The Role of the Reticuloendothelial System in the Host Reaction to Neoplasia*

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The fundamental questions to be dealt with in studies of the cancer process can be broadly divided into two general categories. One involves the events which lead to the origin of the cancer cell, encompassing those intimate intracellular alterations induced by virus, chemical, or physical carcinogen which lead to the formation of cells with neoplastic potential. The other process involves the possible host reaction to the existent tumor or to the agents responsible for its origin. It is with one aspect of the latter process that this paper will be chiefly concerned, specifically with the phenomena usually designated as “Host Defenses.” Reactions on the part of the host, such as vascularization, which are favorable to the success of the tumor will not be discussed. The presence of a host defense against cancer implies that an organism has the capacity to react with or inactivate oncogenic factors or that, following formation of the neoplastic cell, the host possesses mechanisms to limit the growth of such cells or to destroy them altogether. In bacterial or viral infections where host factors clearly influence the disease state produced, the immunological, phagocytic, and inflammatory reactions are of unquestionable importance. Similar factors if operative in the primary tumor-bearing host would lead one to postulate that tumors possess unique antigens capable of eliciting an immune response of varying effectiveness or that the distortion of normal tissue continuity as a consequence of tumor mass or the production by the tumor of substances with irritative properties would induce an inflammatory reaction limiting tumor invasion.

The question of specific tumor antigens has long concerned oncologists and immunologists (30, 37, 39, 82, 83). It is obvious that evidence bearing on this problem is relevant only in the case of primary tumors or in early isologous transplants of such tumors in which the contribution of isoantigens is excluded. Several approaches have been used to detect cancer antigens, only a few of which will be mentioned.

Following immunization of guinea pigs with heterologous tumor tissue and desensitization either in vivo (82, 83) or in vitro (19, 50–52) with normal tissue or serum, a further anaphylactic reaction could be elicited by tumor extracts or sera from patients with cancer. Antisera prepared in foreign species against mouse tumor tissue were studied by agar gel diffusion technics and found to contain an antigen specific for the tumor (83). Björklund et al. (14, 15) reported that antisera prepared in horses with pooled human tumor tissue were able to lyse neoplastic cells in vitro; the lytic activity of these sera could be absorbed with neoplastic but not with normal tissue. The evidence from studies which imply the presence of a specific antigen or of antigenic components common to various tumors of the same species must be interpreted with caution. What appears to be a unique tumor antigen may merely reflect a quantitative antigenic difference between tumor and normal tissues or factors extraneous and unrelated to the tumor cells, such as bacterial or viral agents.

A more desirable approach would be to demonstrate inhibition of tumor growth as a consequence of specific immunization and to relate tumor inhibition with a demonstrable immune response of the host. Studies to demonstrate such antitumor immunity in most experimental tumor systems have been largely unsuccessful (24, 25, 34, 37). Positive results, however, have been reported with some rodent tumors (1, 33, 66). Hirsch et al. (40)
claimed to have prolonged survival time of mice bearing isologous transplants of spontaneous mammary tumors by repeated prior exposure to cycles of tumor growth and removal. The presence of a unique antigenic component (x-antigen) in murine leukemia has been reported by Gorer and Amos (39), who used antisera prepared in homologous strains and absorbed in vivo. With irradiated cells (67) or irradiated cells in Freund's adjuvant, 1 immunization with isologous lymphomas has not as yet been accomplished. Cross-reactivity among leukemias within the same inbred strain has been absent or weak (31). Foley (26) and Prehn and Main (65) have demonstrated that prior growth of methylcholanthrene-induced tumors in various inbred strains of mice, followed by tumor removal, either surgically or by ligation, rendered the mice resistant to further transplants of the same tumor. Révész (67) has produced a similar state of refractoriness to transplants of methylcholanthrene-induced tumor cells by prior immunization of the animals with irradiated cells derived from the original tumor. Finally, Klein et al. (47) have demonstrated a resistance of the primary host to its own methylcholanthrene-induced sarcoma by repeatedly immunizing with irradiated autologous tumor cells and challenging with cells kept in isologous passage from the original tumor.

Indirect evidence based on morphological changes in draining lymph nodes and spleen, and on the presence of inflammatory cells at the tumor site during growth of primary tumors of mice, rats, and humans, can be interpreted as indicating a host response. Parsons (62, 63) and Baruah (3) have noted increased numbers of plasma cells in the lymph nodes and spleens of animals bearing carcinogen-induced tumors. Both sinus histiocytosis in the lymph node draining the tumor site (16, 80) and plasma cell proliferation in draining lymph node and surrounding the tumor (6) have been observed and correlated with a more favorable prognosis. Morphological evidence cannot, however, be interpreted as necessarily indicating a specific immune response, but may have been elicited by products of tumor necrosis or a secondary bacterial or viral infection of the tumor or host.

To understand further the role of the host in the control of tumor growth, we have attempted to alter the development of experimental tumors by conditions which increase natural resistance to experimental infections. Prior treatment with certain agents including endotoxin, zymosan, and infection with Bacillus Calmette-Guérin (B.C.G.) has been found to alter favorably the response of the host to bacterial (23, 41, 69) or viral challenge (28, 58, 61, 79). These agents are capable of enhancing the capacity of the host to form antibody (12, 35) and eliciting alterations in the macrophage elements of the reticuloendothelial system (RES), including heightened phagocytic activity (5, 7, 9), increased bactericidal activity of individual macrophages (46), and greater acid phosphatase activity of the macrophage (75, 76). Previous reports from this and other laboratories have demonstrated that certain transplanted tumors, as well as a virus leukemia, induce a state of RES hyperactivity as measured by the rate of clearance of colloidal carbon from the blood (10, 13, 59, 72) and hyperplasia of phagocytic cells (75, 76). Furthermore, prior treatment with zymosan (17) and B.C.G. (11, 36, 56) has been found to limit the growth of certain transplanted tumors.

