Lack of Significant Oncogenicity of Biological Products in Hamsters

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A variety of biological products which included live and inactivated viral vaccines, inactivated rickettsial and bacterial vaccines, toxoids, and a multiple bacterial antigen product did not appear to be oncogenic for newborn hamsters following a single subcutaneous inoculation. The incidence of spontaneous tumors was approximately 4.7%, and this figure was not significantly altered by the inoculation of any of the test materials.

The Division of Biologics Standards of the National Institutes of Health is charged with the responsibility for the safety, purity, and potency of biological products distributed interstate. In recent years, the concepts of safety and purity have broadened to include both the absence of adventitious agents and particularly the lack of oncogenic potential. Several workers (1, 6, 13) have found that certain viruses are oncogenic for the newborn hamster. Furthermore, some of the cell culture systems which have been used in the past to produce viral vaccines have been shown to contain occasional adventitious agents (6, 8, 11), some of which are oncogenic for hamsters (6, 13). Therefore, a study was designed to screen biological products to determine whether any were oncogenic in the hamster system.

MATERIALS AND METHODS

Inocula. The biological products tested as well as the control preparations used are listed in Table 1. The biological products were final, packaged materials which were obtained commercially and were representative of all the various forms of particular products. Upon receipt, each major product was assigned a code with a letter designation. All products either were obtained packaged in small quantities or were repackaged in such a manner that materials for each day's inoculation were removed from storage, used, and the remainder discarded.

Only fluid diphtheria and tetanus toxoids and pertussis vaccine, combined, was used in this study, since it was found that aluminum adjuvant compounds were toxic for newborn animals.

Study plan. The decision was made that, for each individual biological preparation to be tested, a minimum of 100 animals should survive for longterm observation. Preliminary experiments had indicated that deaths from the inoculation and other causes up to the time of weaning would result in a 25% loss; therefore, an excess of animals had to be inoculated in order to have 100 for each group. Actually, slightly higher losses were encountered which required more inoculations. All animals that survived weaning were evaluated for the study.

Preliminary studies were designed to establish the doses of various vaccines tolerated by the newborn hamsters, for little or no information was available concerning the effects of vaccines on this animal model system. Some products had to be diluted or treated in some manner prior to inoculation into newborn hamsters for the animals to survive. Thus, smallpox vaccine was diluted 1:10 with saline, and yellow fever vaccine was inactivated either by heating at 50 C for 60 min or by treatment with ultraviolet light at 500 ergs/min 2 for 90 sec. Both were inoculated immediately after the procedures were performed.

Preparations of simian virus 40 (SV40) and human adenovirus 12, both previously shown to be oncogenic for the newborn hamster (6, 13), and of fluid from the uninoculated control bottles of cell cultures used to grow each of these viruses were used as control preparations for inoculation into the newborn hamsters. These preparations were obtained from Flow Laboratories, Inc., Rockville, Md. and Microbiological Associates, Bethesda, Md. The titer of the SV40 preparation was 10⁴ tissue culture infectious doses (TCID₅₀) per ml and that of adenovirus 12, 10⁴ TCID₅₀ per ml.

Animals. Each week 25 pregnant Syrian hamsters (Mesocricetus auratus) were obtained from the Animal Production Section, Laboratory AIDS Branch, National Institutes of Health. Only litters containing eight or more animals were used.

Inoculation. Within 24 hours of birth, hamsters were inoculated with 0.1 ml of a biological product or dilutions thereof by the subcutaneous route into
The nuchal region as described previously (12).

**Animal observations.** On the 21st day after birth, the animals were weaned and the sexes separated. No more than eight animals were kept in one cage.

Cages were changed once a week. All cages were autoclaved, washed, and dried before being reused. Sterile sawdust bedding was used. Animals were fed pelleted food for rodents, kale, and carrots which were washed but could not be sterilized.

