different, and each experimental manipulation helps to throw light on the underlying biological processes.

Summary. Repeated injections of rabbit antiserum against the lymphoid cells of mouse thymus (ALS) were found to increase markedly the evidence of tumors in resistant strains of mice neonatally infected with polyoma virus. In this system the effects of ALS are similar to those of early thymectomy. The BALB/c mice receiving repeated injections of ALS and infected intraperitoneally with Moloney leukemogenic virus (MLV) when 21 days old showed an increased incidence of lymphoid neoplasms as compared to that in untreated controls infected at the same age. The majority of tumors in the treated animals were reticulum cell sarcomas, type A, developing subcutaneously at the site of injection of the ALS 3–6 weeks after infection with MLV. Early thymectomy markedly decreased the incidence of leukemias in BALB/c mice infected with MLV when 3 weeks old. In this system the effects of ALS are the opposite of those produced by thymectomy. It is concluded that the thymic elements required for neoplastic conversion by MLV are not eliminated by ALS, which may alter the pathology of the induced tumors by a combination of immunosuppression and capacity to stimulate the proliferation of primitive lymphoid cells.

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Effect of Plasma Cell Tumor on Antibody Production by Mouse Spleen Cells (32658)

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Circulating antibody was shown to be quantitatively reduced in C_3H mice bearing the transmissible plasma cell tumor, X5563, following a primary inoculation with sheep erythrocytes (1). This effect might be due in part to a reduction in the numbers of lymphoid cells synthesizing antibody; to a competitive mechanism depleting the protein precursors of the antibody; to an abnormal utilization of the globulin molecules after their synthesis; or to some combination of these conditions. The present experiments were car-

ried out to see whether numbers of mouse spleen cells participating in the primary synthesis of hemolysin were different in the tumorous mice than in their controls.

A technique for the enumeration of lymphoid cells actively producing hemolytic antibody has been described (2) and a modification of the procedure has been used to demonstrate that the plaques formed during the primary immune response are chiefly due to the elaboration of IgM globulin by the lymphoid cells (3). Several authors (4–6) have confirmed that the plaque method scores only IgM globulin production. A rise in circulating hemolysin titers to peak values was found to parallel the increase in plaque counts in mouse spleen cells and these counts fell rapidly with time after peak circulating titer had been reached (7). Friedman (8) observed that maximum circulating antibody titer and numbers of plaque forming cells occurred on the fifth day after primary immunization in C_3H mice.

Materials and Methods. Seven to 10-week-old C_3H/eb mice[1] of both sexes used in these experiments were housed three or four per cage and were given a constant supply of food and water. The X5563 tumor[2] was aseptically transferred subcutaneously to the right flank of randomly selected animals with a total of 10 transfers being recorded for the purposes of these experiments. Fresh tumor tissue minced in physiological saline was delivered to the host animals in 0.1 ml quantities through a 20 gauge needle. Transplanted tumors were palpable 2–6 days after transplant, and death of the mice with no additional treatment occurred 18–20 days later. Although the rate of growth of the tumors varied among the experimental mice, antigen was not given until the tumor reached 15 mm in largest diameter as measured externally with calipers.

Tumor bearing animals and their nontumorous controls were immunized by an intraperitoneal injection of 0.5 ml of a suspension of washed sheep erythrocytes (SRBC) containing a total of 9×10^8 cells. Five days after immunization the mice were sacrificed with ether, and blood and spleens were removed for subsequent study. Fresh cells from the spleens were plated and plaque counts (PFC) per 10^6 viable cells were made according to the method described elsewhere (2,9). Hemagglutinin titers were determined to provide an indication of the antibody response of the mice. Serial twofold dilutions were made and the greatest dilution yielding unequivocal agglutination of SRBC was recorded as the titer of the serums. After 24 hours' refrigeration of the sera, reaction volumes of 0.5 ml were read. Antibody titers were determined within 2–3 weeks following serum collection. In a few instances PFC counts were determined after a second immunizing injection of SRBC given on the fifth day following the primary immunization.

Results. Plaque forming cell counts made from cells of the spleens of 8 tumorous mice averaged 25.7 ± 15.3 (SD) plaques per 10^6 viable cells compared to an average PFC count of 218 ± 112.5 (SD) for 13 nontumorous controls as shown in the results summarized in Table I, group I. The lower PFC counts in the tumor mice were associated with reduced capacity to form hemagglutinating antibody as shown in the Table, since their serum titers averaged only 11 compared to 138 for the control animals of group I, Table I.

A few of the mice sacrificed 4 days after a second injection of SRBC which had been given on the fifth day after the primary immunization had PFC counts that averaged only 6 for the tumorous and 9 for the control mice as indicated for the mice in group II of Table I. These low PFC counts are in agreement with the fact that plaque formation is attributable mainly to IgM antibody. In independent tests with both tumorous and nontumorous mice, few to no plaques could be demonstrated among spleen cells of animals not immunized with SRBC. The higher average circulating hemagglutinin titers of 134 and 640 for the tumorous and control mice re-

[1] Mice were kindly supplied by Dr. Edward J. Breyere of the Department of Biology, The American University, Washington, D. C.

[2] Initial inoculation of the tumor was in its one hundred twenty-second transfer generation and was kindly supplied by Dr. Michael Potter, Laboratory of Biology, National Cancer Institute, Bethesda, Md.

