same serum to parallel cultures infected with Ilheus virus did not prevent continued propagation of the Ilheus virus. It has recently been reported that infectious canine hepatitis virus grows well in tissue cultures of kidneys from ICH-immune dogs(14).

*Summary.* 1. Bunyamwera virus was grown in roller-slide tissue cultures of human lymph nodes (fibroblastic type cells). Propagation of virus was demonstrated by increases in virus titers in short-term virus growth studies, by long persistence of virus in tissue cultures as contrasted with rapid disappearance in cell-free preparations, and by persistence of virus after serial passages had diluted the original virus inoculum far beyond its minimal infective dilution. 2. Similar but less extensive studies demonstrated the propagation in human fibroblast tissue cultures of West Nile, Egypt 101, Ilheus, and Br I viruses. 3. Bunyamwera virus showed changes interpreted as an adaptation phenomenon during serial passages in tissue cultures. These changes were reversed by serial intracerebral passage in mice. 4. None of these viruses caused appreciable cytopathology under these experimental conditions, even after prolonged growth in tissue culture. 5. Bunyamwera, Egypt 101, and Ilheus viruses were also grown in tissue cultures from various human cancers. It was not possible to determine whether virus was propagating in the cancer cells, or in non-neoplastic stromal cells. 6. Egypt 101 virus propagated in cells from an immune patient, but not in the presence of immune serum.

The authors are greatly indebted to Miss Priscilla J. Goettler, who performed much of the tissue culture technic involved in this study; and to Drs. Hilary Koprowski, Joseph Melnick, Alice Moore, and Kenneth Smithburn who supplied stock viruses.

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Received February 25, 1954. P.S.E.B.M., 1954, v86.

## Specificity of Antineoplastic Phenomenon Induced in Mice by Carcinogenic Agents. (21045)

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Following the injection of methylcholanthrene into mice an antineoplastic (AN) factor appears in their spleens(1). This response appeared to be specific in that the AN factor was not engendered in homozygous mice through inoculations with phenanthrene and with cooking oil (the diluent), and it was not present in spleens from uninoculated mice. The AN phenomenon endured through a period that appeared to coincide with the latent period of carcinogenesis. This supposition was further supported by the absence

of the AN property in the spleens from tumorous mice.

This study has now been extended to observations on the chemical and genetic specificity of the AN phenomenon, using the tissue culture technic described in previous reports(1,2).

*Methods.* Exp. I. Induction of tumors. Groups of 28-30 young C3H mice* were each injected subcutaneously with 0.5 mg of a carcinogenic compound suspended in cooking oil. The compounds included methylcholanthrene (MCA)†; 1,2,5,6-dibenzanthracene (DBA)†; and 3,4-benzypyrene (BP).† These mice were observed until tumors appeared in the injected area. Two biweekly injections with BP and DBA were required to induce tumor formation. Exp. II. Studies on AN reactions with spleens. When tumors appeared in the mice of Exp. I, additional groups of 12-20 C3H mice and of CFW‡ mice were then injected with the same agents as noted above (0.5 mg in cooking oil subcutaneously). At 1 and 2 weeks following the latter injection, 4-5 mice from each group were sacrificed and their spleens were processed as follows: Each spleen was minced with scissors so as to reduce it to pieces approximately 1 mm in diameter. The minced tissue was washed 3 times in Hank's solution and suspended in 4 x the volume with Hank's solution and embryo extract (3 + 1). Each C3H mouse carrying a well established tumor was sacrificed by ether anesthesia. The tumor and spleen tissue from each animal was then individually processed in a manner similar to that described for the spleen. The processed tissues were explanted in coagulating chicken plasma on to the wall of a test tube. Tumor tissue was explanted on one side of the tube and spleen tissue on the opposite wall. One ml of nutrient fluid§ was added to each tube which was then tightly stoppered. The tissues were incubated at 35°C at a 5° angle, and rotated 6 times per hour. The tissues were studied in the following combinations: 1. Tumor plus "homologous" spleen from C3H mice. 2. Tumor and spleens from C3H mice, the latter inoculated 1 and 2 weeks previously with 1 of the carcinogenic compounds. 3. Tumor from C3H mice and spleens from CFW mice, the latter inoculated 1 and 2 weeks previously with a carcinogenic compound. The explants were observed microscopically at daily intervals and changes were recorded as to whether the explants were growing or not. No tissue combination was evaluated unless the spleen tissue showed evidence of viability. Control tubes contained tumor and spleen explants alone. The nutrient fluid was adjusted to optimal pH (7.2) with $NaHCO_3$ when needed.

