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## Search for Virus in Human Malignancies. 2. *In vivo* Studies.* (27712)

A. J. GIRARDI, M. R. HILLEMAN AND R. E. ZWICKEY

*Division of Virus and Tissue Culture Research and Department of Toxicology and Pathology, Merck Institute for Therapeutic Research, West Point, Pa.*

The findings in studies to recover viruses in cell culture systems from human malignant tissues have been recorded(1). This *in vitro* work was paralleled by *in vivo* studies in which newborn mice and hamsters were employed as the indicator system. The results obtained in the latter study are presented here.

*Materials and methods.* The collection and processing of *clinical specimens* and methods for preparation of *tissue cultures* from malignant tissues have been described(1). *Mice* of the ICR/Ha strain were maintained in these laboratories as a random bred strain. For breeding purpose, one male and one female were held as a monogamous family unit from 8 weeks to 10 months of age. The young were weaned at 21 days. The feed consisted of high fat (11%) breeder diet (Ralston Purina). The colony routinely proved free of Salmonella, ectromelia and other common mouse pathogens, and special work carried out in our laboratory indicated freedom from PVM and LCM viruses. The experimental mice and 700 breeder mice were tested and found free of hemagglutination-inhibiting (HI) antibodies for polyoma virus. Pregnant *hamsters* were obtained from Lakeview Hamster Colony, Newfield, N. J. During experimental use, the animal diet consisted of pelleted Rockland Mouse Diet supplemented twice weekly with green peanuts and kale or carrots. *Animal inoculation and observation.* Newborn mice less than 20 hours old were inoculated subcutaneously in the dorsal nuchal area with 0.05 to 0.1 ml of material. The mice were weaned at 21 days of age and the mother was discarded. Hamsters less than 20 hours of age were inoculated subcutaneously with 0.1 to 0.2 ml of sample. The animals were weaned at 18 to 21 days of age, and the mothers were discarded after bleeding for tests for polyoma antibody. During the first 60 to 90 days, each litter of mice or hamsters was housed individually in "sealed" filter cages(2) of special design which precluded airborne cross-infection between cages. All manipulations, such as feeding and palpation of animals, were performed on the individual cages inside a hood equipped with ultraviolet light, continuous exhaust, entrance and exit locks, and rubber sleeves and gloves. Autoclaved water bottles and water were used and the tube was inserted into the cage through a tight-fitting aperture. After the early critical 2- or 3-month period, animals were transferred to ordinary holding boxes with mesh screen lids. The sexes were not segregated. The animals were palpated at least once each week and, in addition, each cage was examined twice daily for dead animals,

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to prevent cannibalism and to permit autopsy. All organs which appeared abnormal at autopsy were examined histopathologically. Once a mass was felt on palpation, the animal was caged separately. Tumors and other tissues for histopathologic observation were fixed in 10% formalin and the paraffin sections were stained with hematoxylin and eosin. *Control animals.* Approximately 54% of the mice received experimental inocula while 46% were injected with placebo or were held as uninoculated controls. About ⅓ of the hamsters served as controls. Control preparations consisted of the suspending medium for malignant tissues (Hanks' balanced salt solution), extracts prepared from tissues of human beings without gross evidence of malignancy, or extracts of cell cultures prepared from such "normal" tissues. For each experimental material, equivalent control materials were inoculated at the same time and in the same manner or uninoculated animals were set aside for control observation purpose. For presentation in the text, the data relating to the control mice were pooled into 4 groups, *i.e.*, A, B, C and D. The time periods during which the various control groups were established are shown in Table III. These are properly designated with the test group to which they correspond. Since the experimental as well as control hamsters were essentially free of malignancy, the data relating to the controls were pooled and summarized.

*Period of observation.* Mice which developed palpable masses or which died were autopsied. Animals which survived were held for 16 to 24 months after which each was bled and inspected at autopsy for evidence of malignancy. All abnormal tissues were examined histologically as described above. The hamsters were generally free of malignancy and hence but few were sacrificed during the observation period. Survivors 20 to 28 months of age were bled, sacrificed and inspected grossly for evidence of malignancy. Suspect neoplastic and otherwise abnormal organs obtained from animals which died spontaneously or which were sacrificed were examined histologically as described above. *Serology.* Sera obtained from the animals were inactivated at 56°C for 45 minutes and tested for inhibition of polyoma virus hemagglutination (HI) without prior treatment with receptor destroying enzyme (RDE). Very few sera gave positive results and these specimens were retested following treatment with RDE to remove non-specific inhibitors of hemagglutination.

