height for the heat-treated lots compared to 101 inches for the control.

An unanticipated result was the pollen sterility in all treated lots. Normal tassels were produced with well-developed florets, but the anthers were small and shriveled and for the most part remained enclosed in the glumes. In view of the fact that high temperatures sterilize the male germ cells in animals from amphibians to mammals, these results are highly significant. This influence on growth is an antivernalization effect and may have wide usefulness in the production of hybrid seed, especially if it is shown by plants other than maize. Further experiments are in progress.

Failure of Sodium Salicylate to Inhibit Hyaluronidase *in Vitro*

ROBERT M. PIKE

Department of Bacteriology and Immunology, Southwestern Medical College, Dallas, Texas

When India ink or Evans Blue was injected with hyaluronidase into the skin of rabbits or human subjects following the administration of sodium salicylate, Guerra (*1*) observed decreased spreading as compared with normal controls. If this result was due to the inhibition of hyaluronidase by sodium salicylate, as concluded by Guerra, it should be possible to demonstrate an antagonistic effect of sodium salicylate on hyaluronidase *in vitro*.

Using the broth culture filtrate of a mucoid group A streptococcus as substrate, it was found that 0.005 ml. of a crude bovine testicular extract completely destroyed 0.5 mg. of hyaluronic acid. The addition to the reacting mixture of 5 mg. of sodium salicylate did not decrease the rate of disappearance of the hyaluronic acid, the amount of which was estimated by precipitation with acidified dilute horse serum (*3*).

In a second experiment, 1:100 dilution of crude testicular extract was prepared in 10 per cent sodium salicylate. This solution, as well as a 1:100 dilution of the same testicular extract without sodium salicylate, was tested for its ability to prevent the mucin clot formation by potassium hyaluronate prepared from human umbilical cord (*2*). Both solutions prevented clot formation, again indicating no inhibition of testicular hyaluronidase by the sodium salicylate.

The action of sodium salicylate on streptococcal hyaluronidase was also examined. A broth culture filtrate of a nonmucoid group A streptococcus as a source of the enzyme was combined with a filtrate of a mucoid group A streptococcus containing hyaluronic acid. The enzyme destroyed 0.5 mg. of hyaluronic acid on incubation overnight. The inclusion of 0.1 per cent sodium salicylate in the system did not alter the reaction. When broth containing 10 mg. per cent of hyaluronic acid was inoculated with a nonmucoid group A streptococcus, the hyaluronic acid was destroyed by the time maximum growth was attained. The addition of sodium salicylate to the broth did not retard the disappearance of the hyaluronic acid except in concentrations that inhibited growth. Sodium salicylate, 0.1 per cent, was slightly bacteriostatic; 0.05 per cent inhibited neither growth nor the destruction of the hyaluronic acid.

These experiments fail to demonstrate any inhibitory effect of sodium salicylate on testicular or streptococcal hyaluronidase.

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The Carcinogenic Action of Smegma[1]

ALFRED PLAUT and ALICE C. KOHN-SPEYER

Pathological Laboratory, Beth Israel Hospital, and *Department of Cancer Research, College of Physicians and Surgeons, Columbia University, New York City*

Carcinoma of penis does not occur in people who have been circumcised in the first weeks of life and is rare in people who have been circumcised in childhood or in early puberty. Circumcision in adult life does not affect the frequency of the disease. Phimosis seems to be a predisposing factor. Penile cancer is frequent where personal hygiene is poor; it is rarer where bathrooms are plentiful. All this has been known for decades, and various opinions have been expressed concerning causal relations. Little has been done, however, to establish experimentally the possible carcinogenic action of smegma. Fishman, Shear, Friedman, and Stewart (*1*) injected filtered suspensions of human smegma subcutaneously into 12 young mice of the A strain. No tumors were obtained either in these mice or in 20 young mice into whose distended vaginas smegma was introduced.

In our experiments mice of the Paris R 3 strain were used. No spontaneous malignant tumors of skin and no cutaneous papillomata have been observed in many thousands of mice of this strain in the Crocker Cancer Research Institute. Some of the mice were bred in the Crocker Institute, others in Beth Israel Hospital. They were kept in wooden cages with wire covers, were fed Rockland pellets, and were given water.

Since human smegma, which was used in the first experiments, could not be obtained in sufficient amounts, horse smegma was substituted. This particular substitution was made because penile cancer is frequent in the horse. Smegma is best obtained from dead horses in rendering plants or from anesthesized animals in a department of veterinary surgery. The smegma was kept dry in glass jars in the refrigerator or at room temperature. Generally it was impossible to discover whether the material came from a stallion or from a gelding. One batch was examined for male and female sex hormones, but none were found.

The nonsaponifiable fraction was prepared in the usual way. An analysis for fatty substances gave the following result: total fat, 45 per cent of dry substance (fatty acids, 32.2 per cent; phosphatids, 3.9 per cent; total cholesterol, 6 per cent; cholesterol esters, 2 per cent).

Cerumen, which resembles smegma in being a skin product that is retained on or near the surface of the body, was used

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as control substance. This could be used because carcinoma of the auditory meatus is very rare in man and animals.