The present study deals further with the relationship of the RES, defined in its broadest sense, to tumor development and growth. The genetic control of phagocytic activity and immune response has been investigated in various inbred strains of mice both under basal conditions and following stimuli which provoke proliferation of RE cells. The response of the RES to tumor growth and virus challenge has been investigated. Attempts to alter tumorigenesis, growth of isologous tumors, and viral infection by agents known to influence the RES will also be reported.

Reticuloendothelial Function in Various Inbred Strains of Mice

The use of inbred strains of mice has been essential to experimentation in cancer research. Extensive studies have been performed on a variety of mouse strains to determine biological characteristics which might accurately correlate with the spontaneous or induced tumor incidence in these strains. It has been suggested that reticuloendothelial function, as defined by dye storage and capacity to form antibody, might be deficient in certain high-tumor strains (21, 70, 71). Davidsohn and Stern have reported that, of eleven strains studied, the five with low tumor incidence demonstrated a more vigorous immune response to heterologous red blood cells than did five of six high-tumor strains. Ipsen (44, 45), investigating the immune response to tetanus toxoid in various strains of mice, has reported that BALB/c and C57BL/6 were better antibody-producers than C3H or DBA/2, and, furthermore, random-breed Swiss mice appeared to be superior to most inbred strains in this respect.

With the availability of more accurate technics...
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...to measure the phagocytic function of the RES, it seemed of importance to investigate further RE function in various strains of mice (57).

Assessment of phagocytic activity has been performed by following the rates of clearance of a standard dose of colloidal carbon (16 mg/100 gm body weight) from the peripheral blood according to the technics described by Biozzi et al. (8). The stabilized preparation of colloidal carbon of homogeneous particle size (about 250 Å) was injected intravenously. Repeated samples of blood were withdrawn from the retroorbital venous plexus, and the carbon concentration in each sample was determined spectrophotometrically. When the logarithms of these values are plotted against time, they are found to fall along a straight line. The slope of this line, K, is a measurement of phagocytic activity of the RES for the dose of carbon injected. Since approximately 90 per cent of the injected colloid is removed from the circulating blood by the phagocytic cells lining the sinusoids of the liver and the spleen, these organs are removed and their weights correlated with clearance rates.

Chart 1 represents a comparison of the rates of clearance of carbon in Swiss Ha/ICR and A strain mice under both basal conditions and 19 days following infection with 1 mg. (net weight) Bacillus Calmette-Guérin (B.C.G.). The response of the RES in various inbred strains to a standardized infection with B.C.G. has been investigated, since this infection causes a sustained hyperactivity of the RES as evidenced by hyperplasia of macrophage elements in the liver and spleen associated with heightened phagocytic activity (7).

Modifications in phagocytic activity and weights of liver and spleen following B.C.G. infection in normal female Swiss Ha/ICR mice are presented in Chart 2. As a rule, no mortality is observed during the course of this infection, and, except for occasional transient weight loss, the dose of B.C.G. used is well tolerated by most strains of mice. The peak of phagocytic activity occurs 2-3 weeks following initiation of infection and then progressively falls. Marked hepatosplenomegaly accompanies the infection, and the weights of these organs remain increased for at least 2-4 months. In our studies we have chosen 18-21 days following infection with B.C.G. as a standard time to compare the response of the different mouse strains.

The following strains of mice were investigated in our various studies: DBA/2, CSH/Bi, JK, BALB/c, C57BL/4 and A. These mice were obtained from Dr. J. J. Bittner, University of Minnesota. In addition, the I, C3H/An, BALB/c and C57BL/C1 (Columbia subline) are maintained by brother-sister mating to an inbred nucleus in our laboratories; two crosses, (Ic X C3H/An)F1 and (C3H/An X BALB/c)F1, are derived from these inbred strains. AKR were obtained from Texas Inbred Mouse Company and Jackson Memorial Laboratories, and Swiss Ha/ICR from Millerton Research Farms, N.Y. Virgin female adult mice, 2-4 months old, were used in these studies. The results of our observations are presented in Chart 3. Each value represents the average of data obtained from four to six mice.

The basal level of phagocytic activity was essentially similar in all inbred strains but generally lower than that found in the random-bred Swiss mouse. No difference could be demonstrated between the basal phagocytic activity of high-tumor or leukemia strains such as C3H, DBA/2, or AKR and the low-tumor strains C57BL/4, JK, or I. The absence of the mammary tumor agent (C3Hf, C3H/Bi foster-nursed) apparently does not alter the phagocytic capacity of the C3H/Bi mouse. Similar to rates of clearance, control values for liver and spleen weights differ little among the various inbred strains; Swiss mice, however, possess somewhat larger organs, even when calculated on a per gram body weight basis. A point deserving emphasis is that basal phagocytic activity and
spleen weight are very sensitive indicators of the past infectious history of the animal. If mice are not maintained under optimally clean conditions, the essential similarity of basal RE function in these various strains can be obscured. The small difference observed in normal phagocytic activity and spleen size between inbred and random-bred mice may reflect either more rigorous precautions in the care of inbred animals or poorer response of these mice to infectious stimuli from their environment. The data concerning reaction to B.C.G. would tend to favor the latter hypothesis.