*Results. Tests in mice.* Table I summarizes the fate of non-sex segregated mice which received preparations from human malignant specimens. Similar data for the mice in the corresponding control groups A, B, C, D which received "normal" control material or which were held uninoculated are presented in Table II. These controls represented 46% (908/1976) of the total of animals observed. There was little or no significant difference in death rate, incidence or histologic type of neoplasia, or time of appearance of neoplasia between the experimental and control groups.

*Deaths in mice.* Forty-six per cent (488/1068) of mice in the experimental group died during the 16- to 24-month observation period without gross evidence of neoplasia; 45% (405/908) of the animals in the control groups succumbed.

*Incidence of neoplasia.* The over-all summary (Table III) shows that 21% (120/580) of the survivors in the experimental group and 25% (127/503) of the controls developed neoplasia. Lack of significant difference was clearly evident when the results of tests with experimental materials conducted at time periods A, B, C and D are viewed as a whole against their appropriate controls. Where the numbers of survivors were small, as was sometimes the case with individual inocula, tumor incidence on occasion appeared high in the test or control group examined. This appeared to be related to animal variation and emphasized the inherent danger of drawing conclusions based on small individual groups.

*Histopathology.* A variety of neoplasms occurred among the experimental and control groups of animals with histologic types as shown in Tables I and II. The data for the experimental hosts did not show any basic difference from the over-all pattern of spontaneous neoplasia which occurred among the

TABLE I. Neoplasia in ICR/Ha Mice Inoculated When Newborn with Extracts of Human Malignant Tissues.

| Clinical material | | | | Observations in mice | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Numbers of animals with histologic type of malignancy | | | | | | | | | | | | Host age when positive (days) | | | |
| Type of malignancy | Specimen: Type | Specimen: No. | Specimen: Treatment | No. weaned | No. non-neoplastic deaths | No. with neoplasia | Mammary carcinoma | Malignant lymphoma | Pulmonary adenocarcinoma | Hepatoma | Myeloid leukemia | Cutaneous squamous cell carcinoma | Undiff. sarcoma | Adenocarcinoma primary site unknown | Cavernous hemangiosarcoma | Adrenal medullary carcinoma | Renal adenocarcinoma | Regressions | Histopathology not done | Earliest | Latest | Average | Control group |
| Stem cell leukemia 1 case (Age: 1 yr.) | Brain | 1 | Crude susp. | 47 | 13 | 9 | 4 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 235 | 530 | 405 | A |
| | " | | Freon | 37 | 16 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 302 | 454 | 396 | C |
| | Kidney | 1 | Crude susp. | 24 | 12 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 301 | 505 | 403 | B |
| | " | | " | 16 | 3 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 474 | 474 | 474 | C |
| | " | | Freon | 35 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | C |
| | Liver | 1 | Crude susp. | 11 | 7 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 363 | - | 363 | A |
| | " | | " | 33 | 9 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 412 | 462 | 437 | B |
| | " | | Freon | 8 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 252 | 356 | 304 | C |
| | Lung | 1 | Crude susp. | 11 | 3 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 400 | 441 | 420 | A |
| | " | | " | 25 | 14 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 278 | - | 278 | B |
| | " | | Freon | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | C |
| | Bone marrow | 1 | Crude susp. | 24 | 15 | 7 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 228 | 773 | 574 | A |
| | " | | " | 20 | 12 | 4 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 196 | 427 | 340 | B |
| | " | | Freon | 11 | 5 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 467 | 467 | 467 | C |
| | Lymph nodes | 1 | Crude susp. | 6 | 4 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 538 | - | 538 | A |
| | " | | " | 25 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | B |
| | " | | Freon | 36 | 16 | 4 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 294 | 482 | 378 | C |
| | Spleen | 1 | Crude susp. | 16 | 8 | 5 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 424 | 536 | 479 | A |
| | " | | " | 26 | 9 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 277 | 474 | 375 | B |
| | " | | Freon | 35 | 13 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 454 | 473 | 463 | C |
| | Thymus | 1 | Crude susp. | 11 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | A |
| | " | | " | 16 | 11 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 367 | 367 | 367 | C |
| | " | | Freon | 28 | 8 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 473 | - | 473 | C |
| Acute lymphocytic leukemia 10 cases (Ages: 2, 3, 4, 4, 4, 6, 6, 6, 7 yrs.) | Plasma | 1 | Crude susp. | 48 | 18 | 12 | 9 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 315 | 502 | 389 | B |
| | " | 1 | " | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | D |
| | Bone marrow | 1 | Crude susp. | 22 | 15 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 453 | 392 | A |
| | " | 3 | " | 105 | 60 | 7 | 4 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 189 | 757 | 478 | B |
| | " | 7 | " | 125 | 60 | 13 | 9 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 181 | 502 | 336 | C |
| Wilm's tumor 1 case (Age: 9 mos.) | Tumor | 1 | Crude susp. | 24 | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 393 | - | 393 | C |
| | " | 1 | " | 28 | 7 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 340 | - | 340 | D |
| Neuroblastoma 2 cases (Ages: 18 days, 3 yrs.) | Tumor | 1 | Crude susp. | 20 | 6 | 3 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 488 | 565 | 539 | A |
| | " | 1 | " | 28 | 17 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 495 | 523 | 514 | B |
| | " | 1 | " | 31 | 9 | 7 | 5 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 259 | 482 | 394 | C |
| Angiosarcoma of liver 1 case (Age: 1 yr.) | Metas. omentum | 1 | Crude susp. | 46 | 20 | 8 | 5 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 266 | 664 | 461 | D |
| Renal cell carcinoma 1 case (Age: 7 yrs.) | Tumor | 1 | Crude susp. | 77 | 47 | 6 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 293 | 769 | 531 | D |
| Total | | | | 1068 | 488 | 120* | 71 | 20 | 6 | 5 | 5 | 3 | 1 | 1 | 1 | 1 | 2 | 4 | 4 | 181 | 773 | 426 | - |
| Per cent | | | | | | 21% | 12% | 3% | 1% | 1% | 1% | 0.5% | 0.2% | 0.2% | 0.2% | 0.2% | 0.3% | 0.7% | 0.7% | | | | |