Weekly observations were made of each animal outside its cage and animals were checked in cages twice daily. Any animal showing signs of abnormality was palpated to determine whether a mass was present in the abdomen. All moribund animals were anesthetized and exsanguinated. Necropsies were performed as soon as possible after death. Complete gross examinations were carried out on all animals when possible. Surviving animals were observed for 18 months and then sacrificed. All animals were autopsied and all tissues and organs which were removed, as well as remnants of the carcasses, were preserved in 10% buffered Formalin.

Histopathological examination of the inoculation site, heart, lungs, thymus, lymph nodes, liver, spleen, pancreas, adrenals, kidneys, bone marrow, reproductive organs, and representative areas of the gastrointestinal tract was performed.

**RESULTS**

Of the total of 13,466 newborn animals which were inoculated during the major study and the 624 unoinoculated controls, 9,558 (68%) were weaned. Of these, 7,117 (74%) were available for histopathologic examination over the 18-month observation period. Many were lost because of autolysis. Of the 1,543 animals available from the preliminary study, 1,150 (75%) also had complete histopathological examinations. Thus, a total of 8,267 animals were available for study.

Tumors were found in 228 of the 7,643 animals inoculated with test materials and examined histologically, an incidence of 3%. Of these, 80 were adrenal cortical adenomas and the remainder (1.9%) were a variety of other tumors, as listed in Table 3. There were 19 tumors in the surviving unoinoculated control animals (403), an incidence of 4.7%. Of these, 10 were adrenal cortical adenomas and 9 (2.2%) were of other types (Table 2). A more detailed description of the histological findings will be described in a subsequent report. However, the most frequently encountered tumor types other than adrenal cortical adenomas were malignant lymphomas, islet cell adenomas of the pancreas, and undifferentiated carcinomas of the thyroid. The tumors occurred in all organ systems and in animals inoculated with all products. None could be said to be associated with the inoculation site or with inoculation of any particular product (Table 3). Table 4 summarizes the total tumor incidence in these hamsters.

The incidence of tumors after inoculation of SV40 virus and adenovirus 12 was similar to those previously reported (11), namely, 76% and 66%, respectively. These tumors were fibrosarcomas and undifferentiated sarcomas, respectively, as has been reported (6, 12). Thus, the types of tumors seen in animals inoculated with test materials were not the same as those induced by the viruses.

The incidence of tumors for each biological product is presented in Table 2. Ninety-five per cent confidence limits for percentages of tumors occurring were also computed. The adrenal cortical adenomas were excluded, for these benign tumors have been reported to occur spontaneously in hamsters with considerable frequency (10). When the percentages of tumors for each major group were compared statistically with those of the unoinoculated control animals by using the binomial approximation method, no statistical differences ($P = 0.05$ level) were found which indicated that the

**Table 1. Biological substances used for inoculation**

| Products inoculated                        | No. of licensed manufacturers' products tested |
|--------------------------------------------|-----------------------------------------------|
| Vaccines                                   |                                               |
| Measles virus vaccine, live attenuated     | 6                                             |
| Measles virus vaccine, inactivated          | 2                                             |
| Smallpox vaccine                           | 6                                             |
| Yellow fever vaccine                       | 1                                             |
| Rabies vaccine                             | 2                                             |
| Poliovirus vaccine, live, oral             | 3                                             |
| Poliomyelitis vaccine                      | 2                                             |
| Cholera vaccine                            | 4                                             |
| Pertussis vaccine                          | 3                                             |
| Typhus vaccine                             | 2                                             |
| Mumps vaccine                              | 2                                             |
| Rocky Mountain spotted fever vaccine       | 1                                             |
| Typhoid and paratyphoid vaccine            | 4                                             |
| Toxoids                                    |                                               |
| Tetanus, plain                             | 4                                             |
| Diphtheria and tetanus, plain              | 1                                             |
| Multiple antigens                          |                                               |
| Diphtheria and tetanus toxoids and         | 3                                             |
| pertussis vaccine combined, plain          |                                               |
| Control materials                          |                                               |
| SV40                                       |                                               |
| Human adenoavirus 12                       |                                               |
| Growth medium from cell culture control    |                                               |
| bottles used for SV40 production           |                                               |
| Growth medium from cell culture control    |                                               |
| bottles for adenoavirus 12 production      |                                               |
distribution of tumors in inoculated groups was similar to those of the controls. Hence, their occurrence was to be expected and not related to the inocula.