Following B.C.G. infection differences in response of various strains, unsuspected from basal studies, became apparent. B.C.G.-infected Swiss Ha/ICR mice develop rates of clearance 6–15 times above normal, whereas most inbred strains respond by a four- to sevenfold increase. One inbred strain, AKR, showed as vigorous a response as Swiss Ha/ICR to B.C.G. infection with respect to phagocytic function. What relevance this heightened reactivity may have to their high incidence of leukemia deserves further study. The livers of the various inbred strains do not greatly enlarge following B.C.G. infection. The Swiss Ha/ICR mouse, in contrast, develops considerable hepatomegaly. The smallest increase in spleen weight occurred in C3H/Bi and DBA/2 mice, whereas marked splenomegaly, in some cases over 1 gm., is found in C57BL/6, BALB/c, and Swiss Ha/ICR. A study of the spleen response to B.C.G. infection in two F1 crosses—(C3H/An × BALB/c)-F1 and (DBA/2 × Swiss Webster)F1—revealed a response intermediate to that of the parent strain. Histologically, granuloma formation was observed in the liver and spleen in all B.C.G.-infected mice. In contrast to the multiple and large granulomata found in C57BL/6, BALB/c, and Swiss Ha/ICR mice, C3H/Bi mice develop smaller granulomas. This finding correlates well with our observation that mice of this strain are less resistant to B.C.G. and may die during the course of the infection.

Experiments were also conducted to correlate immune response to Brucella abortus with RES function in these various strains. Results presented

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**Chart 2.**—Effect of B.C.G. infection (1 mg. wet weight I.V.) on phagocytic function as measured by rate of carbon clearance and liver and spleen weights in female Swiss Ha/ICR mice. (From Old et al., Ann. N.Y. Acad. Sci., 88:264, 1960.)
in Table 1 show that DBA/2 and C3H produce lower titers of antibody to Brucella than do C57BL/6 and Swiss HA/ICR mice. Although the differences are not marked, the lower immune response seen in DBA/2 and C3H correlates with their relatively smaller increase in spleen size after B.C.G. infection, as seen in Chart 3.

From these studies and the reports of others (21, 44, 45, 70, 71), it is apparent that there are genetic factors which determine the functional characteristics of the RES and its capacity to respond to infectious or antigenic challenge. As might be expected, random-bred Swiss mice respond more vigorously to the challenges used in these studies than do most inbred strains. Whereas variability in response is less marked within individual inbred strains as compared with Swiss HA/ICR mice, differences among various inbred strains in terms of spleen response to B.C.G. and immune response to Brucella are apparent. The consistently poorest response has been observed in DBA/2 and C3H strains, a fact confirming the observations of Davidsohn and Stern (21) and Ipsen (44, 45) with other systems. Since these two strains of mice are known for high spontaneous tumor incidence, it would be tempting to correlate tumor susceptibility to deficient reticuloendothelial function. However, the host and the presence or absence of the mammary tumor agent, such a direct correlation appears as yet unwarranted.

**Response of the Reticuloendothelial System to Tumor Growth and Certain Virus Infections**

The observed increase in phagocytic activity of the RES, measured by carbon clearance, during the course of various infectious processes suggested that application of these techniques to the study of experimental tumors might reveal similar changes. This approach would allow experimental confirmation of the long-postulated relationship between tumor development and growth of the RES (Stern and Willheim [73]). Biozzi et al. (10) have observed that growth of a transplanted rat tumor (Guerin carcinoma) is associated with increased phagocytic activity of the RES. Similarly, hyperactivity of the RES has been reported during growth of a number of other transplantable rodent tumors (59, 72). In contrast, little or no modification in phagocytic activity in mice bearing isologous transplanted tumors or spontaneous tumors was found (18, 59). These findings were interpreted as evidence of participation by the RES in the homograft reaction of the host to specific isoantigens of the tumors.

Further studies have been made to explore the mechanism of activation of the RES by transplanted tumors and of factors other than the homograft reaction which might be involved in these phenomena. It has been found that a variety of transplantable tumors or tissues from hosts bearing such tumors contain a transmissible agent(s) which has the capacity to elicit a similar effect on the RES as the growth of the intact tumor cells (60).

As an example of the changes in phagocytic function of the RES occurring during the course of a transplanted tumor growth, the result of an
experiment with Sarcoma 180 (S-180) in Swiss 
Ha/ICR mice is presented in Chart 4. As can be 
seen, alterations in phagocytic activity, as meas-
ured by carbon clearance (16 mg/100 gm body 
weight), occur surprisingly early, at a time when 
the tumor is barely palpable. Another early feature 
associated with tumor growth is the development 
of splenomegaly. Both the spleen weight and 
phagocytic activity return to normal levels prior 
to death of the animal.