*Results.* Tumors appeared in the mice at approximately 5 weeks following MCA inoculation and 12 weeks following inoculations with DBA and BP. The tumors were solid fibrosarcomas, subcutaneously localized and growing without evidence of metastasis.

The spleens from tumorous mice were frequently enlarged and engorged with blood, while those from more recently injected mice (Exp. II) were small, solid, and contained prominent Malpighian corpuscles.

Explants of tumor tissue proliferated as fibrocytes extending into the periphery, and forming a "halo" of cells around the original explant within 24-48 hours. Occasionally, the plasma clot was digested in one area thereby giving the appearance of a "signet ring" growth. Explants of spleen tissue expanded by quick migration of loose cells. Within 24-48 hours, lymphoid cells and fibrocytes surrounded the periphery of the explants. The type of cell around each explant was related to the presence of a Malpighian corpuscle in the explant, lymphoid cells predominating when such was present.

The fate of MCA-induced tumor explants with homologous spleen and with spleens from mice recently inoculated with MCA has been described(1). The tumors grew profusely with their homologous spleens; whereas, with the latter spleens noted above, the explants failed to grow. Recent inoculations of C3H mice with DBA and with BP engendered no such AN effect in their spleens against MCA-

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† Eastman Kodak Co.

‡ Carworth Farms, New City, N. Y.

§ Hank's solution; ox serum ultrafiltrate, embryo extract, penicillin, and streptomycin.

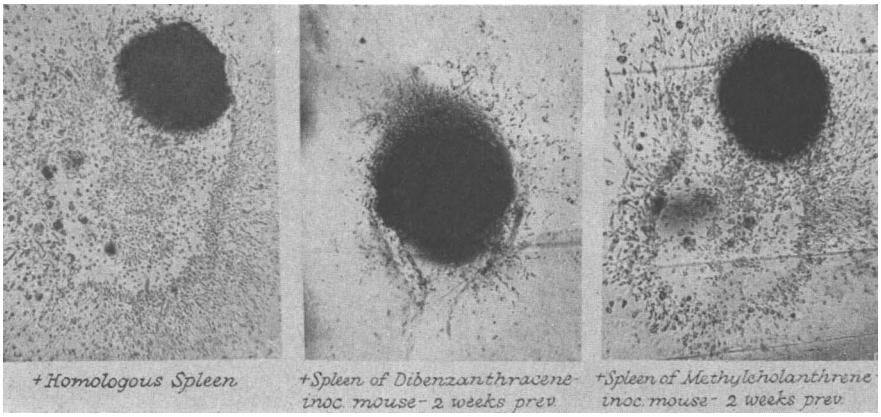


FIG. 1. Tissue cultures of 1,2,5,6-dibenzanthracene-induced mouse tumor—5 days. ×32.

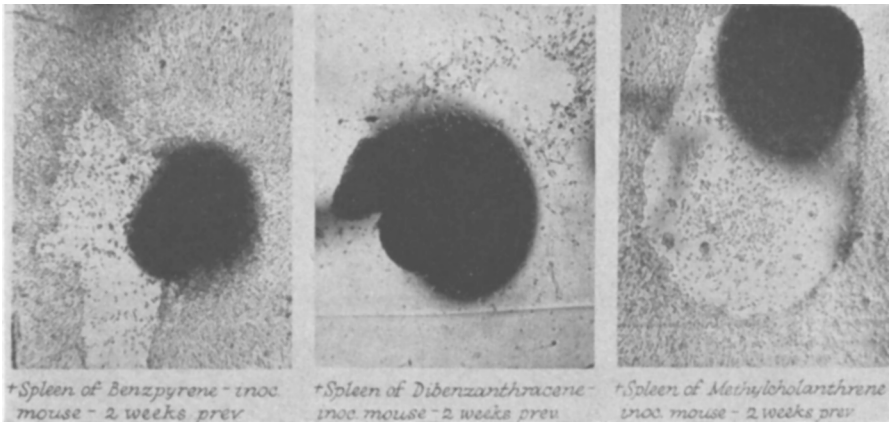


FIG. 2. Tissue cultures of 1,2,5,6-dibenzanthracene-induced mouse tumor—5 days. ×32.

