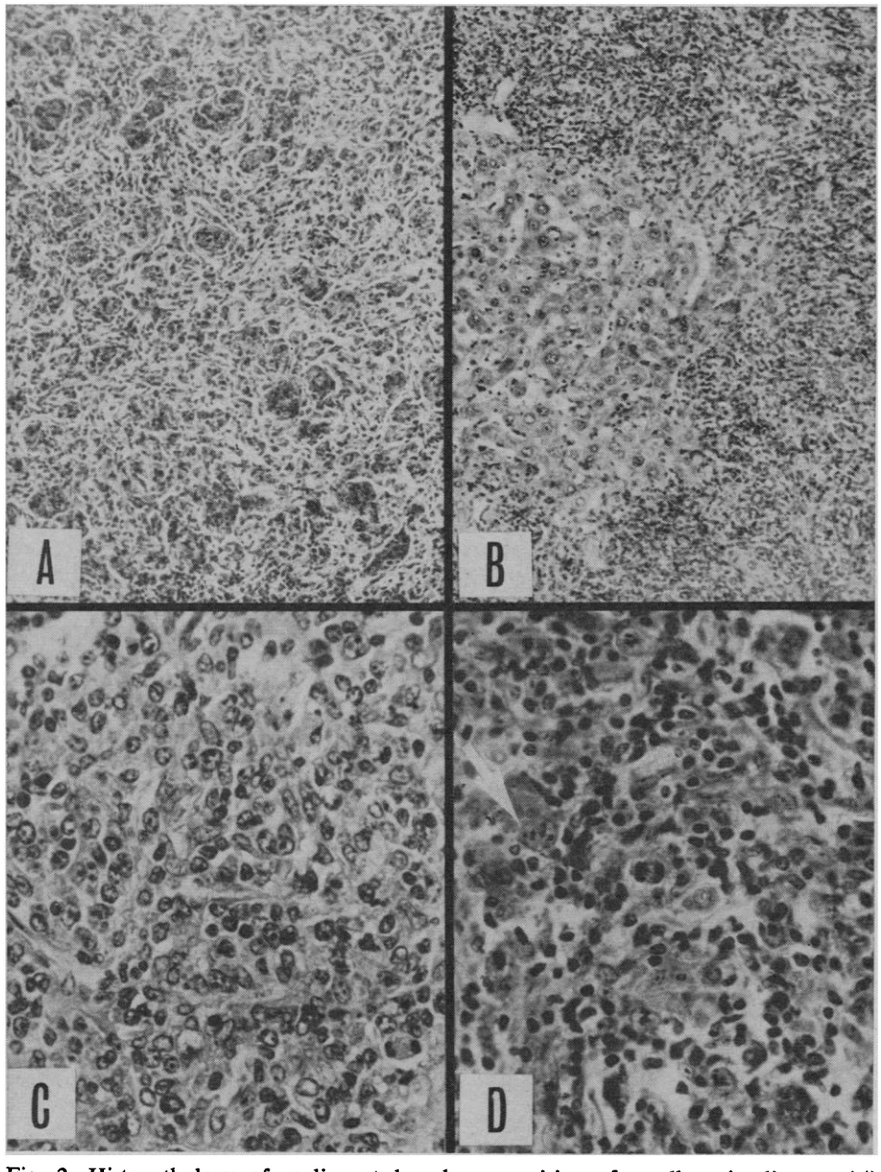

Fig. 2. Histopathology of malignant lymphomas arising after allogenic disease. (*A*) Retroperitoneal tumor. Nests of lymphocytes surrounded by sheets of histiocytes (approximately × 70). (*B*) Invasion of the liver by tumor (approximately × 70). (*C*) Lymph nodes. Numerous bizarre reticulum cells replacing the normal elements (approximately × 320). (*D*) Spleen. Malignant tumor consisting of reticulum cells and lymphocytes. White arrow points to a binucleated cell with prominent nucleoli, resembling a Reed-Sternberg cell. Note the mitotic figure (approximately × 320). Hematoxylin-eosin stain.