A finding in the course of other experiments that 
animals bearing S-180 demonstrated a greater re-
sistance to Mengo virus challenge, a fact which 
could possibly be interpreted as interference by 
another viral agent, led us to suspect the presence 
of a transmissible agent in the tumor and tissue of 
S-180-bearing mice. It was found that homoge-

data from Old et al., Ann. N.Y. 
Acad. Sci., 88:264, 1960.)
phosphatase activity of liver macrophages is practically abolished, and only an occasional cell or cell remnant with acid phosphatase activity is evident (Figs. 1–3). By 4 days, however, the number of Kupffer cells in the liver is markedly increased as demonstrated both by acid phosphatase and clearance of carbon. At this time, both liver and, in particular, spleen weights are increased, and a leukocytosis of 15–20,000 WBC/cu mm without change in the differential is apparent during the first 3 weeks following infection. From the 7th day to the 4th week, the heightened phagocytic activity of the RES falls to normal or slightly above normal activity. The spleen, which increases to approximately 3–8 times its normal weight during the first week of infection, does not appear to return to normal weight for many months. Related to the persistence of the splenomegaly, the S-180 agent can be recovered from the tissues of infected animals as long as 4 months following infection. In Table 2 some characteristics of the agent or agents isolated from tumor or tissues of S-180-bearing animals are presented. With the use of identical technics for isolation and demonstration of the S-180 factor, agents with similar characteristics have been recovered from a variety of other transplanted tumors (Table 3). In contrast, the tumor or tissues from animals bearing spontaneous or carcinogen-induced tumors, or early isologous transplants of these tumors, have

3 Another technic which can be used to investigate macrophage elements of the RES is the Gomori technic for histochemical demonstration of acid phosphatase activity (30). It has been shown that the macrophage possesses a particularly high concentration of acid phosphatase (30, 35), and in previous studies (75, 76) the number of acid phosphatase-containing phagocytic cells in the liver as well as their acid phosphatase content correlated well with the functional state of the RES as measured by carbon clearance.

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**Chart 5.**—Effect of infection with an agent(s) isolated from Sarcoma 180 on phagocytic activity of the reticuloendothelial system and spleen, liver, and body weight in female Swiss HA/ICR mice.
thus far proved negative for RES-stimulating activity. Those tumors which have been shown to transmit an S-180-like agent, with the exception of L1210,\(^4\) have all been found to cause increased phagocytic activity of the RES during tumor growth. Whereas growth of Ca-755 in C57BL/4 backcross mice\(^6\) causes a greatly enhanced phagocytic activity of the RES and the appearance of a transmissible agent causing splenomegaly, neither of these phenomena can be observed when the tumor is grown in the isologous host, C57BL/C1 (Columbia subline). Isologous transplants of tumors that do not significantly modify the phagocytic activity of the RES can, themselves, be infected with the S-180-derived agent by growth in a previously infected host and, in subsequent passage, provoke marked enhancement of RES activity. The growth and behavior of such "infected" tumors, however, do not change.

Findings such as these suggest that a re-evaluation of the significance of increased phagocytic activity as an indication of participation by the RES in the homograft reaction is necessary. To determine the contribution of the isoantigens of the homograft in these phenomena, tumor systems which possess no transmissible agent demonstrable by technics used to isolate the S-180 agent have to be employed. An experiment was performed with a tumor induced by methylcholanthrene in female BALB/c mice (Meth. A). This tumor in its eighth or ninth transplant generation was implanted as bilateral passage pieces into isologous BALB/c and two homologous strains, C3H/An and Swiss Ha/ICR. In contrast to long-transplanted tumors which have lost strain specificity, this tumor will grow only transiently in homologous hosts. Animals in this experiment were studied at 6–7 days following tumor inoculation, since the tumor reaches maximum size at this time in homologous hosts, and such an interval is sufficient for the growth of S-180 to cause a high degree of RES hyperactivity. The results of these experiments (Table 4) show a small but significant increase in rates of carbon clearance as well as an increase in weights of liver and spleen in both the isologous and homologous host. Observations concerning this particular carcinogen-induced tumor (Meth. A female BALB/c) to be presented in the last section of this paper demonstrate the marked antigenicity of this tumor in isologous hosts. This may account for the small but definite effect on the RES of isologous tumor growth observed in these experiments. In no way, however, were these

### TABLE 2

**SOME CHARACTERISTICS OF A TRANSMISSIBLE AGENT(S) ISOLATED FROM SARCOMA 180**

1. Recoverable from all tissue of S-180 tumor-bearing animals
2. ID\(_{50}\) 4.8–6 days following infection
3. ID\(_{50}\) 2.6–7 days to 2 months following infection
4. Filterable—Sela 0.2 filter
5. Reduction of acid phosphatase activity in liver macrophages 24 hours following infection
6. Reduction in lethality of Mengo virus infection
7. Increased rates of carbon clearance
8. Proliferation of liver macrophages
9. Splenomegaly
10. Leukocytosis
11. Increased alkaline phosphatase sinusoidal lining of liver
12. Severe anemia and death in irradiated C3H mice (250 r)
13. Unaltered to accelerated S-180 or Ehrlich Ascites growth in infected mice

ID\(_{50}\) refers to negative log or infective dilution.

### TABLE 3

**RECOVERY OF AGENT(S) FROM VARIOUS TISSUES OF TUMOR-BEARING ANIMALS INDUCING SPLENOMEGALY IN SWISS MICE**

<table>
<thead>
<tr>
<th>Active</th>
<th>Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoma 180 (Swiss Ha/ICR)</td>
<td>Adenocarcinoma 755 (C57BL/C1)</td>
</tr>
<tr>
<td>Ehrlich ascites (Swiss Ha/ICR)</td>
<td>3,4,9,10-Dibenzpyrene-induced fibrosarcomas</td>
</tr>
<tr>
<td>Adenocarcinoma 755 (C57BL/4 backcross)</td>
<td>1st transplant generation (BALB/c) ([1×C3H]F(_1))</td>
</tr>
<tr>
<td>Leukemia 1210 (DBA/2)</td>
<td>8-Methylcholanthrene-induced fibrosarcomas</td>
</tr>
<tr>
<td></td>
<td>5th transplant generation ([C3H×BALB/c]F(_1))</td>
</tr>
<tr>
<td></td>
<td>7th transplant generation (BALB/c)</td>
</tr>
<tr>
<td></td>
<td>3,4,9,10-Dibenzpyrene-induced fibrosarcoma (Swiss Ha/ICR)</td>
</tr>
<tr>
<td></td>
<td>Spontaneous mammary adenocarcinomas (Swiss Webster) ([C3H/An] [(IXC3H] F(_1)]</td>
</tr>
<tr>
<td></td>
<td>Spontaneous lymphatic leukemia (DBA/2)</td>
</tr>
</tbody>
</table>

\(^4\) Response of the RES to growth of L1210 in isologous DBA/2 has not as yet been studied in our laboratories.

