In the course of studies dealing with metabolic effects of ethylenephosphoramide chemotherapy upon host and tumor tissues (2, 3, 14, 15), certain cytopathogenic effects were noted in sections of treated Walker 256 rat tumors. The changes consisted of the appearance of large, polymorphic giant cells with bizarre nuclear and cytoplasmic configurations and unusual staining properties. Changes similar to these have been noted in human, experimental animal, and plant tissues as a result of cell injury by a variety of physical and chemical agents, including viruses and x-ray irradiation (6-8, 11, 13, 16). To our knowledge, however, such changes have not been reported, heretofore, as an effect of ethylenephosphoramide chemotherapy. Because the study of these changes may contribute toward an understanding of mechanisms of cell injury, the present preliminary report is made.

MATERIALS AND METHODS

Four separate experiments were performed on male Sprague-Dawley rats weighing between 130 and 200 gm. Animals numbered ten to eleven per group. An 18 per cent casein diet and a modified pair feeding regimen were used (2, 14). The following drugs were tested:

MEPA = N-(3-oxapentamethylene)-N',N''-diethylene phosphoramide
PDP = N,N'-diethylene-N''-phenylphosphoramidine
MPDP = N,N'-diethylene-N''-methyl-N''-phenylphosphoramidide

The plan of experiments was as follows: Minced Walker 256 tumor was transplanted from carrier animals to subcutaneous tissues in flank areas of hosts by means of a trocar. After 3 or 4 days, tumor takes were apparent, and intraperitoneal injections of aqueous drug solutions were begun. Experimental groups consisted of drug-treated tumor-bearing animals. Control groups consisted of untreated tumor-bearing animals and of untreated tumor-free animals. Schedules of drug administration varied. The lowest unit dose was 0.3 mg/kg/day (MEPA), and the highest was 10.0 mg/kg/week (PDP). Various intermediate dosages were used in combinations involving daily, alternate daily, thrice weekly, and once weekly injections. Details of individual studies have been presented in previous publications (14). Animals were sacrificed, at time intervals to be noted, by means of gas asphyxiation. Tissues were dissected out, fixed in 10 per cent formalin, and processed for hematoxylin and eosin staining.

In a few experiments not included in this report, the Flexner-Jobling tumor was studied. Cytopathological changes noted in the Walker 256 tumor have not been seen in the Flexner-Jobling tumor, although the latter is far more sensitive to this class of drugs.

RESULTS

In this paper only histologic changes seen in the Walker 256 tumor are presented. The results of histologic study of nontumor tissues from animals used in these experiments have been reported elsewhere (14, 15). Data dealing with effects of ethylenephosphoramides upon tumor and host metabolism have also been published (1, 3-5, 10).

Figures 1 and 2 are photomicrographs of tumor sections from untreated control animals.

Figure 1 shows a section of untreated tumor 4 days following inoculation in the subcutaneous tissues of the host. The tumor cells were densely packed, and their nuclei were large and hyperchromatic. The tumor tissue was surrounded by edematous connective tissue, and there was moderate infiltration of mononuclear cells in and about the tumor and its vascular supply.

In Figure 2 a tumor section is shown, also from an untreated animal 14 days after transplantation.
The tumor cells are seen to be infiltrating adjacent muscle bundles. Cells are densely packed, so that the tissue has a syncytial appearance. Details of individual tumor cells are shown in Figure 3.

Figure 3 is a higher magnification of a section of a 14-day post-transplantation untreated tumor and demonstrates its distinctive morphologic characteristics. Nuclei are ovoid or irregular and possess sharply defined, deeply stained membranes. Deeply basophilic nucleoli and granular chromatin material are evident, the latter often being marginalized. Mitotic figures are fairly frequent; three may be seen in this field. The cytoplasm stains with varying density and is "frothy" in appearance. Occasional polymorphonuclear leukocytes and mononuclear cells are present. Connective tissue appears to be scant.

