A Consideration of the Mechanism by Which Squamous-Cell Carcinomatoid Tumors in the Chicken Spontaneously Regress*

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Rigdon and Brashear (9) in 1954 reported the development of squamous-cell carcinomas in the skin of chickens following the local application of methylcholanthrene. Several investigators have objected to the terminology used in designating this lesion, since it spontaneously regressed and no metastases occurred. Cancer research investigators have declined to label a neoplasm as malignant unless there are metastases or unless it can be transplanted. These characteristics, of course, have proved very useful as "ground rules"; the future may establish whether these concepts are correct or not. An increasing number of malignant tumors which regress spontaneously is being found in man and animal (1, 3, 4, 7, 8, 11). The morphological characteristics of these ulcers in the chicken, with squamous epithelial cells deeply infiltrating the underlying stroma, frequently with intercellular bridges and epithelial pearls, have served as a stimulus for us to evaluate critically the mechanism by which epithelial cells proliferate, infiltrate the underlying stroma, and metastasize. The failure of such lesions to metastasize in the chicken may be related to the absence of a lymphatic system, such as that present in man.

Recently we have been interested in the histologic features of these regressing tumors in the chicken and the effect of cortisone on them. These characteristics are now reported.

MATERIALS AND METHODS

The technics used to produce these squamous-cell carcinomatoid tumors have been reported (9).

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1 H. P. Rusch, personal communication, 1955.

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The 0.25 per cent solution of methylcholanthrene in acetone was applied to the skin of the body beneath the right wing and the undersurface of this wing. Four chickens were first treated with methylcholanthrene when 4 days of age, six when 35 days of age, two when 38, and five when 77 days of age, as shown in Table 1. Two-tenths of a ml. of the acetone solution of methylcholanthrene was used on the younger chickens and 1 ml. on the older birds. The quantity was gradually increased from the lower to higher level as the birds increased in size. If ulcerations occurred during the time the methylcholanthrene was being applied, the treatment was discontinued for a few days while the lesions healed. The applications of the methylcholanthrene were applied to some chickens 6 days/week for 30 days and to the others 5 days/week, for a total of 30 applications. The feathers were plucked once/week on chickens 729, 731, 734, 741, and 748, beginning after the last application of methylcholanthrene and thereafter during the remainder of the experiment. The feathers were never plucked from chickens 782, 10380, 10283, 10285, 10288, 10294, and 10297. The feathers were plucked from the treated areas of skin on chickens 10876, 10885, 10896, 10897, and 1097 during the time methylcholanthrene was applied and thereafter until the birds were sacrificed. The five White Leghorns and the one Rhode Island Red chicken were obtained when 1 day of age from a commercial hatchery. Eleven single-combed White Leghorn chickens were obtained when 34 days of age from the Poultry Department of Texas Agricultural and Mechanical College. Table 1 gives the age and sex of each chicken, the experimental day (time from the first application of methylcholanthrene) when the tumor was either removed or a biopsy was taken, and the time that cortisone was first given.

Cortisone acetate, 25 mg/ml/bird, was given intramuscularly to seven chickens (Table 1). The injections were made daily except on Saturdays.
and Sundays. Many photographs were made to record the progression and regression of the lesions.

The pathologic specimens were fixed in a 4.0 per cent solution of formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin. Selected sections were stained by the following techniques: Mallory's aniline blue, Masson's trichrome stain, and Wilder's reticulum stain. Only histologically proved squamous-cell carcinomatoid tumors are included in this study, in which 44 occurred in seventeen different chickens. The largest number of tumors observed in any one chicken (10876) was eight.

RESULTS

The first squamous-cell carcinomatoid tumor was observed on the 23d experimental day in chicken 741. The longest interval between the first application of methylcholanthrene and the development of this tumor was 128 days (chicken 12287). The time of the appearance of the 44 tumors, referable to the time of the first application of methylcholanthrene, was more or less uniformly distributed between the above two extremes. The lesions when first observed were either nodules or ulcers, usually about 2 mm. in diameter (Fig. 2). The nodules subsequently ulcerated.

| TABLE I |

<table>
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<th>AGE OF CHICKENS WHEN TREATED WITH METHYLCHOLANTHRENE AND TIME WHEN TUMORS WERE REMOVED</th>
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* WL, White Leghorns; RI, Rhode Island Reds; A&M, single-combed White Leghorn.
† 0.25 per cent solution of methylcholanthrene in acetone.

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maximum size that these tumors attained varied from 8 to 19 mm. The base of the ulcers was granular. The periphery was hyperplastic, and the edges undermined, especially in those chickens given cortisone (Fig. 1). The crater of the lesion rapidly filled with necrotic material, which could be easily removed with forceps. This feature was most conspicuous in the cortisone-treated chickens. Healing seemed to progress rapidly from both the base and the periphery; a typical scar resulted.