\(^6\) C57BL/4 backcross produced by Dr. J. J. Bittner, University of Minnesota.
effects comparable to those seen with growth of tumors from which transmissible agents active on the RES can be recovered.

Several important questions are raised by the finding of a transmissible agent(s) from tumor-bearing hosts, which causes activation of the RES.

1. Are the phenomena observed the result of infection by a single agent or multiple agents?

2. Do similar agents occur in tumors in other species?

3. What, if any, is the relationship of these agents to oncogenesis?

Since these agents have been found in tumors which have undergone prolonged passage through innumerable hosts, the possibility of infection with virus unrelated to the tumor is apparent. Transmissible agents have been found in a number of transplanta
table tumor systems (20, 22, 48, 49, 68, 74) without demonstrable relationship to the origin of the tumor. One such agent (or agents), which is characterized by causing elevated plasma lactic dehydrogenase levels, has been isolated from a wide variety of transplantable tumors by Riley and associates (68).

It is most probable that the agent(s) described in our experiments bears no relationship to tumorigenesis. Animals infected with S-180 agent and observed up to 6 months have as yet developed no tumors. Nevertheless, experiments to explore the possible oncogenic activity of the S-180-derived agent injected into newborn mice are in progress. It may be relevant to this problem that a known oncogenic agent causing a reticulum-cell leukemia in mice, the Friend virus (27), induces similar striking alterations in the phagocytic activity of the RES (Chart 6) (59). Rates of carbon clearance fall approximately 50 per cent 24 hours after inoculation of this agent and then show progressive elevation during the early phase of the leukemia.

If the agents described in the present study bear no relationship to tumorigenesis, these findings would emphasize further the need for caution in interpreting data concerning morphological, biochemical, and immunological alterations in the tumor or hosts growing such tumors.

The Reticuloendothelial System and Virus Infection

Since it is now well established that a variety of tumors have a definite viral etiology, basic information on host factors which might influence virus diseases becomes relevant to a discussion of the cancer problem. In addition, certain virus infections, including the Friend virus leukemia or that infection induced by the agent derived from S-180,
fect on macrophages in vitro in cultures containing a mixed cell population (2).

Findings such as these suggest that agents which induce proliferation of phagocytic cells, or create within the macrophage an intracellular environment unfavorable for virus replication, might modify the course and lethality of virus infection. Endotoxins, substances known to heighten natural resistance to bacterial challenge as well as to enhance the phagocytic activity of the RES, have been reported to offer a degree of protection in certain virus infections (28, 79). In addition, certain agents such as trypan red (81) and atabrine (42, 43), which are phagocytized by cells of the RES, also alter mortality following virus infection.

To further assess the role of the RES in virus infections, we have studied Mengo virus lethality in mice. Mengo virus produces encephalitis and death in normal Swiss mice within 2.5–5 days following injection by a variety of routes. The consistent and rapid lethality as well as the uniform susceptibility of mice of varying ages have made this virus useful as an experimental model. Infection with B.C.G. with resultant RES hyperplasia has been found to render mice more resistant to Mengo challenge (Table 5) (58). Similar protective activity has been observed with other agents active on the RES (61). A complete study of these effects and their possible mechanism will be published elsewhere.

The lethality of Mengo virus in mice previously infected with Friend virus (FV), or given inoculations of S-180, or the agent derived from S-180, was also studied. The results of these experiments are presented in Tables 6 and 7. A significant protection to Mengo challenge, as reflected in the

![Chart 6](http://cancerres.aacrjournals.org)
LD₅₀, is seen as early as 1 day following infection with FV or the S-180 agent. This resistance to Mengo decreases with time, even though both FV and the S-180 agent can be recovered from the tissues many months after inoculation. Attempts to demonstrate a difference in resistance to Mengo challenge between CSII mice with the mammary tumor agent and foster-nursed C3H without the agent proved unsuccessful.

Since macrophages are a site of virus localization in vivo, and since (specifically in the case of infection with FV and S-180 agent) the RES appears to be rapidly affected, it might be assumed that these viruses also localize and infect RE cells at an early stage. The possibility exists, therefore, that the observed interference in these last experiments occurs at the level of the macrophage. It is also of interest that infection with an oncogenic agent (Friend virus) has been shown to alter the outcome of infection with a neurotropic virus.

**TABLE 5**

**INCREASED RESISTANCE TO MENG0 VIRUS CHALLENGE INDUCED BY B.C.G. INFECTION**

<table>
<thead>
<tr>
<th>Days following B.C.G. infection</th>
<th>B.C.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.8</td>
</tr>
<tr>
<td>1</td>
<td>6.8</td>
</tr>
<tr>
<td>8</td>
<td>5.8</td>
</tr>
<tr>
<td>16</td>
<td>5.0</td>
</tr>
<tr>
<td>26</td>
<td>5.6</td>
</tr>
<tr>
<td>Control</td>
<td>L.D₅₀*</td>
</tr>
<tr>
<td>7.0</td>
<td>7.2</td>
</tr>
<tr>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>6.5</td>
<td>7.6</td>
</tr>
</tbody>
</table>

* L.D₅₀-log dilution I.V. challenge.