* Based on no. survivors (580)

placebo and uninoculated control groups. One possible exception was the occurrence of myeloid leukemia among 5 experimental animals and its absence from the controls. Although this difference is not statistically significant, tissues from the affected animals are being studied further with the hope for isolation of an agent which might be related to the original clinical malignancy. Pulmonary adenocarcinoma and hepatoma occurred both in experimental and control groups but with somewhat greater frequency in the former group.

Regressions of palpable masses were noted among 4 animals in the experimental groups and among 4 in the controls. There were also a few instances in which histopathologic examination was not made of palpable masses which progressed, and the animals finally died. They were classified as malignancy of undiagnosed type. The small numbers of regressions and of histologically undiagnosed cases did not influence the final conclusions. In a small number of cases, mice exhibited more than one kind of neoplasm. This accounts for those instances in Tables I and II where the sum of the individual neoplasms is greater than the total number of animals with neoplasia.

*Time of appearance.* Animals in the ex-

**TABLE II. Occurrence of Neoplasia in Control ICR/Ha Mice.**

| Control group | No. weaned | No. non-neoplastic deaths | No. with neoplasia | Observations in mice: Histologic type of neoplasia: Mammary carcinoma | Malignant lymphoma | Pulmonary adenocarcinoma | Hepatoma | Myeloid leukemia | Cutaneous squamous cell carcinoma | Undiff. sarcoma | Adenocarcinoma primary site unknown | Cavernous hemangiosarcoma | Adrenal medullary carcinoma | Renal adenocarcinoma | Regressions | Histopathology not done | Host age when positive (days): Earliest | Latest | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Placebo controls** | | | | | | | | | | | | | | | | | | | |
| A | 49 | 34 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | | 314 | 629 | 478 |
| B | 61 | 36 | 8 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | | 303 | 655 | 472 |
| C | 70 | 34 | 17 | 14 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 202 | 702 | 412 |
| D | 95 | 61 | 8 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 183 | 502 | 318 |
| Total %* | 275 | 165 | 39 | 29 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 183 | 702 | 415 |
| | | | 35% | 26% | 4% | 1% | | | | | | | | | 2% | 3% | | | |
| **Uninoculated controls** | | | | | | | | | | | | | | | | | | | |
| A | 166 | 70 | 32 | 22 | 6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 278 | 772 | 471 |
| B | 172 | 50 | 31 | 24 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 281 | 753 | 473 |
| C | 170 | 63 | 17 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 201 | 531 | 384 |
| D | 125 | 57 | 8 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 351 | 735 | 512 |
| Total %* | 633 | 240 | 88 | 60 | 15 | 0 | 1 | 0 | 3 | 0 | 0 | 2 | 1 | 1 | 2 | 5 | 201 | 772 | 458 |
| | | | 22% | 15% | 4% | | 0.3% | | 0.8% | | | 0.5% | 0.3% | 0.3% | 0.5% | 1% | | | |