**DISCUSSION**

This study was undertaken to determine whether biological products currently in use have oncogenic potential. The study was particularly apt in view of the oncogenicity of some viruses which are widespread in nature.

Similar previous studies of biological products have been less extensive than the present report. Thus, McLeod et al. (10) reported that no tumors developed when randomly bred hamsters were inoculated with tissue culture preparations of live polioviruses (types I and II), vaccinia virus, and measles virus, using three routes of administration. Girardi et al. (5, 7) reported similar results in the Lakeview strain of hamsters after two routes of administration of tissue culture preparations of the same viruses reported by McLeod as well as influenza, mumps, and rabies viruses. McLeod gives no information about the occurrence of spontaneous tumors in control groups, and Girardi reports an incidence of less than 1%. In the study reported here, the occurrence of tumors in uninoculated control animals was 4.7% and in animals inoculated with control materials was zero. When adrenal cortical adenomas were not considered, the occurrence was 2.2% in uninoculated animals. The overall occurrence of tumor formation in 7,729 animals inoculated with various biological products was 3%. Of these, 1.2% were classified as adrenal cortical adenomas and 1.8% as other tumors. Fortner and his colleagues (2–4) have reported an occurrence of over 60% of spontaneous tumors, whereas Kirkman (9) reported an overall incidence of 11.3% after observation for 2.5 to 3.5 years. Eliminating adrenal cortical adenomas reduced that percentage to 3.7%. The tumor formation which we observed over the 18-month period appeared to be of the same order of magnitude as that reported by Kirkman.

Based on the experience of others, the incidence of spontaneous tumor formation in the uninoculated controls, and the fact that the types of tumors seen in our study are similar to those reported to occur spontaneously, we believe that the tumors in the inoculated animals were spontaneous and not related to the inocula. Although two investigators (6, 7, 10) did not report any tumor formation in hamsters when they inoculated vaccine strains of live viruses, neither did they report any spontaneous tumor formation in uninoculated animals.

The results of our study indicate that human biological products in wide use today are not oncogenic in newborn hamsters; however, it
Table 3. Types of tumors seen in 18-month observation period of newborn hamsters

| Site                        | Type of tumor                                      |
|-----------------------------|----------------------------------------------------|
| Endocrine                   | Adrenal cortical adenoma                           |
|                             | Adrenal cortical carcinoma                         |
|                             | Adrenal medullary tumor                             |
|                             | Unclassified malignant adrenal tumor                |
|                             | Undifferentiated carcinoma, thyroid                 |
| Reticuloendothelial system  | Malignant lymphoma                                  |
| Pancreas                    | Islet cell adenoma                                  |
| Genitourinary tract         | Tubular adenoma, kidney                            |
|                             | Thecal cell tumor, ovary                            |
|                             | Granulosa cell tumor, ovary                         |
|                             | Dermoid cyst, ovary                                 |
|                             | Carcinoma, vagina, cervix, uterus (including squamous cell and adenocarcinoma) |
|                             | Leiomyoma, uterus                                   |
|                             | Leiomyosarcoma, uterus                              |
|                             | Lobular hyperplasia, mammary gland                 |
| Soft tissues and bone       | Osteogenic sarcoma                                  |
|                             | Fibrosarcoma                                       |
| Respiratory tract           | Tracheal carcinoma                                  |
|                             | Bronchial carcinoma                                 |
|                             | Pulmonary mucin-producing adenoma                   |