**EFFECT OF B.C.G. INFECTION ON TRANPLANTED HOMOLOGOUS AND ISOLOGOUS TUMOR GROWTH, SPONTANEOUS TUMORS, M ETHYLCHOLANTHRENE CARCINOGENESIS, AND LEUKEMOGENESIS**

This section will deal with experiments in which the effect on tumor growth of heightened nonspecific resistance, as measured by bacterial or viral challenge, was investigated. As a means to accomplish this effect, B.C.G. infection was again chosen, since this agent causes marked and sustained modification in a variety of factors involved in host resistance. Mice infected with B.C.G. have been found more resistant to bacterial challenge (10, 23, 41), to infection with a neurotropic virus (58, 61), and to ionizing radiation; these mice also exhibit an enhanced immune response to various antigens (12, 35) and possess a hyperactive RES (7, 41, 59) with macrophages showing an increased acid phosphatase activity (75, 76) and an improved capacity to kill phagocytized, virulent Salmonella strains (46). Our first studies were carried out with various long-transplanted tumors, including S-180 and Ehrlich ascites in Swiss Ha/ICR mice and Ca-755 in C57BL/C1. Favorable effects were observed by us with these tumor systems (56, 59) and similarly by Biozzi et al. (11) and Halpern et al. (36) with Ehrlich ascites in mice and Guérin carcinoma in rats infected with B.C.G. As an illustration of this protection, the effect of initiating infection with various doses of B.C.G. on the rate of regression and mortality due to S-180 growth in adult female Swiss Ha/ICR mice is presented in Table 8. The greatest degree of protection is obtained with 1 mg. wet weight B.C.G. per mouse and rapidly falls off with lower doses.

In the present studies an attempt has been made to extend these observations to both the development and growth of carcigen-induced and spontaneous primary tumors and early isologous transplants of such tumors. On the whole, our results have been in agreement with the findings of others.
who have attempted to induce immunity to these tumors by various means; B.C.G. infection has had little or no effect on the progression of spontaneous mammary tumors or on isologous transplants of such tumors, with one exception, whereas the growth of early transplants of several carcinogen-induced tumors has been inhibited by previous B.C.G. infection.

B.C.G. infection induced at various times following the development of spontaneous mammary adenocarcinomas in a large number of Swiss Webster and C3H/An mice has not altered the growth of these tumors nor prolonged the survival time of the animals. Growth of isologous transplants of such tumors in B.C.G.-infected mice was indistinguishable from that of controls (Chart 7). An exception to these results has been the finding that first-generation transplants of certain (I X C3H/An)F1 spontaneous mammary adenocarcinomas were inhibited when grown in mice infected 7 days previously with B.C.G. (Chart 8).

In the main, however, the negative results observed with most spontaneous mammary tumors are not surprising, since the enhancement of immune mechanisms could not be expected to affect a system where no well defined tumor antigenicity has been observed to date. We therefore initiated studies on carcinogen-induced tumors which have been reported to possess antigens capable of immunizing the isologous (~6, 65, 67) and autologous host (47).

Tumors were induced in a variety of mouse strains by one subcutaneous injection of 0.05 or 0.1 mg. of either 3-methylcholanthrene (MC) or 3,4-9,10-dibenzpyrene (DBP) dissolved in sesame oil. Trocar pieces of the induced tumors were transplanted to isologous mice of the same sex as the primary tumor donor, allowed to grow for a period until reaching an average tumor diameter of approximately 1–1.5 cm., and then ligated. These animals and controls were challenged at times varying from 2 days to 1½ months following ligation with a second trocar piece of the same tumor maintained in isologous passage. The results of our experience with carcinogen-induced tumors in various mouse strains are presented in Table 9. In a total of 47 tumors definite inhibition of tumor growth or complete regression was observed in 58 per cent of cases studied. Tumors induced by 3-methylcholanthrene appeared to be more often antigenic than those induced by 3,4-9,10-dibenzpyrene. As the latent period for tumor development has been observed to be shorter and the total incidence higher in the case of primary DBP-induced tumors than in those induced by 3-methylcholanthrene, these differences may possibly be related to the variations in tumor antigenicity as noted above. Thus, less antigenic tumor cells would be expected to establish themselves with less difficulty.

The observed antigenicity of carcinogen-induced tumors is in agreement with the findings reported by Foley (~6), Prehn and Main (65), Révézé (67), Prehn (64), and the Kleins et al. (47). In addition, our data add one more carcinogen, 3,4-9,10-dibenzpyrene, to the two other carcinogens, 3-methylcholanthrene and dibenzanthracene, which have been shown to induce tumors antigenic to their isologous hosts.

