progressively increasing doses during the first 23 days of life, the total dose was 150 to 275 μg/kg. The animals were sacrificed at the age of 39 days.

C. The mothers were not treated. The animals were treated as under schedule B, the total dose was 2.5 to 15 μg/kg in 23 days. There were eight animals in this group. They were sacrificed at 23 or 28 days of age.

Approximately an equal number of embryos and young rats from mothers that received no treatment were sacrificed for control studies. Also, litter mates of young rats receiving Piromen in both groups B and C were given injections of 0.9 percent sodium chloride as a further control measure.

Sections from representative levels of the brain and spinal cord of both the injected and control material were stained by the hematoxylin and eosin, Nissl, phosphotungstic-acid hematoxylin, Cajal gold sublimate, and Hortega silver carbonate methods. A comparison between the injected and the control animals showed no differences in the condition of either glial or neuronal tissues.

Even though large doses of Piromen were given at a time when gliogenesis was taking place, we could discern no effect from its administration in the manner described. We are unable to answer the possible questions whether Piromen crosses the placental barrier and reaches the central nervous system of the embryos, or whether an adequate concentration of Piromen is produced in the central nervous system by intraperitoneal or subcutaneous administration.

Reference

1. W. F. Windle, C. D. Clemente, D. Scott, Jr., and W. W. Chambers. *Induction of neuronal regeneration in the central nervous system of animals* (Tr. Amer. Neur. Assoc., 1952), pp. 164–170.

Received December 7, 1953.

Development of Lymphoid Tumors in Nonirradiated Thymic Grafts in Thymectomized Irradiated Mice[1]

Henry S. Kaplan and Mary B. Brown

Department of Radiology, Stanford University School of Medicine, San Francisco, California

Lymphoid tumors and lymphatic leukemias arising either spontaneously or in response to exogenous agents (x-rays, estrogens) tend to originate in the thymus in several mouse strains (*1–3*), and their incidence may be drastically reduced by thymectomy (*1–6*). That the effect of thymectomy is not due simply to removal of potentially malignant cells was demonstrated by Law and Miller (*7*), who found that lymphatic leukemia incidence in methylcholanthrene-treated dba mice could be restored to control levels by autologous or homologus thymic implants, although the implants were seldom involved by the leukemic process. This observation was recently confirmed when Kaplan, *et al.* (*8*) noted a significant increase in lymphoid tumor incidence in thymectomized, irradiated C 57 black mice bearing homologous thymic implants.

FIG. 1. Arrow points to lymphoid tumor mass replacing thymic implant. All other lymphoid tissues were microscopically normal. (×3)

An experiment was then set up to test the influence of such factors as the age of the thymic implants and the time of their implantation on tumor response. The complete experimental design is not pertinent to the present report, which is concerned only with those groups that were treated essentially as follows: C 57 black mice, segregated by sex, were thymectomized by our usual technique (*5*) at 30 to 40 days of age and were started on whole-body irradiation 2 to 7 days later. They received four doses of 168 r each at 8-day intervals; physical factors were: 120 kv, 9 ma, 0.25 mm Cu + 1.0 mm Al added filter, 30 cm mouse-target distance, 32 r/min. Thymus glands were rapidly excised from intact, nonirradiated 33-day-old C 57 black donors and were immediately implanted subcutaneously in the right axillary region of each recipient mouse. Most grafts were made within 1 hr after the last irradiation, but mice of two groups received implants 1 and 8 days, respectively, after irradiation.

Tumors were observed as early as 4 mo after thymic implantation, but most of these had disseminated by

[1] This investigation was supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, and the Jane Coffin Childs Memorial Fund for Medical Research.

the time they were detected, and it was not possible to determine their site of origin. More recently, however, a small firm nodular mass was palpated at the site of thymic implantation in three animals (two male, one female) in whom grafts had been made promptly after irradiation, about 5 mo earlier. These mice were immediately sacrificed and carefully autopsied. Each bore a nodular opalescent whitish mass in the subcutaneous tissues of the right chest wall, below the axilla, which had the gross appearance of a lymphoma. Neither the immediately adjacent right axillary lymph nodes nor the more remote superficial nodes were enlarged. On opening the thorax, there was no visible residue of the autologous (excised) thymus. The mediastinal nodes were not enlarged, and the lungs appeared normal. There was no microscopic abnormality of the abdominal viscera, except for the usual post-radiation atrophy of the ovaries and uterus of the female mouse.