One of the characteristic features of the graft versus host reaction is a marked proliferation in lymphoid organs of morphologically primitive cells. Although the origin of these cells may vary with species and organ, there is convincing evidence that in the mouse spleen they are of host origin (*10*). There is as yet no satisfactory explanation of this paradoxical finding. On theoretical grounds, it might be expected that F_1 hybrid cells would not actively participate in the graft versus host reaction. Since the particle-clearing function of the reticuloendothelial system is highly exaggerated in allogenic disease (*11*), it is conceivable that host macrophages and other phagocytic cells proliferate in response to tissue injury induced by the graft. In any event, these observations raise the possibility that host cells, stimulated by parental spleen cells or the damage they cause, might undergo a neoplastic transformation in the course of a chronic graft versus host reaction. This interpretation is consistent with the transplantation characteristics of the tumor we observed.

Another important feature of the graft versus host reaction is extensive destruction of lymphocytes. This raises the possibility of the release of oncogenic material from these cells. Fialkow *et al.* (*12*) have recently found that fibroblasts cultured in vitro develop gross chromosomal changes, particularly hyperploidy, on the addition of a crude extract of allogenic peripheral blood lymphocytes. Chromosomal changes were not observed when the lymphocyte extract was added to autologous fibroblasts. Their experiments suggest the possibility that chromosom-

Table 2. Discriminant spleen-cell assays in tumor-bearing mice. Parental cells were undetectable in mouse No. 258; all others were chimeras. Of these, all except mouse No. 553 contained parental cells which were specifically tolerant of BDF_1 antigens.

Animal No.	Spleen index	
	BDF_1	BAF_1
241	1.0	1.6
249	1.0	1.4
258	1.0	1.2
531	1.0	1.8
537	1.0	1.4
550	1.2	1.9
553	1.5	1.7
554	1.2	1.7
556	1.2	1.6
572	1.1	1.4
576	1.0	1.6
587	1.1	1.6

al abnormalities could occur in the course of the graft versus host reaction as the result of an as yet to be identified product of the breakdown of lymphocytes. Such chromosomal defects might be the first step in the development of malignancy.

Finally, the very strong possibility of the release of an oncogenic virus during the graft versus host reaction must be seriously considered. The parental line used in the present experiments, C57Bl/6, is heavily infested with mouse leukemia virus (*13*). This virus is present in a latent form and requires some external stimulus, such as x-irradiation, for its transformation into active oncogenic material (*14*). It is entirely possible that the extensive cellular reaction which develops during the graft versus host reaction has a role analogous to that of total body x-irradiation in causing the release or activation of an oncogenic virus. There are, however, several differences between the graft versus host reaction–associated tumor and mouse lymphoid neoplasms known to be of viral origin. (i) In the case of virus-induced mouse leukemia the thymus is commonly affected, but in the tumors which developed during the graft versus host reaction the thymus never contained neoplastic cells. It is of interest to note, however, that on transplantation to isogenic recipients, the graft versus host reaction–associated tumor characteristically involved the thymus. The reason for this change in the behavior of the neoplasm is unknown. (ii) Thymectomy generally, but not invariably, inhibits the development of virus-induced mouse leukemia (*15*). We have some evidence that thymectomy of BDF_1 hosts does not prevent the development of graft versus host reaction–associated lymphomas. (iii) In those situations where thymectomized F_1 hybrid mice were exposed to total body x-irradiation and transplanted with parental thymic tissue, a tumor developed which was almost always of donor origin (*16*). These experiments have been interpreted as indicating that the thymus is an obligatory target organ of oncogenic viruses that are activated by total body x-irradiation. In our studies, nonirradiated hybrid mice were injected with parental spleen cells and the tumor was of host origin. These differences, while of importance, do not rule out a viral etiology for the tumors we observed.

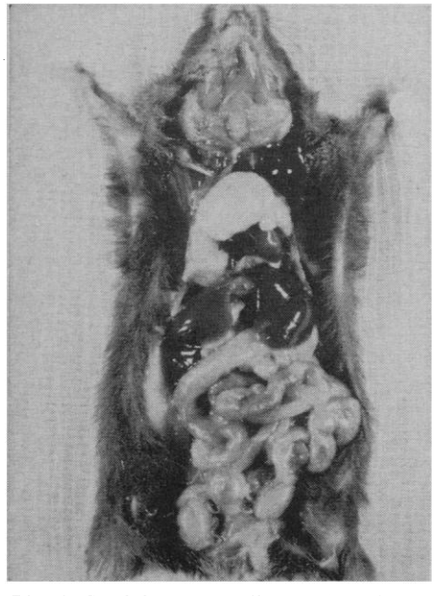

Fig. 3. Recipient of malignant lymphoma cells that arose in a BDF_1 mouse which had recovered from allogenic disease. There is a large tumor replacing the thymus.