induced tumors. Furthermore, CFW mice similarly inoculated with MCA failed to engender the AN factor in their spleens when cultured with MCA tumors from C3H mice (Table I).

DBA-induced tumor explants from C3H mice were attenuated and destroyed by spleens of C3H mice recently injected with DBA in contrast to the profuse tumor growth when explanted in combination with the homologous spleen (Fig. 1 and 2). The AN factor for DBA tumors in C3H mice was also absent from the spleens of C3H mice recently injected with MCA and with BP, as well as in CFW mice inoculated with all three of the carcinogenic agents (Table I).

BP-induced tumor explants from C3H mice were destroyed when explanted with spleen tissue from C3H mice recently inoculated with BP, whereas tumor growth was profuse with homologous spleen explants (Fig. 3). The AN phenomenon for BP-induced tumors was not demonstrable with spleen tissue from C3H and from CFW mice recently inoculated with DBA and with MCA.

When the growth of spleen tissue was incompatible with tumor growth, the cytopathogenic sequence was the same in all tumors. Usually the cells failed to grow out from the explant or if some growth was observed on the first day, the cells were fragmented and destroyed by the second day,

*Discussion.* We have here presented evidence indicating that the AN phenomenon is chemically and genetically specific. The findings support the previously reported observations that the response of mice to a recent injection with carcinogenic agent was a trans-

TABLE I. Effect of Spleen on Growth of Tumor Explants from $C_3H$ Mice.

| Tumor induced by: | Growth of tumor with homologous spleen | Effect of spleens from C3H and CFW mice inoculated 7-14 days previously with: MCA C3H | MCA CFW | BP C3H | BP CFW | DBA C3H | DBA CFW |
|---|---|---|---|---|---|---|---|
| MCA* | 13/13† | 1/25‡ | 9/9 | 4/4 | 0 | 2/4 | 0 |
| BP | 11/11 | 8/8 | 0 | 5/20 | 6/6 | 11/12 | 0 |
| DBA | 9/9 | 5/6 | 0 | 5/5 | 0 | 0/6 | 2/2 |

* MCA, 20-Methylcholanthrene; BP, 3,4-Benzpyrene; DBA, 1,2,5,6-Dibenzanthracene.
† Number growing/tumors tested.
‡ Growth of tumor/spleens examined; 0, not tested.

ient AN effect(1). The specific AN phenomenon was demonstrable in a high percentage of mice inoculated with MCA, DBA and BP one and two weeks previously. This reaction has not yet been observed with spleen-tumor tissue combinations from the same mouse, regardless of the cause of the tumor (Table I). The initial AN response described above disappears with the appearance of the tumor. The intervening time, representing the latent period, might conceivably be altered by varying the quantity of inoculum, its route of inoculation, the age, and the genetic strain of mouse employed(3).

The spleen has been described by others as one of the most prolific of the antibody producing organs in the body(4,5). Antibody production may be the basis for the AN phenomenon observed; but the relationship between a carcinogenic agent and antibody response still needs clarification. The antibody response to a conventional antigenic stimulus can be detected 5 days after inoculation(6,7). Although we have not yet defined the exact mechanism responsible for the phenomenon, it becomes detectable 5 days after inoculation of the carcinogenic compounds.

When mice are inoculated with homotransplants of well established, transmissible tumors, the latent period is thereafter very short. Although this has not been studied extensively, some experiments with the technics described above indicate that the AN response following tumor transplantation disappeared 8 days after the tumor was inoculated. The mechanism whereby a host is thus rendered susceptible to a homologous tumor may represent an accelerated development of what eventually evolves through the action of a carcinogenic agent.