The tumor masses, superficial, mediastinal, and mesenteric lymph nodes, spleen, lungs, one kidney and adrenal, and a portion of the liver were fixed in Bouin's fluid, sectioned, and stained with hematoxylin and eosin. Microscopic study revealed typical lymphosarcomas, identical with those previously described (*9*), replacing one or both lobes of the thymic implants, with invasion beyond the capsule and into the intercostal muscles in one instance. There was no evidence whatever of tumor infiltration in any of the other tissues. To date, tumors grossly confined to the implant have been observed in more than a dozen animals of which four received a thymic graft 1 day, and one as late as 8 days, after irradiation (Fig. 1).

It seems clear that the site of origin of these lymphoid tumors was in the thymic implants, which received no radiation at any time. To the best of our knowledge, this represents the first definitive instance of the induction of a malignant tumor in a tissue that has not been exposed to the carcinogenic agent responsible for the neoplastic change. The observation establishes the existence of a completely indirect mechanism of induction of lymphoid tumors in systemically irradiated mice, in which the role played by the thymus appears to be a dual one. In some strains, although not in all, the thymus is the site of origin of the malignant lymphoid cells, and in all strains thus far studied, it appears to contribute an influence necessary for the development of lymphomas and lymphatic leukemias, even when it is not involved by the tumor process (*7*).

References

1. D. P. McEndy, M. C. Boon, and J. Furth. *Cancer Research* **4**, 377 (1944).
2. W. U. Gardner, T. F. Dougherty, and W. L. Williams. *Ibid.* 73.
3. H. S. Kaplan. *J. Natl. Cancer Inst.* **8**, 191 (1948).
4. J. Furth. *J. Gerontol.* **1**, 46 (1946).
5. H. S. Kaplan. *J. Natl. Cancer Inst.* **11**, 83 (1950).
6. L. W. Law and J. H. Miller. *Ibid.* 253.
7. ———. *Ibid.* 425.
8. H. S. Kaplan, M. B. Brown, and J. Paull. *Cancer Research* **13**, 677 (1953).
9. H. S. Kaplan. *Ibid.* 7, 141 (1947).

Received December 7, 1953.

Hemagglutination by *Clostridium botulinum* Type D

M. Sterne

Wellcome Research Laboratories, Beckenham, Kent, England

Lamanna (*1*) showed that hemagglutination of red cells by supernatants of *Clostridium botulinum* type C cultures was specific in the sense that the reaction could be inhibited by antiserum to type C, but not by antisera to types A and B. This result is additional evidence of the considerable differences existing between these types, as exemplified by their cultural, biochemical, and toxigenic dissimilarities. However, the greater similarity of types C and D suggested that a common hemagglutinating factor might occur, and, accordingly, a number of experiments were carried out to test this possibility.

Table 1 summarizes the results of a typical experiment with a type-D "toxin."

TABLE 1. Typical experiment with a type-D "toxin."

Antiserum used	Results (end-point of dilution of serum showing inhibition of hemagglutination)
Cl. botulinum type D	Complete inhibition to dilution of 1/384
Cl. botulinum type C	Complete inhibition to dilution of 1/768
Cl. perfringens	Partial inhibition to dilution of 1/12
Cl. edematiens	Partial inhibition to dilution of 1/12
Cl. septicum	No inhibition
6 Normal Sera	No inhibition

The *Cl. botulinum* type-C and type-D antisera used in the tests on hemagglutination inhibition were from hyperimmunized horses and had high antitoxic titers. Although it is not unusual for high-titer type-C antisera to contain a small amount of antibody to type-D toxin, and vice versa, this particular type-C antiserum contained no detectable antibody to type-D toxin. The type-D toxin was a solution of a dry, partially purified filtrate of a *Cl. botulinum* type-D culture and was employed in the test at twice the concentration that gave complete agglutination of sheep cells in the system used.

Clearly, hemagglutination of sheep cells by the type-D filtrate was inhibited by antisera to both types D and C. It is noteworthy that type C (the heterologous) antiserum was the more potent in this respect—a finding that was constant in all the experiments with these antisera. This particular type-C antiserum contained no demonstrable antibody to the toxin of type D.

It is also of interest that high concentrations of a *Cl. perfringens* and a *Cl. edematiens* antiserum gave a partial inhibition of hemagglutination, although the