Method

A buried skin tunnel, as described in a previous publication (*2*), was made in midline in the middorsal region. Its walls consisted of skin and panniculus carnosus. The smegma, or the cerumen, was kneaded with water into a pulp thin enough to pass through the nozzle of a 2-cc. syringe (bore admitting catgut #3). No needle was used. The nozzle of the syringe was pushed gently into the caudad opening of the tunnel, and the plunger was pushed slowly until the material appeared at the cephalad end. When the skin tunnel broke down (which happened in about 40 per cent of the operations), the substances were injected under the skin or, in the case of the nonsaponifiable fraction, painted on the shaved skin. Benzol was painted on the skin or in the tunnel of 26 control mice. The injections were given every three weeks and sometimes every two weeks. The nonsaponifiable fraction was applied every week or alternated weekly with benzol, in an effort to check the regrowth of hair.

Of the 800 mice used, 250 died too early. Four hundred were treated with smegma. In 190, smegma was put into the skin tunnel; 122 received subcutaneous injections of smegma; and in 88, the suspension of the nonsaponifiable fraction was painted into the tunnel or on the skin surface. Most of the 150 mice which served as controls were treated with cerumen. The mice were operated upon when one month old. The average age at which treatment was started was 65 days for the smegma-treated mice and 68 days for the controls.

Results

There was no significant difference in the survival rates of treated and control mice up to the 400th day of life: 85 and 88 per cent, respectively, after 200 days; 74 and 80 per cent after 300 days; 65 and 57 per cent after 400 days. After 500 days, 47 per cent of those treated with smegma were alive as compared with 30 per cent of the controls. From the 600th day on, there was a marked difference (26 and 6 per cent, respectively), and, on the 700th day, the survival rates were 12 and 1½ per cent. The survival rate of the mice treated with the nonsaponifiable fraction was higher than that of the mice treated with whole smegma.

Microscopic sections of skin and inner organs were available for 212 of the 400 mice treated with smegma and for 80 of the 150 controls. Lung adenomas were found 15 times in the sections of the treated mice and twice in the controls. For lymphoid tumors and leukemia the figures were 15 and 5. Mammary carcinoma occurred 21 times in 105 treated female mice and 3 times in 40 female controls. The mammary tumors did not appear at an earlier age than is usual in the Paris strain. There were eight other spontaneous tumors, six appearing in the mice treated with smegma (adenocarcinoma of kidney, anal papilloma, two so-called hepatomas, one less differentiated carcinoma of liver, one inguinal rhabdomyosarcoma) and two in the controls (hepatoma, perianal subcutaneous spindle-cell sarcoma).

No tumor was found at the site of treatment in any control mouse. The one subcutaneous spindle-cell sarcoma found was situated far away from the site of treatment. *Tumors occurring in smegma-treated mice at the site of treatment included:* four papillary warts, two hornifying squamous-cell carcinomas (one with metastases), one undifferentiated skin carcinoma, and one spindle-cell sarcoma with metastases. Three of the papillary warts were cytologically regular; the fourth, in places, was carcinoma-like. The intrathoracic metastases of one carcinoma contained horn pearls.

There was much variation in the time interval between the starting of treatment and the first evidence of tumor: 36, 82, 122, and 247 days for the warts; 130 days for the sarcoma; 220 and 423 days for the carcinomas. (In the case of one carcinoma the figure is not available.) Thirty-six days is a short but not impossible interval.

The percentage of positive cases was about the same, whether whole smegma (6 in 190) or the nonsaponifiable fraction (3 in 88) was used. Of the four warts obtained, three followed the smegma treatment; of the four truly malignant tumors, two arose after use of whole smegma and two after the nonsaponifiable fraction. No tumors developed at the site of subcutaneous injection of smegma in 122 mice.

Discussion

Considering the nature of the substance used, no strong carcinogenic action (high percentage of positive results) was expected. That any tumors resulted from treating a skin surface with a product of skin epithelium or skin glands might be surprising. At the site of application of a similar substance (cerumen) in 150 mice, no tumors were found.

Among other differences between the smegma-treated mice and the controls, the higher incidence of mammary cancer is noticeable. This point cannot be stressed, however, because of the small number of female controls.

The difference in the incidence of lung adenoma is somewhat suggestive. One may perhaps assume, in the light of the work done by Shimkin and Leiter and by Shimkin and Lorenz (*3*), that the mice absorbed out of the smegma a substance of a certain tumor-forming power.

There is nothing to indicate the possible nature of the supposed carcinogenic factor in smegma. It can hardly be just a result of decomposition in stagnating skin products. If this were the case, one should expect cancers in, for example, the umbilicus of obese people or in dermoid and epidermoid cysts.

The chemical composition of smegma has been studied very little and not recently. For decades histologists have disagreed on the question of whether the glans and the inner layer of prepuce contain sebaceous glands and in what numbers. According to Stieve (*4*), only occasional glands occur, and these play no part in the formation of smegma which, in his opinion, is a product of skin desquamation.

Provided our results can be duplicated and improved, this may be the first experimental production of cancer by external application of an external product of the animal body. There is, however, no way of knowing how far the skin epithelium was intact at the time the tumors developed.

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