* Based on No. of survivors (110,393).

TABLE III. Summary. Neoplasia of all types in test mice compared with controls.

| Group | Inoculum | Results: Numbers: Weaned | Non-neoplastic deaths | Survived | Developed neoplasia | % with neoplasia of: Total weaned | Survivors |
|---|---|---|---|---|---|---|---|
| A | Experimental | 168 | 74 | 94 | 31 | 18 | 33 |
| 12/29/59 to 1/20/60 | Placebo | 49 | 34 | 15 | 6 | 12 | 40 |
| | Uninoc. | 166 | 70 | 96 | 32 | 19 | 33 |
| B | Experimental | 334 | 165 | 169 | 33 | 10 | 20 |
| 2/ 1/60 to 2/18/60 | Placebo | 61 | 36 | 25 | 8 | 13 | 32 |
| | Uninoc. | 172 | 50 | 122 | 31 | 18 | 25 |
| C | Experimental | 410 | 172 | 238 | 41 | 10 | 17 |
| 3/ 1/60 to 3/30/60 | Placebo | 70 | 34 | 36 | 17 | 24 | 47 |
| | Uninoc. | 170 | 63 | 107 | 17 | 24 | 16 |
| D | Experimental | 156 | 77 | 79 | 15 | 10 | 19 |
| 5/13/60 to 6/15/60 | Placebo | 95 | 61 | 34 | 8 | 9 | 24 |
| | Uninoc. | 125 | 57 | 68 | 8 | 6 | 12 |
| All groups, A-D | Experimental | 1068 | 488 | 580 | 120 | 11 | 21 |
| | Placebo | 275 | 165 | 110 | 39 | 14 | 35 |
| | Uninoc. | 633 | 240 | 393 | 88 | 14 | 22 |

perimental group developed neoplasia between days 181 and 773 (average 426 days); in control groups, between days 183 and 772 (average 445 days). No significance was attached to the average time difference of 19 days.

*Sex comparison.* The majority of neoplasms in the experimental and control groups occurred in females (Table IV). This was due, principally, to the greater frequency of mammary neoplasia in females compared with males. Differences in occurrence of the other kinds of malignancy as related to sex were so small as not to be significant. The actual numbers of male and female survivors are not known, hence it is not possible to judge the significance of the apparent greater percentage (24%) of neoplasia in male experimental animals than in male placebo (15%) and uninoculated (16%) controls.

TABLE IV. Occurrence of Neoplasia, According to Kind of Neoplasm and Sex, in Experimental and Control ICR/Ha Mice.