Table 4. Incidence of tumors in hamsters

| Inoculum                  | Per cent with tumors | Per cent with adrenal cortical adenomas | Per cent with other tumors |
|---------------------------|----------------------|----------------------------------------|---------------------------|
| All biological products   | 3.0                  | 1.1                                    | 1.9                       |
| Uninoculated              | 4.7                  | 2.5                                    | 2.2                       |
| Control culture fluids    |                      |                                        |                           |
| from bottles used for     |                      |                                        |                           |
| production of SV40 and    |                      |                                        |                           |
| adenovirus 12             | 0                    | 0                                      | 0                         |

should be borne in mind that only a single lot of vaccine or toxoid of each manufacturer was used. Ideally, several lots of each product of each manufacturer should have been used. Practically, this was and still is impossible because of the magnitude of such a study. Furthermore, two products, yellow fever vaccine and smallpox vaccine, could be used only by inactivating the live virus or diluting the product. One other product (diphtheria and tetanus toxoids and pertussis vaccine, combined) was inoculated as the fluid product rather than the more widely used aluminum adjuvant adsorbed material, for otherwise the newborn animals would not survive. It also should be noted that the inactivation procedures used for yellow fever vaccine probably inactivated any adventitious and possibly oncogenic agents, such as avian leukosis viruses, that might be present in this vaccine. All these facts must be considered in drawing conclusions concerning these products.

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LITERATURE CITED

1. Ahlstrom, C. G., and N. Forsby. 1962. Sarcomas in hamsters after injection with Rous chicken tumor material. J. Exp. Med. 115:839-852.
2. Fortner, J. G. 1957. Spontaneous tumors including gastrointestinal neoplasms and malignant melanomas in the Syrian hamster. Cancer 10:1153-1156.
3. Fortner, J. G. 1961. The influence of castration on spontaneous tumorigenesis in the Syrian (golden) hamster. Cancer Res. 21:1491-1498.
4. Fortner, J. G., A. G. Mahy, and G. R. Schrod. 1961. Transplantable tumors of the Syrian (golden) hamster. I. Tumors of the alimentary tract, endocrine glands and melanomas. II. Tumors of the hematopoietic tissues, genitourinary organs, mammary glands and sarcomas. Cancer Res. 2:161-254.
5. Girardi, A. J., M. R. Hilleman, and R. E. Zwickie. 1964. Tests in hamsters for oncogenic quality of ordinary viruses including adenovirus type 7. Proc. Soc. Exp. Biol. Med. 115:1141-1150.
6. Girardi, A. J., B. H. Sweet, V. R. Slotnick, and M. R. Hilleman. 1962. Development of tumors in hamsters inoculated in the neonatal period with vaccinating virus, SV-40. Proc. Soc. Exp. Biol. Med. 109:649-650.
7. Girardi, A. J., V. M. Larson, and M. R. Hilleman. 1965. Further tests in hamsters for oncogenic quality of ordinary viruses and mycoplasma, with correlative review. Proc. Soc. Exp. Biol. Med. 116:173-179.
8. Hull, R., J. R. Minner, and C. C. Mascoll. 1958. New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery of additional agents both from cultures of monkey kidney tissues and directly from tissues and excreta. Amer. J. Hyg. 68:3144.
9. Kirkham, H. 1962. A preliminary report concerning tumors observed in Syrian hamsters. Stanford Med. Bull. 20:163-166.
10. McLeod, D. L., and A. W. Ham. 1963. Search for oncogenic properties in various viruses found in man. Positive results with adenovirus types 12 and 18. Can. Med. Ass. J. 89:799-805.
11. Malherbe, H., and R. Harvin. 1957. Seven viruses isolated from the vervet monkey. Brit. J. Exp. Pathol. 38:539-541.
12. Rabson, A. S., R. L. Kirschstein, and F. J. Paul. 1964. Tumors produced by adenovirus 12 in mastomys and mice. J. Nat. Cancer Inst. 32:77-87.
13. Trentin, J. J., V. Sebe, and G. T. Taylor. 1962. The quest for human cancer viruses. Science 137:835-841.