There was some variation in the histologic appearance of the carcinomatoid lesions that developed in the different chickens and also within the same bird. The lesion was always of the squamous-cell type. Intercellular bridges were conspicuous in some, and others showed typical epithelial pearls (Fig. 9). The extent of infiltration of tumor cells into the underlying stroma varied. Polymorphonuclear leukocytes usually filled the crater of the ulcerated lesion. Some of the lesions showed a diffuse infiltration of the neoplastic tissue by leukocytes. Leukocytes, mononuclear cells, and groups of lymphocytes were found routinely in the dermis about these lesions. Polymorphonuclear leukocytes frequently were restricted to the periphery of the tumor growth (Fig. 8). In such lesions there was extensive degeneration of the squamous epithelial cells at the periphery of the tumor (Figures 6 and 8). The nuclei of the cells were pyknotic, and the cytoplasm stained deeply with eosin (Figs. 7 and 8). Many of the cells were markedly shrunk, and others were fragmented. The degree of degeneration varied in the different lesions, both within the same chicken and in different birds. Some of these cellular changes were present in the younger tumors; however, the older lesions showed the more extensive degenerative changes.

The second histologic feature of interest in these regressing tumors occurred in the reticulum. There was a marked increase in the reticulum within the area of the squamous cells when compared with the surrounding dermis (Fig. 5). The largest amount of reticulum seemed to be located at the margin of the tumor growth in the areas where the epithelial cells showed the most extensive degenerative changes. Here the leukocytes were also most conspicuous. The reticulum usually stained black, while the collagen in the dermis stained light brown or a golden brown color. This increase in reticulum resulted from the presence of thin strands, although there were some wide bands of similar staining material.

Ultimately, the squamous cells within the tumor completely degenerated, the leukocytes phagocytized the debris, collagen filled in the area, and epithelial cells from the epidermis at the margins of the ulcer extended over the denuded surface to form a typical scar.

Cortisone was given to seven chickens. Either a biopsy was taken or the lesion was completely removed before the cortisone was first given. Several of the chickens developed one or more squamous-cell tumors after the cortisone injections were begun. Macroscopically, several of these tumors appeared to grow at a faster rate than that of similar tumors in noncortisone-treated birds. This rapid growth of the tumors is illustrated in chicken 10376 (Fig. 1). The lesion measured 4 mm. when the first biopsy was removed on the 82d experimental day. At this time cortisone was first injected. Nine days later the lesion measured 8 mm., and the second biopsy was removed. The margin of these ulcers appeared to be higher in the cortisone-treated than in the noncortisone-treated birds.

Microscopically there was a variation in the type of epithelial growth in the cortisone-treated chickens like that observed in the noncortisone-treated birds (Figs. 9 and 10). There were no changes observed within the epithelial cells of the cortisone-treated chickens to indicate a difference in the rate of growth from that observed in the noncortisone-treated chicken. The same type of degeneration was also present in the epithelial cells and about the periphery of the lesion. If there was a quantitative difference in the number of degenerating cells in the cortisone and noncortisone-treated chickens, we did not observe it. The amount of degeneration within the tumor also varied in the noncortisone-treated chickens.

The most conspicuous difference observed between the cortisone-treated and nontreated chickens was in relation to the leukocytic and lymphatic reaction about the periphery of these tumors. The number of these cells was usually smaller in the cortisone-treated birds (Figs. 3, 9, and 10). Apparently the number of leukocytes and lymphocytes decreased after the first injection of cortisone. This is well illustrated in chicken 10376. Figure 3 shows the lesion before cortisone was given (81st experimental day), and Figures 9 and 10 show the lesion 21 days later. Chickens that developed tumors after cortisone administration usually had fewer inflammatory cells around the growth than birds that did not receive this steroid.

**DISCUSSION**

Squamous-cell ulcerated lesions that develop in the skin of chickens following the local application of methylcholanthrene spontaneously regress (9).
This regression is associated with, and apparently results from, the degeneration that occurs in the epithelial cells. The cells at the periphery of these tumors show the most extensive degeneration. Accompanying these degenerative changes in the epithelial cell is a local increase in both leukocytes and mononuclear cells. There is also a proliferation of reticulum in the margin of these tumors. This degenerative process, beginning at the periphery, is a progressive one, ultimately resulting in a complete regression of the ulcerative growth.

Cortisone acetate, as used in this experiment, did not significantly influence this process of regression. The same type of change was present in the epithelial cells in the cortisone- and non-cortisone-treated chickens. Some of the tumors may have grown more rapidly in the cortisone-treated birds; however, this variation in the rate of growth did not appear to be significant. Cortisone did decrease the cellular reaction about these tumors. Such an effect might have been anticipated, since many investigators have observed a decrease in the cellular reaction about inflammatory foci when cortisone was given (5, 6, 10, 13). The degenerative changes that occur in the lymphocytes within the dermis of these chickens treated with cortisone will be considered in detail in a separate study now being made.