| Type of neoplasia | No. of neoplasia in different groups: Experimental ♂ | Experimental ♀ | Placebo ♂ | Placebo ♀ | Uninoculated ♂ | Uninoculated ♀ |
|---|---|---|---|---|---|---|
| Mammary carcinoma | 2 | 69 | 2 | 27 | 3 | 57 |
| Malignant lymphoma | 10 | 10* | 2 | 2 | 6 | 9* |
| Pulmonary adenocarcinoma | 4 | 2 | 1 | 0 | 0 | 0 |
| Hepatoma | 3 | 2 | 0 | 0 | 0 | 1 |
| Myeloid leukemia | 3 | 2** | 0 | 0 | 0 | 0 |
| Cutaneous squamous cell carcinoma | 1 | 2 | 0 | 0 | 2 | 1 |
| Undifferentiated sarcoma | 0 | 1 | 0 | 0 | 0 | 0 |
| Adenocarcinoma—primary site unknown | 1 | 0 | 0 | 0 | 0 | 0 |
| Cavernous hemangiosarcoma | 1 | 0 | 0 | 0 | 1 | 1* |
| Adrenal medullary carcinoma | 1 | 0 | 0 | 0 | 1 | 0 |
| Renal adenocarcinoma | 2† | 0 | 0 | 0 | 1 | 0 |
| Regressions | 1 | 3 | 0 | 2 | 0 | 2 |
| Histopathology—not done | 1 | 3 | 1 | 2 | 0 | 5 |
| Totals | 30 (24%)‡ | 94 | 6 (15%)‡ | 33 | 14 (16%)‡ | 76 |

* *Each* asterisk designates an animal with 2 malignancies; one mammary, one as indicated.
† An animal with 2 malignancies; one malignant lymphoma, one renal adenocarcinoma.
‡ % males, of total, with neoplasia.

TABLE V. Fate of Mice Which Developed Palpable Masses but Were Not Sacrificed.

| Mouse group | No. animals with masses | Deaths. Days post first detection of mass | | | Regressions of masses | |
|---|---|---|---|---|---|---|
| | | Earliest | Latest | Avg | No. animals | Time, days |
| Experimental | 25 | 6 | 73 | 37 | 2 | 24, 35 |
| Control—Total | 21 | 8 | 91 | 39 | 2 | 28, 35 |
| Placebo | 6 | 8 | 54 | 23 | 1 | 35 |
| Uninoc. | 15 | 8 | 91 | 45 | 1 | 28 |

*Fate of tumor-bearing mice.* Although most animals were sacrificed shortly after appearance of a palpable mass, occasional animals were allowed to survive. The tumors were generally highly malignant biologically and resulted in death in all but 4 animals within 6 to 91 days (Table V). In 4 animals there was regression with apparent recovery.

*Transplantation experiments.* Tumor-bearing mice from different groups were selected as donors of tissue for transplant studies. These included 6 mammary carcinoma and 1 malignant lymphoma from the experimental group, 2 mammary carcinoma from the placebo animals, 3 mammary carcinoma from the uninoculated control animals, and 4 mammary carcinoma from the stock breeders. The tumor tissues were minced in Hanks' balanced salt solution and inoculated *via* trocar into the dorsal subcutaneous tissues of groups of five 21-day-old weanling ICR/Ha mice. Observations for appearance of tumors were continued until the animals were 6 months old. Only 2 of the 80 mice developed tumors. One of these had received mammary carcinoma material from an uninoculated control animal and the other had been given tumor tissue of the same type derived from a stock breeder animal. The transplant tumors developed 2.5 and 4 months later, at an age somewhat less than generally required for spontaneous mammary carcinoma to arise.

*Influence of breeding on tumor occurrence.* The mice in the litters in the experimental investigations were not separated by sex. These animals eventually gave birth to litters which were discarded within 1 week post-natum. Thus, the mothers were not allowed to nurse their young for the usual 21-day period and the possibility was considered that such "stagnation of the mammary ducts"(3) might have influenced the development of mammary carcinomas. To test this possibility, a group of mated stock breeder females which had had several litters under the conditions of the breeding colony were set aside without males at 6 to 7 months of age and evaluated for incidence of mammary malignancy when 1 year old compared with the incidence of similar malignancy in the test animals at the same age. The incidence of mammary tumors was nearly alike and it appeared that removal of litters from the lactating mothers did not affect appreciably the development of mammary carcinoma (Table VI).

It did appear, however, that sex segregation might affect the development of mammary carcinoma in ICR/Ha mice. A group of 151 mice was segregated, according to sex, at 21 days of age and was observed for 13 months for development of malignancies. No tumors developed in any of the 77 virgin females or in any of the 50 virgin males which survived the observation period. All but 2 of the 24 animals which died were autopsied and none had gross evidence of malignancy. The time elapsed and the numbers of animals observed are not yet sufficient to render a definitive judgment of the role of segregation on tumor incidence. The data suggest, however, that retention of virginity in female mice may be compatible with reduction in mammary tumors.

