OBSERVATIONS ON THE INJECTION OF HUMAN WART (VERRUCA VULGARIS) EXTRACTS INTO RABBITS WITH BENZPYRENE PAPILLOMAS ON THE EAR

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The possible rôle of viruses in the etiology of malignant neoplasms has long been a subject for discussion and experimentation. Since a review of this literature is beyond the scope of this brief paper, it must suffice to mention previous pertinent reports.

In 1936 Rous and Kidd found that intravenous injection of Shope rabbit papilloma virus activated tar warts on rabbits' ears to malignant degeneration. Andrewes, Ahlström, Foulds, and Gye in 1937 showed that rabbits injected intramuscularly with tar and then intravenously with the infectious fibroma virus (Shope) developed generalized fibromatosis, a phenomenon not observed in animals not treated with tar. Similar results were obtained when 3:4 benzpyrene was substituted for tar. Lacassagne and Nyka in a small series of animals observed that intravenous injection of Shope papilloma virus intensified the growth of cutaneous benzpyrene tumors (carcinoma) in rabbits. This effect was not obtained in rabbits in which the hypophysis was destroyed.

In the following series of experiments the writers endeavored to determine whether injections of saline extracts from the common human wart, verruca vulgaris, might intensify the growth of and hasten malignant degeneration of benzpyrene warts on the ears of rabbits. Verruca vulgaris is classified as a virus disease and has been shown to be transmissible in man by injection of Berkefeld filtrates of the lesions.

The rabbits employed were adults of mixed breeds obtained from a dealer. Upon the external surfaces of both ears of each rabbit a 0.3 per cent benzene solution of benzpyrene was dropped three times a week. The changes taking place on the exposed cutaneous surfaces showed great variation in so far as benign lesions were concerned. Some animals had no more than a few small warts after 300 days. Others developed sessile warts, cutaneous horns, frill horns (Rous), and in two instances large pedunculated complex papillomas on both ears, 2 to 3 cm. in diameter, which later regressed spontaneously. Successive crops of lesions appeared and some regressed during the period of benzpyrene application. When small lesions appeared on the ears or, as in one case, when there was marked hyperkeratosis, the wart extracts were injected intravenously and benzpyrene applications were discontinued.

Extracts of the human warts were obtained as follows: The wart-bearing area of skin was swabbed with alcohol and this was permitted to dry completely. By means of a safety-razor blade, the protruding surfaces of the warts were removed; no local anesthetic was employed. Care was taken to

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**Table I: Animals Developing Carcinoma**
(Benzpyrene applied three times a week on external aspects of both ears)

<table>
<thead>
<tr>
<th>White 3</th>
<th>Gray</th>
<th>Gray 5</th>
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<tbody>
<tr>
<td>84th day: 3.5 c.c. human wart extract intravenously. Keratosis present both ears</td>
<td>200th day: Benzpyrene discontinued. Few small scattered warts both ears; 3 c.c. human wart extract injected</td>
<td>220th day: Benzpyrene continuing. Oval lesion 1 cm. in diameter on left ear, outer surface, with raised rolled edges</td>
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<td>100th day: 2 c.c. human wart extract</td>
<td>236th day: Increase in size of all warts and appearance of new ones. One rapidly growing lesion near base of right ear</td>
<td>250th day: Lesion has perforated to inner aspect of ear. Grossly typical squamous-cell carcinoma</td>
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<tr>
<td>110th day: Several small papillomas both ears</td>
<td>278th day: Large ulcerating lesion at base of right ear, 3.5 cm. in diameter with rolled raised edges; typical gross picture of squamous-cell carcinoma. Biopsy: Squamous-cell carcinoma</td>
<td>274th day: Benzpyrene discontinued</td>
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<tr>
<td>145th day: Benzpyrene discontinued. Rather vigorous increase in size of all papillomas</td>
<td>318th day: Half of base of right ear destroyed</td>
<td>304th day: Lesion increased in size to 2 cm. in diameter. Animal died. Microscopic examination: Squamous-cell carcinoma</td>
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<tr>
<td>160th day: 10 c.c. human wart extract</td>
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<tr>
<td>184th day: 1 c.c. human wart extract. Continued growth of some papillomas and regression of others. Lesion at margin and near tip of left ear suggesting carcinoma</td>
<td>254th day: Increase in size of above</td>
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<tr>
<td>254th day: Increase in size of above</td>
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<tr>
<td>272nd day: Lesion has gross appearance of carcinoma; raised rolled borders, ulceration, 1.5 cm. in diameter. Biopsy: Squamous-cell carcinoma</td>
<td>274th day: Benzpyrene discontinued</td>
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<tr>
<td>332nd day: Lesion has spread down ear toward base, and has destroyed whole thickness of a portion of the ear</td>
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Cut as deeply as possible so that the portion removed would consist of more than the thickened horny layer of skin. The several small disc-like pedicles thus obtained, ranging in size from 3 to 5 mm. in diameter and 1 to 2 mm. in thickness, were placed in a small mortar and ground for several minutes with sand, sterile normal saline solution being added immediately (10 to 12 c.c.). The cloudy suspension was then injected intravenously in the rabbits.

