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*).

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Hemagglutination by *Clostridium botulinum* Type D

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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

donors of these sera had never, so far as was known, been exposed to botulinum antigens and possessed no demonstrable botulinum antitoxins.

The results summarized here are additional evidence of the closer relationship existing between *Cl. botulinum* types C and D than that between types A, B, and C. And they emphasize, in addition, that the lethal toxin and hemagglutinin are not the same.

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Nuclear Emulsions for Electron Microscopy[1]

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The identification of radioactive mineral grains using "Nuclear Research" emulsions and a petrographic microscope is limited to those grains within the resolving power of the microscope. Frequently there is need for the higher resolving power of the electron microscope to make accurate size measurements and study the morphology of these grains.

A technic has been devised to obtain emulsions thin enough to allow transmission of the electron beam, yet containing a sufficiently heavy and uniform concentration of silver bromide crystals to permit the recording of alpha-particle tracks. The technic makes use of Ilford C2 emulsions in gel form. A small quantity of the gel is heated to 50° C in a glass beaker, according to the directions of the manufacturer. When the gel is fluid, approximately 0.025 ml is removed from the beaker with a blood pipet and deposited on a 1 × 3 in. Formvar-coated glass slide. The finely ground sample, the amount used being determined by trial, is mixed with the gel which is then spread uniformly over the surface of the slide by means of a curved stainless steel spatula.[2] An alternative method of adding the sample is to sprinkle the mineral grains over the moist surface of the gel after the slide has been coated. This latter method reduces the possibility of forming silver tracks by pressure of the mineral grains in the silver bromide crystals as the gel is spread over the surface of the glass slide. The volume of gel placed on the slide takes into account loss due to adhesion to the spatula. It is extremely important that the entire procedure, from the removal of the measured amount of gel to the formation of the film on the slide, be carried out rapidly before the gel starts to set or fogging due to pressure of the spatula will result. This time period was found to be less than 1 min. With practice, it is a relatively simple matter to obtain emulsions of consistent thickness. After the emulsion has been prepared it is stored in a light-tight cabinet for a predetermined length of time. For highly active uraninite, an exposure of from 3 to 4 days yielded numerous alpha tracks. The exposed emulsions are developed for 2 min in Eastman Kodak D-8 developer, fixed for 15 min in a standard fixer, and then washed and dried. They are then scored with a sharp blade to divide them into ½-in. squares. The squares of emulsion are floated from the glass slide by placing the slide in a Formvar solvent, ethylene dichloride. After washing in fresh solvent, they are picked up on sections of stainless steel screen and are carefully placed in steam for 1 to 2 min to obtain better adhesion of the gelatin to the screen. At this point suitable areas for the electron microscope may be chosen by examination in an optical microscope and marked. Disks ⅛ in. in diameter are cut from the marked areas by means of a punch. The thickness of an average emulsion prepared in this manner was measured by shadow-casting sections on collodion substrates and was found to be approximately 0.2 μ.

If it is desired to attempt identification of the mineral grains in the emulsions by selected area diffraction in the RCA or Philips electron microscope, it is advisable to remove the gelatin. This can be done in an enzyme if the emulsion is fixed in a 30-percent solution of sodium thiosulphate containing no hardener. Sections of the developed emulsion are picked up on collodion substrates supported on the stainless steel screens. These are carefully immersed in a freshly-prepared 0.5 percent solution of Taka-Diastase at room temperature for 10 min. Some emulsions may require a more concentrated solution. They are then washed

FIG. 1. Electron micrograph of a Carnotite particle with an alpha track. The small opaque particles scattered throughout the field are silver grains caused by normal fogging of the emulsion and are not associated with radioactive particles. The diameter of the Carnotite particle is approximately 3 μ.

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[2] Sold under the trade name, Scoopula, by Fisher Scientific Co., Pittsburgh, Pa.