*Tests for polyoma virus antibody.* None of the sera of mice bled just prior to autopsy showed the presence of polyoma antibody in HI tests for polyoma when tested at a 1:50 dilution. *Tests in hamsters.* Table VII summarizes results of the tests in hamsters inoculated when newborn with (a) extracts of human malignant tissues, (b) extracts of cell

TABLE VI. Comparison of Incidence of Mammary Carcinoma in Stock Breeders and in Test Animals at 1 Year of Age.

| Group | No. weaned | No. non-cancer deaths | Results: Mammary carcinoma / Total survivors | |
|---|---|---|---|---|
| | | | Numbers | % |
| Stock breeders (normal suckling) | 468 | 162 | 13/306 | 4.3 |
| Test animals (litters removed at <7 days) | 1976 | 541 | 86/1435 | 6.0 |

cultures of human malignant tissues, or (c) crude extracts of cell cultures of grivet monkey kidney inoculated with specimens from human cases of malignancy. Abnormal tissues from nearly all of the animals which died were observed histopathologically, although cannibalism prevented autopsy in a few animals. Of the total of 1270 hamsters weaned, 399 (31%) were controls and 871 experimental hosts.

Only 4 neoplasia developed among the hamsters and there was no significant difference in occurrence of neoplasms among the animals which had been inoculated with any of the various test materials.

Serum samples obtained from the hamster mothers at time of weaning the litters and from the animals at time of autopsy were tested for mouse polyoma antibody at a dilution of 1:10 and all were found to be negative.

*Discussion.* The remarkable successes(4) which have attended investigations of viral etiology of neoplasia in animals have stimulated efforts to demonstrate viral etiology in malignancies in man. The method for such investigation employed most extensively has been the passage of primary or "cell culture enriched" extracts of human malignant materials in experimental animals. The demonstrated lack of strict species-specificity for oncogenic viruses of animals(4) has provided a basis for hope that human malignant agents might be demonstrable in laboratory animals. Although "positive" results in studies of human materials have been recorded by several workers(4-11, cf. 12), none of the experiments can be regarded as having resulted in recovery of a viral agent of proved oncogenic quality for man. There are many difficulties in the animal experimental approach, the more important being spontaneous appearance of malignancies in test animals, possible evoking of oncogenic principles indigenous to the animals resulting from injections of foreign substance, and inability to conduct tests in the human species which would permit fulfillment of Koch's postulates.

The present study was an attempt to recover, in animals, viruses or virus-like oncogenic agents from cases of leukemia and other malignancies of man. For this purpose, both mice and hamsters were used. The ICR/Ha strain of white Swiss mice was specially chosen because of its ready availability, its known freedom from polyoma virus infection, and because of the reported successes by Grace *et al.*(5) in similar investigations with this same strain of mice. The animals were inoculated during the neonatal period, when the known animal tumor viruses generally effect their greatest percentage of "takes"(4). The human malignant tissues were from leukemias as well as from various solid tumors. These tissues, as in the *in vitro* studies reported earlier(1), were prepared as crude extracts or were treated with Freon to effect removal of hypothetical virus inhibitors such as antibody. In certain instances, the human malignant tissues were grown as, or were passed in, cell cultures for the purpose, hopefully, of "evoking" or "enriching" the oncogenic agents.

Most important was the care taken to establish uninoculated or placebo-inoculated controls from the same groups of animals and at the same time as the experimental groups were established and to observe all animals for an adequate period. Of importance also was the fact that all animals were held in special filter cages(2) during the first 2 to 3 months, the period of greatest danger of cross-infection. These cages precluded airborne or direct or indirect contact transfer of agents between the various groups of study animals.

TABLE VII. Neoplasia in Hamsters Inoculated When Newborn with Extracts of Human Malignant Materials or of Fluids from Cell Cultures.