The effect of B.C.G. infection on the growth of early isologous transplants of these tumors was next investigated. As might be expected from the reports on the variable antigenicity of carcinogen-induced tumors, the effect of B.C.G. infection on tumor growth was not consistent. The detailed results of these studies are compiled in Table 9; 45 per cent of tumors were significantly inhibited; the growth of 10 per cent of the tumors appeared enhanced, whereas 45 per cent of tumors did not seem to be affected when grown in B.C.G.-infected isologous hosts. As an example of the latter situation, the growth of a transplant of a DBP-induced tumor in male BALB/c mice, as shown in Chart 7, was not influenced by previous B.C.G. infection. It was found that previous growth and removal of this tumor in normal mice did not appear to immunize the isologous hosts. As an example of a favorable response, Chart 9 illustrates the be-
behavior of a first-generation transplant of a female BALB/c DBP-induced tumor grown in isologous female mice. The development of this tumor in B.C.G.-infected mice was clearly inhibited, and four of ten of the tumors completely regressed. All ten control tumors progressed to large size and had caused death by the 112th day in 60 per cent of the animals. Similar effects of B.C.G. infection are seen in Table 10 and Chart 10. These two tumors, DBP (I × C3H)F1 female and Meth. A BALB/c female, were found capable of immunizing the isologous host. Although in our experience there has been a correspondence between tumor "antigenicity" and inhibition of growth in B.C.G.-in-  

**Chart 7.**—Rate of transplanted isologous tumor growth in normal and B.C.G.-infected mice. B.C.G. infection initiated 2-3 weeks prior to implantation. Figures on graph refer to number of survivors.
and Prehn (64) with carcinogen-induced fibrosarcomas have demonstrated that tissues other than the fibrosarcoma from the same animal failed to immunize against subsequent tumor challenge. Such data suggest that the tumor antigenicity observed arises as a consequence of the carcinogenic process and is not the result of residual strain heterozygocity. One might, therefore, expect that such tumors are also antigenic in their primary host. Evidence of an immune response to the carcinogen-induced tumor by the autochthonous host has been recently presented by Klein et al. (47).

In view of these findings it would seem pertinent to investigate whether the incidence or growth of carcinogen-induced tumors in the primary host could be affected by B.C.G. infection. Experiments to demonstrate such an effect, however, are difficult to design, since the problem arises as to the optimal time to initiate B.C.G. infection with relationship to the carcinogenic stimulus. Moreover, considering the range of antigenicity observed in the tumors produced by a given carcinogen, one might expect that enhancement of the immune response of the host might only have the effect of suppressing the more antigenic cells with neoplastic potential. Eventually growth of less antigenic or nonantigenic tumor cells could conceivably occur. A net result of this selection process would be a delay in tumor appearance after B.C.G. infection without affecting the ultimate tumor incidence.

Experiments to explore these possibilities were carried out in adult Swiss Ha/ICR female mice. 3-Methylcholanthrene dissolved in sesame oil was injected into the right thigh muscle of twenty to 25 Swiss Ha/ICR mice for each dose of carcinogen studied. A dose response study to determine the critical dose range for tumorigenesis was made and is presented in Chart 11. Two types of experiments with B.C.G. were performed, one in which ani-

### TABLE 9

**Comparative Effect of Specific Immunization and Prior Infection with B.C.G. on Isologous Transplants of Carcinogen-Induced Tumors**

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Sex</th>
<th>Strain</th>
<th>Ligation/challenge</th>
<th>B.C.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. tumors</td>
<td>&gt;60</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>♀</td>
<td>C57/Bl</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>C3H/An</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>BALB/c</td>
<td>4</td>
<td>2**</td>
</tr>
</tbody>
</table>
|                   |♀   | (IX C3H)F1             | 1         | 1* | 0 | 0 | 1 | 0 | 1* | 0 | 0 | 1 | 0 | 1*
|                   |♀   | (C3H X BALB/c)F1      | 3         | 3*** | 1 | 1 | 0 | 3 | 0 | 2* | 1 | 0 | 1 |
| 3,4,9,10-Dibenzpyrene |♂   | I                      | 1         | 0 | 1 | 0 | 0 |          | 0   | 0 | 1 | 0 | 1 |
|                   |♂   | BALB/c                  | 9         | 1* | 1 | 7 | 0 | 5 | 1* | 2 | 2 | 0 | 1 |
|                   |♂   | (IX C3H)F1             | 2         | 1* | 0 | 0 | 1 | 2 | 1* | 0 | 1 | 0 | 1 |
|                   |♂   | (C3H X BALB/c)F1      | 3         | 0 | 2** | 1 | 0 | 4 | 0 | 2 | 1 | 1 |
| Total:            |     |                         | 47        | 12 | 13 | 20 | 9 | 2 | 29 | 2 | 11 | 13 | 3 |
|                   |     |                         | (35%)    | (28%) | (43%) | (9%) | (4%) | (7%) | (38%) | (45%) | (10%) |
| Summary           |♀   | Methylcholanthrene      | 16        | 9 (50%) | 3 (10%) | 4 (25%) | 0 | 7 | 0 (0%) | 4 (28%) | 2 (14%) |
|                   |♂   | Diphenyrene             | 15        | 2 (13%) | 4 (27%) | 8 (38%) | 1 (7%) | 11 | 2 (18%) | 3 (30%) | 4 (30%) |
|                   |♀   | Diphenyrene             | 16        | 1 (6%) | 6 (38%) | 8 (50%) | 1 (6%) | 11 | 0 (0%) | 3 (31%) | 7 (54%) |

* **, and *** refer to number of groups where complete tumor regression was observed in 30 per cent or more of challenged animals.
mals were infected 2 weeks prior to carcinogen injection (Chart 11), and experiments in which B.C.G. infection was initiated at a time corresponding to the emergence of the first tumors (Chart 12). B.C.G. infection prior to carcinogen injection did not consistently alter the rate of tumor appearance or the final incidence. When infection was initiated 86 days following carcinogen injection, a definite delay in the appearance of tumors was observed, although the final cumulative incidence was eventually similar to that seen in noninfected animals. The B.C.G.-infected animals did show a slight transient weight loss (Chart 12), which does not seem sufficient to account for the delay in tumor incidence. Similar delays in appearance of leukemia in B.C.G.-infected AKR mice (Chart 13) and in the development of spontaneous mammary adenocarcinomas in B.C.G.-infected (I X C3H)F1 mice (Chart 14) have also been observed.