TABLE I. Plaque Counts per 10^6 Viable Spleen Cells in C_3H/eb Mice Bearing X5563 Tumors and Their Nontumorous Controls Compared with Circulating Hemagglutinin Titers.

Group[a]	Treatment	No. of mice	Average			
			Body wt (gm)	Tum. wt./Body wt. × 100	PFC 10^6 cells	Hemagg. titer
I	Tumor	8	29.2	18	25.7 ± 15.3 (SD)	11
	No tumor	13	18.3	—	218 ± 112.5 (SD)	138
II	Tumor	5	32.2	31	6	134
	No tumor	3	17.3	—	9	640

[a] Group I mice were singly immunized intraperitoneally with 9×10^8 SRBC 5 days before sacrifice; Group II mice received a second equal immunization 5 days after the first and were sacrificed 4 days later.

spectively (group II, Table I) are in keeping with the anamnestic response.

Results summarized in Table I show that body weight of the tumorous mice averaged about 30% higher than their controls, and that at sacrifice the tumors represented about 18% of the body weight. Spleens taken from the tumor mice averaged over twice the weight of those taken from the control animals. Increased body weight in the tumor bearing mice was due partly to increased blood volume and partly to the solid tumor mass. Entrapped blood would account to some extent for the observed increase in spleen weight. In spite of the more than twofold increase in spleen size the numbers of PFC in tumor bearing mice were only about 15% of the number recorded for nontumorous controls.

Discussion. Results of experiments just reported show that the presence of a plasma cell tumor in C_3H/eb mice singly immunized with sheep erythrocytes has a markedly depressing effect on the number of plaques formed when the spleen cells are permitted to react with the antigen on agar. The great reduction in PFC count observed during the anamnestic response both in tumorous mice and controls is attributable to the fact that plaques due to IgG antibody, characteristic of the secondary response, require special procedures for their enumeration (3). Serum hemagglutinin titers after secondary immunization averaged lower in the tumorous mice than in the controls yet were appreciably higher than the titers recorded for the group I mice of Table I and are in agreement with results on hemolysin formation already reported for C_3H mice bearing the X5563 tumor (1). Hemagglutinating antibody titers have been observed to reach peak values 10 days after primary immunization with SRBC compared to 5 days for peak hemolysin titer in mice (3).

Abnormal gamma globulin produced by the neoplastic cells of this tumor is electrophoretically similar to normal globulin (10, 11). The results reported in Table I suggest that competition may occur in the production of IgM globulin by plasma cells in such a way that normal gamma globulin production is reduced in the tumorous mice. On the basis of this possibility, IgM precursors removed by the neoplastic cells may be competitively consumed to the partial exclusion of the demands of the normal globulin producing clones. One possible consequence of this process might be that fewer normal cells would be stimulated to produce IgM antibody and thus result in the low titer and PFC counts observed in the tumorous mice of group I, Table I. Observations made with the group II mice of Table I indicate that production of the IgG globulin proceeds reasonably well as evidenced by the higher circulating antibody titers possibly because of different precursors' requirements for the IgG globulin and because a larger population of normal cells may be synthesizing globulin in the anamnestic phase.

A second possibility for the observed suppression of IgM antibody in the presence of the tumor may relate to the production of the abnormal gamma globulin by some type of feedback mechanism. Myelomas occurring in inbred strains of mice produce a range of gamma ss and gamma 1A globulins (11). The gamma ss and gamma 1A are both IgG globulins. Suppression of IgM globulin production through passive transfer of IgG globulin has been reported (5) as an indication of a probable feedback control on IgM production. If such a mechanism were operating in the experiments reported in the present study it could be concluded that the abnormal IgG globulin produced by the neoplastic plasma cells caused an inhibition of IgM production. The inhibition by the cells of the tumor would correspond to the feedback control of the IgG globulin on normal IgM antibody and in this way help to account for the lower circulating antibody titers in the tumorous mice of group I, Table I.

Summary. The plasma cell tumor, X5563, grown in C_3H/eb mice has been shown to reduce significantly the numbers of hemolytic plaques demonstrable among spleen cells of tumorous animals as compared to their controls 5 days after a primary intraperitoneal immunization with sheep erythrocytes. At the same time amounts of circulating hemagglutinin are also greatly reduced in the presence of this tumor during the primary response. Few plaques were found among spleen cells of mice receiving a second similar immunization whether the mice were tumorous or not although reasonably high circulating hemagglutinin titers were observed in these mice. From these results it was concluded that the X5563 tumor affects the production of IgM globulin to a more marked degree than it does IgG globulin production. Possible competitive processes are suggested to account for the observed suppressive effect of the tumor on antibody production.

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Teratogenic Activity of Six Disazo Dyes in the Wistar Albino Rat* (32659)

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The first disazo dye reported to be teratogenic was trypan blue (1). Since that time, additional structurally related dyes also have been found to possess teratogenic activity in rats (2–4). The mechanism of teratogenic action of the disazo dyes is not known. Possible mechanisms of teratogenic action have been reviewed by Beck and Lloyd (5).

This report presents observations of similarities and dissimilarities in the teratogenic action of six disazo dyes: trypan blue (TB), Evans blue (EB), Niagara blue 4B (NB4B), Niagara sky blue 6B (NSB6B), Congo red (CR), and Niagara blue 2B (NB2B).

Materials and Methods. Virgin females

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