The chemical and genetic specificity of the AN phenomenon conforms with the reports of others(8,9) regarding the susceptibility of a specific species and strain for the optimal transmission of a tumor: the genetic strain

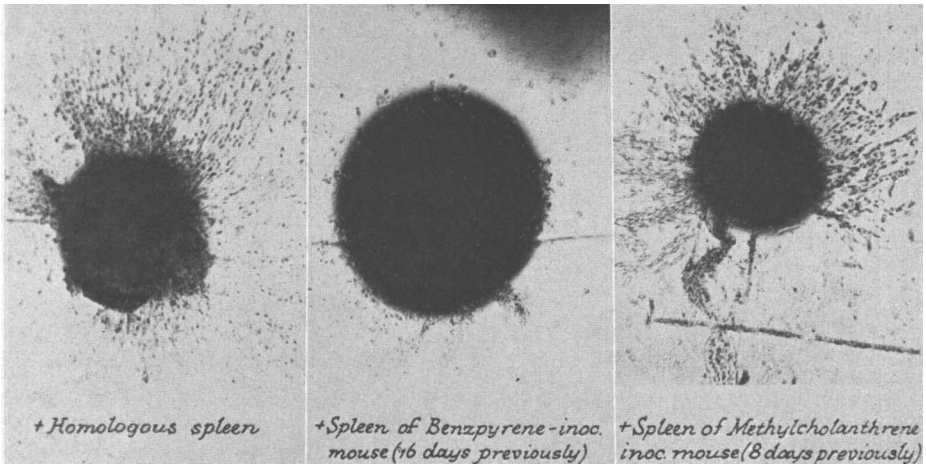


Fig. 3. Tissue cultures of benzpyrene-induced mouse tumor—2 days. ×52.

from which a tumor emerged providing the most fertile soil for its subsequent propagation.

*Summary.* When mice were inoculated with 3 carcinogenic compounds, they developed a transient antineoplastic (AN) response which was demonstrable by tissue culture technic. This AN phenomenon appeared to be chemically and genetically specific. The duration of the AN response to the 3 agents may represent the latent period of carcinogenesis, since the AN phenomenon disappeared when the tumor emerged. The interpretation of the AN phenomenon on an immunological basis is discussed.

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Received March 22, 1954. P.S.E.B.M., 1954, v86.

## Topical Protection against Inflammation by a Preceding Local Inflammation in Adrenalectomized Rats.* (21046)

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Acquisition of topical protection against inflammatory reaction to blood-borne irritants such as egg-white or dextran has been shown in the rat-paw after local pre-irritation with various irritants(1). Further, it has been demonstrated that thermally pre-irritated tissues display topical protection against a subsequent strong thermal irritation leading to inflammation and necrosis in normal tissues (2). One of the authors reviewed earlier data on acquired topical protection against tissue injury and studied mechanisms underlying the acquisition of topical protection against blood-borne irritants and against thermal and X-irradiation injury(3). Recently, an interesting contribution concerning this subject was made by Arteta(4). He observed that adrenalectomy prior to local pre-irritation (peptone injection in the rat-paw) prevented the development of topical protection; thus, the pre-irritated paw displayed apparently undiminished inflammatory reaction to egg-white intraperitoneally injected 24 hours after pre-irritation. However, topical protection could be demonstrated in adrenalectomized rats, if cortisone was intraperitoneally injected 1 hour after the local pre-irritation, the egg-white being given 24 hours later. On the basis of this finding, Arteta advanced the theory that a topical pre-irritation evokes a General Alarm Reaction, and that "the steroids of suprarenal source which are produced during this reaction may move from the blood to the inflamed tissue due to the increase of capillary permeability which takes place in the inflamed area, accumulating there and modifying the tissue's conditions and the degree of resistance of the capillary wall." The preferential accumulation of antiphlogistic steroids in inflamed areas seems to be an intriguing suggestion in connection with the mode of action of these steroids. Some hesitation was felt, however, in accepting this view as an explanation of the mechanism underlying acquired topical protection. Therefore, further investigations were made on the problem of acquired topical protection to intraperitoneally injected egg-white and to thermal

* Supported in part by a grant from the Ministry of Health of the Province of Quebec.

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