The exact mechanism by which B.C.G. infection favorably affects tumor development has not been determined. While it is conceivable that many of the effects of B.C.G. which lead to enhanced host resistance to bacterial or viral challenge may be involved in the alteration of tumor growth, it appears most probable that this effect is the result of a more vigorous or accelerated capacity to reject tissues containing antigens not present in the host. Observations by Dr. H. Balner (personal communication) on the rejection of isologous male skin by female C57BL would lend support to this interpretation. Female mice previously given injections intracutaneously or intra-

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**TABLE 10**

**GROWTH OF A THIRD-GENERATION ISOLOGOUS TRANSPLANT OF A 3,4-9,10-DIBENZ-PYRENE-INDUCED FIBROSARCOMA IN NORMAL AND B.C.G.-INFECTED FEMALE (I X C3H)F1 MICE**

B.C.G. infection initiated 8 days prior to tumor challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Days following tumor challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Controls:</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.25</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>8.75</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>6.25</td>
</tr>
</tbody>
</table>

| B.C.G., 1 mg: | | | | | | |
| 1 | 6.75 | 8.75 | 19 | 29 | 36.75 | 173 |
| 2 | 5.75 | 5.75 | Regressed | Regressed | | |
| 3 | 6.25 | 10 | 8.75 | 35 | 30.5 | 32.5 |
| 5 | Nodule | 11 | 9.75 | 43 | 29.5 | 35.75 |
| 6 | 5 | 9.5 | 12.25 | 28.5 | 35.75 | 42.5 |
| 7 | 6.5 | 12 | Regressed | Regressed | | |
| 8 | 6 | 7.75 | 20.5 | 35.75 | 30.75 | 181 |
| 10 | Nodule | Nodule | Regressed | Regressed | | |

Results expressed as average tumor diameter in mm.
† refers to day of death following tumor implantation.
Table:

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Days Following Tumor Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
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<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
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</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

**Chart 9.**—Growth of a 1st-generation isologous transplant of a 3,4,9,10-dibenzpyrene-induced fibrosarcoma in normal and B.C.G.-infected female BALB/c mice. B.C.G. infection initiated 7 days prior to tumor challenge.

**Chart 10.**—Growth of a 3rd-generation isologous transplant of a 3-methylcholanthrene-induced fibrosarcoma in normal and B.C.G.-infected female BALB/c mice. B.C.G. infection initiated 7 days prior to tumor challenge.
venously of live B.C.G. demonstrated a more rapid rejection of the male isograft. The modest effect of B.C.G. infection on tumor development, together with the more definite inhibitory effect of this infectious agent on the growth of transplants of carcinogen-induced tumors in isologous hosts, further strengthens the evidence that antigens exist in certain tumors and are capable of eliciting an immune response. Through analysis of our data correlating incidence of 3-methylcholanthrene-induced tumors in various strains with the frequency with which such tumors can be established in isologous passage (Table 11), a possible correlation between these two characteristics became apparent. If the failure to establish successful passage of a tumor in isologous hosts can be interpreted as an indication of tumor antigenicity, such data might suggest that the tumor cells produced

### TABLE 11

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. mice inj. S.C. with 5-methylcholanthrene</th>
<th>No. developing tumors at 8 months</th>
<th>No. established passage/no. attempted passage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/An</td>
<td>10</td>
<td>9</td>
<td>5/5</td>
</tr>
<tr>
<td>BALB/c</td>
<td>10</td>
<td>10</td>
<td>4/4</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>3</td>
<td>6/8</td>
</tr>
<tr>
<td>(C3H×BALB/c)F1</td>
<td>10</td>
<td>10</td>
<td>1/7</td>
</tr>
<tr>
<td>(C3H×BALB/c)F1</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Defined as growth for more than two transplant generations.

![Chart 11](chart11.png)

**Chart 11.**—Per cent tumor incidence at 9 months in adult female Swiss Hsd/ICR mice injected with various doses of 3-methylcholanthrene in thigh musculature. Effect of B.C.G. infection (1 mg. wet weight) initiated 14 days prior to carcinogen injection on total tumor incidence.

![Chart 12](chart12.png)

**Chart 12.**—Composite rate of carcinogen-induced tumor development in control and B.C.G.-infected Swiss Hsd/ICR adult female mice. B.C.G. infection (1 mg. wet weight) initiated on the 86th day following 3-methylcholanthrene (0.05, 1.0, 1.5 mg.) injection.
by the action of carcinogens might be more antigenic in certain strains and thus account for the lower tumor incidence in these strains.

The mechanism by which specific antigens are induced during the course of chemical carcinogenesis remains the most important question to be resolved in this particular area of tumor immunology. Although no evidence exists to indicate the processes by which such new antigens might originate, it may be significant that carcinogen-induced tumors, in contrast to viral-induced tumors and tumors of unknown etiology, present chromosomal abnormalities (4, 38, 78). In addition, the fact that each tumor consists of cells with a variety of chromosome numbers raises the question whether all cells of a given tumor possess the same antigen or antigens, or whether patterns of antigens differ from cell to cell in the same tumor. These questions deserve careful investigation. At the present time, the study of carcinogen-induced tumors appears to us to offer one of the most promising experimental approaches in cancer immunology.

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