Nineteen animals received tri-weekly applications of benzpyrene and injections of human wart extract. Of these, 6 were observed for 500 days, having been treated with benzpyrene for periods of 150 to 240 days; 4 were observed 400 days, having received benzpyrene applications for 180 to 240 days; 9 were observed from 330 to 375 days, having been treated with benz-
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FIG. 1. WHITE RABBIT 3

A. Appearance of ears after tri-weekly applications of benzpyrene during a period of 145 days. Human wart extract was injected on the 84th and 110th days.

B. Appearance 160 days after A. Several benign lesions have increased in size and near the tip of the left ear is a small carcinoma. Human wart extract was again injected on this date.

C. Appearance 54 days after B. The lesion on the left ear has progressed, with destruction of part of the ear.

pyrene over periods of 120 to 330 days. One of the group observed 500 days and one of the group observed 330 to 375 days developed carcinoma (see Table I and Figs. 1 and 2).

The control series, treated with benzpyrene alone or combined with injections of human gastric juice from cancerous stomachs or extracts of normal skin or viscera, numbered 40, including 2 animals observed for 500 days; 1 for 400 days; 22 for 365 to 375 days; 9 for 280 to 300 days, and 6 for 240 to 280 days. The period of benzpyrene application ranged from 120 to 375 days. One carcinoma developed in this control series in the group observed from 280 to 300 days (see Table I and Fig. 3).

The animals which developed malignant neoplasms are described in Table I.

DISCUSSION OF RESULTS

Malignant neoplasms of the skin of rabbits' ears are apparently not very readily produced by application of benzpyrene. Schürch and Winterstein observed 12 rabbits for 362 to over 400 days in which bi-weekly applications of 0.3 per cent benzene solution of benzpyrene were made to the inner surfaces of the ears. By the 362nd day 10 of them had warts but after 400 days only one animal had a squamous-cell carcinoma.

In our group of 40 control animals observed from 240 to 500 days, one developed carcinoma by the 220th day of benzpyrene application. In the experimental group of 19 animals 2 had squamous-cell carcinomas. In these two animals, furthermore, benzpyrene was applied for only 145 days in one case and for 200 days in the other case. At the termination of the applications no gross evidence of malignant growth was present. In the rabbit which
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FIG. 2. GRAY RABBIT

A. Appearance of ears 200 days after tri-weekly applications of benzpyrene. Human wart extract was injected intravenously and applications of benzpyrene were discontinued at this time.

B. Appearance 36 days after A, showing increase in size of several benign lesions, and appearance of new ones. W indicates a rapidly growing lesion which appears to be a carcinoma.

C. Appearance 42 days after B. Lesion, W, has increased considerably in size, and has the typical appearance of ulcerating carcinoma.

D. Appearance 40 days after C. Carcinoma has progressed, with destruction of portion of ear. Note large cutaneous "horn" on upper part of left ear.

received benzpyrene for 145 days human wart extracts were injected during the period of such applications and subsequent to their termination. In the other animal the human wart extract was injected only once, and that on the last day of benzpyrene application.

While the animals employed in these experiments are too few to permit of definite conclusions, it is felt, nevertheless, that the results obtained are suggestive of the fact that extracts of human warts (verruca vulgaris) containing the etiologic factor of these lesions, a virus, may on occasion hasten the malignant degeneration of benzpyrene warts in rabbits. These results are recorded because of previous work by others with viruses, mentioned above, and because of the recent use of the ultracentrifuge in purification of viruses.
A. Small carcinoma, E, near tip of left ear. Picture taken after tri-weekly application of benzpyrene over a period of 220 days.

B. Thirty-seven days later. Carcinoma has increased in size and almost perforated the ear.

In conclusion it might be mentioned that subcutaneous implantations of fragments of human warts in the ears of 8 rabbits failed to produce warts in the overlying skin during a period of 50 to 120 days. That the extracts of human warts employed in the above experiments were nevertheless infective was demonstrated by the development of a wart in 90 days in the skin of the dorsum of the left thumb of one of us (A. B.) as a result of an intracutaneous inoculation of the extract at the time some of the intravenous injections were being made in the rabbits.

**SUMMARY**

Observations are recorded which suggest that the virus of the common human wart (verruca vulgaris) may on occasion hasten the malignant degeneration of benzpyrene warts on rabbits' ears.

**REFERENCES**


**ADDENDUM**

Since this report was submitted for publication the 59 rabbits mentioned above have been observed for an additional 150 days. Among the 40 control animals another carcinoma developed, on the 610th day of observation, during the first 200 days of which period benzpyrene was applied. Among the 19 experimental animals there appeared 2 more carcinomas: one 200 days after the injection of wart extract which was made at the termination of a period of 240 days of benzpyrene application; the other 170 days after injection of wart extract, which was made at the termination of a period of 280 days of benzpyrene application. Final observations thus show 4 carcinomas among 19 experimental animals and 2 carcinomas among 40 controls.