| Clinical material | | | | | Occurrence of neoplasms |
|---|---|---|---|---|---|
| Type of malignancy | Type | Specimen No. | Treatment | No. weaned | (No., kind, and time of appearance) |
| | | *Series A. Extracts of human malignant tissues* | | | |
| Stem cell leukemia, 1 case (age: 1 yr) | Brain | 1 | Crude susp. | 17 | |
| | Thymus | 1 | " " | 15 | |
| | Kidney | 1 | " " | 18 | |
| | " | | Freon 3X | 9 | |
| | Liver | 1 | Crude susp. | 19 | |
| | " | | Freon 3X | 7 | |
| | Spleen | 1 | Crude susp. | 13 | |
| | " | | Freon 3X | 1 | |
| | Lung | 1 | Crude susp. | 3 | |
| | " | | Freon 3X | 11 | |
| | Bone marrow | 1 | Crude susp. | 14 | |
| | " " | | Freon 3X | 6 | |
| | Lymph nodes | 1 | Crude susp. | 14 | |
| | " " | | Freon 3X | 8 | |
| Acute lymphocytic leukemia, 9 cases (ages: 2, 4, 4, 6, 6, 6, 6, 7, 7 yr) | Plasma | 1 | Crude susp. | 7 | |
| | Bone marrow | 8 | " " | 127 | 1 malignant lymphoma, 631 days |
| Chronic lymphocytic leukemia, 1 case (age: 59 yr) | Buffy coat | 1 | " " | 17 | |
| Chronic myelogenous leukemia, 1 case (age: 44 yr) | Bone marrow | 1 | " " | 27 | |
| Wilm's tumor, 3 cases (ages: 9 mo, 4, 5 yr) | Tumor | 3 | " " | 79 | |
| | | | Crude filt. | 87 | 1 hemangioma, 625 days |
| | | | Freon 2X | 65 | 1 hemangioma, 550 days |
| Neuroblastoma, 3 cases (ages: 18 days, 3, 5 yr) | Tumor | 3 | Crude susp. | 41 | |
| Totals—18 cases | — | 25 | 37 preparations | 605 | |
| | | *Series B. Extracts of cell cultures of human malignant tissues* | | | |
| Cystosarcoma phylloides, 1 case (age: 13 yr) | Tumor | 1 | Filt. culture fluid | 22 | 1 adrenal medullary adenoma, 621 days |
| Breast cancer, 1 case (age: 56 yr) | "Normal" lymph node | 1 | *Idem* | 21 | |
| | "Normal" breast tissue | 1 | " | 24 | |
| Cancer, intestine, 2 cases (ages: 72, 74 yr) | Metastatic nodes | 1 | " | 13 | |
| | Tumor | 1 | " | 32 | |
| Totals—4 cases | — | 5 | 5 | 112 | |
| | | *Series C. Crude extract, GMK passage material* | | | |
| Stem cell leukemia, 1 case (age: 1 yr) | Brain | 6th pas. | Crude | 22 | |
| | Liver | " " | " | 22 | |
| | Lymph nodes | " " | " | 27 | |
| | Spleen | 5th " | " | 12 | |
| | Thymus | 6th " | " | 21 | |
| Acute lymphocytic leukemia, 1 case (age: 6 yr) | Plasma | 5th " | " | 22 | |
| Chronic lymphocytic leukemia, 1 case (age: 59 yr) | Urine, high speed supt. | 6th " | " | 28 | |
| Totals—3 cases | — | 7 | 7 | | |
| | | *All groups and controls* | | | |
| All experimental | | 37 | 49 | 871 | 4 neoplasia, 550-631 days |
| Control: Placebo | | | | 122 | 1 adrenal cortical adenoma, 592 days |
| Uninoculated | | | | 277 | 1 renal adenocarcinoma, 726 days |

The possibility was considered that an oncogenic agent in the human malignant tissues might manifest itself in experimental animals in such ways as early death, production of lesions of non-malignant quality, or induction of frank malignant disease. Therefore, an autopsy was performed on every animal which died (other than those which were cannibalized) or was sacrificed, and every organ which appeared abnormal was examined for histological evidence of malignancy. The numbers of animals used per group were kept as large as practicable to permit adequate comparisons. Moreover, it was necessary to view incidences of malignancy in the experimental groups of animals against the total background of occurrence of spontaneous malignancies in the control animals.

The present studies carried out in the ICR/Ha mice revealed a high level of spontaneous malignancies, especially mammary carcinoma, and the lack of definitive evidence for induction of neoplasia by injection of materials of human malignant origin. In all, 70% of the neoplasia of all groups of mice was mammary carcinoma. These results are in striking contrast to those of Grace *et al.*(5) in similar studies in the same ICR/Ha strain of mice. These workers recorded a similar high incidence of malignancies, especially of mammary carcinoma, (90% of total of malignancies) in the mice which had received extracts of human malignant tissues. By contrast, the controls were recorded as showing a remarkably low incidence of any sort of malignancy, including mammary carcinoma.