The cytopathogenic action of ethylene phosphoramide treatment is seen in tumors from animals treated by each of the drugs listed in "Materials and Methods." Such tumors were examined 7, 14, and 25 days following transplantation corresponding to 4, 10, and 21 days, respectively, after initiation of drug treatment. We shall present primarily features of the cytopathological changes referred to in the introduction. These were basically the same irrespective of duration of treatment, dose, or drug used.

In Figure 4 is shown a photomicrograph of a tumor section prepared from an animal treated for 4 days with PDP. This section illustrates the characteristic features of the cytopathogenicity of the drug. One can easily note the presence of large, bizarre, polymorphic giant cells. These cells are quite numerous and usually distributed throughout the entire tumor. Some of these giant cells are enormous (100–200 μ length), and all show profound nuclear and cytoplasmic distortion. At times the cells possess single, large nuclei; at other times they are multinucleated or contain clumped nuclear masses. The nuclear material is intensely basophilic. The distinct nuclear outlines and sharp nuclear membranes of the cells of untreated tumors (see Figure 3) are rare in these giant cells. Often the nuclear material appears to diffuse indefinitely into the cytoplasm, and the latter seems tinged with basophilic material. One or more intensely basophilic nucleoli are usually present in each cell. Other nuclear changes include vesiculation or vacuolation, which is often marked. In some the vesicles are small and numerous; in others they are large and few in number. Most of these changes can be seen in Figures 5 and 6. Evidence of distorted mitotic configurations is frequent, as shown in Figure 6, in which an abnormal metaphase–telophase sequence may be seen as if the chromosome groups, upon migrating to opposite poles of the cell, had failed to separate cleanly so that a bridge of threadlike chromosomal material still connected them. The cytoplasm of giant cells is also noteworthy. Mention has been made of its basophilic tinge in certain cells. Many giant cells exhibit indistinct cell boundaries (Figures 5 and 6), and the marginal cytoplasm is drawn out in a diffuse or frayed fashion. Cytoplasmic vesicles or vacuoles are also frequent.

Phenomena similar to these were not seen in the Flexner-Jobling tumor or in tissues of tumour-free drug-treated controls.

Other well defined effects of drug treatment upon the tumors may be seen in Figure 4. Tumor cells per se were far fewer in number in the treated tumors (compare Fig. 4 with Figs. 1 and 2 at the same magnification). Also, as noted in Figure 4, an abundant connective tissue stroma was present in treated tumors, as well as infiltration of mononuclear cells in much greater abundance than in nontreated tumors. The mononuclear infiltration was seen primarily within the tumor rather than in the surrounding connective tissue. There was also considerable increase in capillaries and lymphatic spaces which honeycomb the tumor mass in treated cases.

**DISCUSSION**

Among the physical and chemical agents capable of inducing cytologic abnormalities similar to those associated with the appearance of the giant cells described above are radiation (16), high temperature (13), oxygen lack (7), hydrocortisone (8), podophyllin (9), ethylenediamine tetraacetic acid (11), and viruses (6). To these agents may now be added the ethylene phosphoramides. Since the giant cells described in this paper were seen only in drug-treated tumors and since they displayed unmistakable signs of cellular injury, it is believed...
that they were expressions of cytopathogenic effects resulting from phosphoramide treatment. Because basophilic staining of nuclear material represents the combination of hematoxylin with phosphoric acid groups of nucleic acids (12), the intense nuclear basophilia and frequent cytoplasmic basophilia suggests that in these cells nucleic acid metabolism has been altered. Derangements in nuclear and cytoplasmic sol-gel equilibria were also suggested by the changes in these cells, as well as evidences of interference with the mitotic process. Biochemical and physico-chemical studies are now in progress in an attempt to gain additional information concerning the nature of these phenomena.

SUMMARY

Cytopathogenic effects following treatment of Walker 256 tumors with ethylenephosphoramide drugs have been described. The changes consisted of the appearance of bizarre, polymorphic, giant cells with distorted nuclear and cytoplasmic configurations and unusual staining properties. Among specific cellular processes that may be affected are those concerned with nucleic acid metabolism, mitosis, and nuclear-cytoplasmic sol-gel equilibria.

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Cytopathogenic Effects of Ethylenephosphoramides upon the Walker 256 Rat Tumor

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