Different investigators have observed different effects of cortisone on neoplastic growths. Baserga and Shubik (2) concluded that "cortisone has a general inhibitory action that is slight and temporary on transplantable tumors of the lymphoid series and no action on transplantable tumors of an epithelial origin." Sulzberger et al. (12) observed in mice given cortisone and external applications of methylcholanthrene an increase in the incidence of epidermal tumors. The rate of growth of a transplanted mouse mammary carcinoma was reduced by very large doses of cortisone.

The morphologic changes that are present in these regressing squamous-cell carcinomatoid tumors have stimulated some theoretical possibilities for further investigation. Obviously, a degenerative process occurs in the epithelial cells and progresses until all the tumor cells have disappeared. This degenerative change is most pronounced and seems to begin at the periphery of the growth. Leukocytes infiltrate the area to phagocytize the debris. The role of the lymphocytes in these regressing neoplasms is not clearly appreciated by us at this time. If they do play some role in an immune mechanism in the chicken, it is not obvious to us now. Cortisone does reduce the number of lymphocytes about these neoplasms in the chicken, but the degeneration within the cells continues until the lesion completely disappears. Reticulin is present in large amounts at the periphery. The question naturally arises—where does it come from? Costero has suggested that reticulin may be laid down on pre-existing reticulin fibrils. The stimulus for such proliferation according to Costero may result from cellular degeneration.

SUMMARY

Squamous-cell carcinomatoid tumors occurred in the skin of chickens previously treated with methylcholanthrene. These neoplasms spontaneously regressed until they completely disappeared. This regression was accompanied by degeneration of the epithelial cells, which is most marked at the periphery of the growth. Leukocytes and mononuclear cells infiltrated the periphery of these tumors, apparently to phagocytize the cellular debris. A proliferation of reticulin also occurred at the periphery of these regressing neoplasms. Cortisone acetate resulted in a marked depletion of leukocytes and mononuclear cells about these tumors; however, degeneration of the epithelial cells was essentially the same in cortisone- and non-cortisone-treated chickens.

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1 I. Costero, personal communication, 1955.
Fig. 1.—Chicken 10887. A typical squamous-cell carcinoma in the skin followed the local application of a 0.25 per cent solution of methylcholanthrene in acetone. This lesion was a papule 2 mm. in diameter on the 88th experimental day, at which time a biopsy was taken. The lesion progressed in size, and 21 days later it measured 8 mm., at which time this photograph was made. Subsequently, the lesion regressed, and it was completely absent on the 124th experimental day.

Fig. 2.—Chicken 10876. Several nodules 1–3 mm. in diameter appeared in the skin at approximately the same time. This figure shows three of these squamous-cell lesions. These tumors were first observed on the 101st experimental day, and the bird was sacrificed on the following day. Cortisone had been given for 21 days preceding the time that these lesions were photographed.

Fig. 3.—Chicken 10876. An ulcer 4 mm. in diameter occurred in the methylcholanthrene-treated area of skin. A biopsy taken on the 81st experimental day showed it to be a squamous-cell tumor. Note the leukocytes at the periphery. The tumor continued to grow, and a second biopsy (Fig. 4) was taken 21 days later. H & E. X240.

Fig. 4.—Chicken 10876. A portion of the same tumor as shown in Figure 3, photographed 21 days later. There is extensive degeneration of the tumor cells. Cortisone was given for 21 days before the bird was sacrificed. H & E. X240.

Fig. 5.—Chicken 781. Reticulin increased in the squamous-cell tumors as the lesions regressed. The increase in reticulin was first observed at the periphery of this neoplasm. It accompanied the progressive degeneration of the tumor cells and the infiltration of leukocytes into the margin of the tumor. Wilder’s reticulum stain. X817.

Fig. 6.—Chicken 10876. Same tumor as shown in Figure 4. This shows the degenerative changes in the epithelial cells at the periphery of this tumor. There are essentially no leukocytes infiltrating this area because the chicken had been given cortisone for the preceding 21 days. H & E. X240.
Figs. 7, 8.—Chicken 10876. Same lesion as shown in Figure 4. This lesion was removed on the 102d experimental day. Figure 7 shows well preserved epithelial cells in this tumor, while Figure 8 shows degenerative changes in the cells at the periphery of the same tumor. This is a typical change whether or not cortisone is given. H & E. ×1425.

Figs. 9, 10.—Chicken 10876. Two squamous-cell tumors on the 102d experimental day. Note the variation in the histologic pattern and the few inflammatory cells at the periphery of these tumors. Figure 10 shows the tumor cells at the periphery to be pyknotic when compared with the tumor cells deep within the neoplasm. H & E. ×150.
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