The appearance, in the present studies, of myeloid leukemia in 5 of the experimental animals contrasted with none in the controls, and the slightly more frequent occurrence of pulmonary adenocarcinoma and hepatoma in the former group are interesting observations, but without statistical significance. As might be expected, female mice exhibited a far greater occurrence of mammary carcinoma than did males. There was no apparent sex difference in the mice as to other types of malignancy. The mammary carcinomas were highly malignant biologically and generally terminated in death of the animal. As expected for non-isologous mice, these tumors generally failed to take on transplantation to new mice.

Bagg(13) and Fekete and Green(14) have proposed that prevention of nursing among female mother mice may produce a stagnation of the mammary ducts and may be a factor in the genesis in mammary carcinoma. Our findings did not support this theory. Instead, they are in agreement with those of Bittner (3) who noted no such effect. Thus, mother mice from which the litters were removed within 7 days post delivery showed no greater incidence of mammary carcinoma during a one-year period than did females which were permitted to suckle their young for the full period and which were segregated from the males at 6 to 7 months of age. The observation in these studies of a lesser tumor incidence among segregated virginal mice compared with breeding animals is of considerable interest as a possible method for providing animals of low spontaneous tumor incidence.

The findings with hamsters generally complemented the results of the tests in mice in that these animals likewise failed to demonstrate viral agents in human malignant specimens. The hamsters presented an obvious advantage over the mice in the low incidence of spontaneous malignancy, although the relative sensitivity of these animals to oncogenic agents in general might be lower than in mice.

The negative results presented here are in general agreement with those of Moore(15) who was unable to show any statistically significant difference in occurrence of tumors in mice which had received human malignant tissue extracts compared with controls. As in the *in vitro* studies(1) reported previously, the negative findings recorded here should not be construed as positive evidence for a non-viral etiology in malignancies of the human species. Instead, they serve to emphasize the difficulties likely to continue to be encountered in virus in human cancer studies until some major breakthrough in technology provides simple and precise methods for demonstrating the agents.

*Summary.* Extensive investigations were carried out in carefully controlled studies in newborn white Swiss mice (ICR/Ha) and

hamsters to recover viruses or virus-like oncogenic agents from human malignant tissues. Altogether, 84 preparations from clinical specimens were tested. The human specimens were from leukemias and solid tumors and were tested as crude, filtered or fluorocarbon-treated extracts, as fluids from cell cultures of human malignant tissues, or as fluids from grivet monkey kidney cultures inoculated with clinical materials. There was a considerable incidence of spontaneous neoplasia, especially mammary carcinoma, among the mice but a very infrequent occurrence of malignancy in hamsters. Prevention of nursing among the mother mice did not increase the incidence of mammary carcinoma. Sex segregation of mice with retention of a virginal status appeared, in a small study, to diminish tumor incidence among females considerably below that of freely breeding animals. There was no statistically significant difference in tumor incidence among the experimental animals inoculated with specimens derived from human malignant tissues compared with the controls except for a few cases of leukemia which occurred in the experimental group and in a slightly more frequent occurrence of hepatoma and pulmonary adenocarcinoma among these animals. The need for adequate numbers of animals, for proper control procedures, and for extensive histologic examination of all abnormal animal tissues in studies of the role of transmissible agents in human malignancy was emphasized. The negative findings in these investigations were not interpreted as positive evidence for a non-viral etiology in malignancies of man.

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## Catabolism of Glucose and Gluconate in Intact Rats. (27713)

CHIH H. WANG, LAWRENCE P. SNIPPER,* OYA BILEN AND BETTY HAWTHORNE
(Introduced by Vernon H. Cheldelin)
*Departments of Chemistry and Foods and Nutrition and Science Research Institute, Oregon State University, Corvallis*

The mechanism for the catabolism of hexose in intact rats has been previously examined in several laboratories. Bloom *et al.*(1) concluded that the Embden-Meyerhof-Parnas (EMP) pathway is the principal, if not exclusive, mechanism for glucose utilization since

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