The proposals of Tyler (*3*), Kaplan and Smithers (*4*), and Green *et al.* (*5*) that the graft versus host reaction is a potentially useful model of malignant lymphomas has been amply supported by our results. They are also strengthened by the finding that newborn C3H ($H\text{-}1^a$) mice injected with spleen cells from isogenic resistant adult C3H ($H\text{-}1^b$) mice developed a high incidence of lymphoma (*17*). Under these conditions a long-term, low-grade graft versus host reaction could ensue. Finally, the observation of Mellors (*18*) that NZB/Bl mice, which spontaneously develop autoimmune hemolytic anemia and glomerulonephritis, also develop malignant lymphomas is an additional indication that immunologic and neoplastic proliferations may actually be different phases of the same fundamental process.

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References and Notes

1. W. Dameshek and R. S. Schwartz, *Blood* **14**, 1151 (1959).
2. D. Metcalf, *Brit. J. Cancer* **15**, 769 (1961).
3. A. Tyler, *J. Nat. Cancer Inst.* **25**, 1197 (1960); *Biological Interactions in Normal and Neoplastic Growth* (Little, Brown, Boston, 1962), pp. 533–571.
4. H. S. Kaplan and D. W. Smithers, *Lancet* **1959-II**, 1 (1959).
5. I. Green, M. Inkelas, L. Allen, *ibid.* **1960-I**, 30 (1960).
6. If the recipient of tissue transplanted from a genetically dissimilar individual is immunologically competent, it will be sensitized by antigens present in the graft. The resulting host versus graft reaction destroys the transplant. The rejection of a foreign skin graft exemplifies this response. The graft versus host reaction develops under the following circumstances: (i) the recipient is unable to reject the graft, (ii) the graft contains immunologically competent cells, and (iii) the tissues of the host are antigenic to the graft. These conditions are fulfilled when lymphoid cells from an inbred mouse are transplanted to its F_1 hybrid. The graft versus host reaction, as observed in the living animal, is a complex syndrome. It has been called "runt disease" because its principal manifestation is emaciation or impaired growth. Because it is induced by allogenic (homologous) cells, it is also called "allogenic (homologous) disease." In the newer terminology of tissue transplantation, "allogenic," referring to a graft between genetically dissimilar members of the same species, has replaced "homologous" [see M. Simonsen, *Prog. in Allergy* **6** (1962), 349 (1962) and P. S. Russel and A. P. Monaco, *The Biology of Tissue Transplantation* (Little, Brown, Boston, 1965) for reviews].
7. R. S. Schwartz and L. Beldotti, *Transplantation* **3**, 79 (1965).
8. M. Simonsen, J. Engelbreth-Holm, J. Jensen, H. Poulsen, *Ann. N.Y. Acad. Sci.* **73**, 834 (1958).
9. M. Simonsen, *ibid.* **87**, 382 (1960).
10. J. G. Howard, D. Michie, M. Simonsen, *Brit. J. Exp. Pathol.* **42**, 478 (1961); A. J. S. Davies and S. M. A. Doak, *Nature* **187**, 610 (1960).
11. J. G. Howard, *Brit. J. Exp. Pathol.* **42**, 72 (1961).
12. P. J. Fialkow and S. M. Gartler, data presented at the 57th annual meeting of the American Society for Clinical Investigation, 3 May 1965.
13. L. Gross, *Oncogenic Viruses* (Pergamon, New York, 1961).
14. M. Lieberman and H. S. Kaplan, *Science* **130**, 387 (1959).
15. H. S. Kaplan, *J. Nat. Cancer Inst.* **11**, 83 (1950); J. F. Duplan, *Pathol. Biol. Semaine Hop.* **11**, 917 (1963).
16. H. S. Kaplan, M. B. Brown, J. Paull, *Cancer Res.* **13**, 677 (1953); L. W. Law and M. Potter, *Proc. Nat. Acad. Sci. U.S.* **42**, 160 (1956).
17. R. L. Walford and W. H. Hildemann, unpublished experiments cited by R. L. Walford, *Experimental Gerontology (London)* **1**, 67 (1964).
18. R. Mellors, *Blood*, in press.
19. Some of these experiments were reported at the 57th annual meeting of the American Society for Clinical Investigation, 3 May 1965. Supported with funds from PHS grant No. CA 0468-07.

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