Effect of Cortisone on Mast Cell Tumors (Mastocytoma) of the Dog. (19475)

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Included among the many effects of the adrenal cortical hormones on the organism is their ability to produce a marked reduction in the number of normal tissue mast cells. This has been observed with the use of ACTH in man(1) and with the use of cortisone in rats(2). Spontaneous tumors composed of neoplastic mast cells are of frequent occurrence in the cutaneous tissues of the dog(3). These growths may be benign or malignant, solitary or multiple, and range in size from nodules several centimeters in diameter to those weighing 300 or more g. They may be of unicentric or multicentric origin, and metastases are not uncommon in the regional lymph nodes, spleen, and liver in the malignant varieties. The microscopic structure consists of atypical histogenous mast cells whose cytoplasm contains metachromatic basophilic granules. The cells exhibit all gradations of cellular maturity and immaturity with fine and coarse granules which often can be correlated with the degree of malignancy of the various neoplasms. In the skin tumors the mast cells form nodular or diffuse sheet-like collections that heavily infiltrate the corium and subcutaneous tissue.

In view of the known effect of cortisone in causing a conspicuous reduction in the number of normal tissue mast cells, this agent was employed in the treatment of a dog with malignant multiple mast cell tumors.

Material and methods. The animal was an 8-year-old male white Spitz dog brought to my animal hospital for treatment. Three subepithelial tumors, 2 in the left shoulder region and one in the right upper lip, had been observed for a period of several weeks. The nodules ranged from 1.3 to 2.2 cm in diameter and were elevated from 4 to 6 mm above the surrounding skin. Under local anesthesia the nodules were surgically excised on 9/5/51. Examination of the animal on 12/1/51 revealed recurrence as manifested by the presence of 8 skin nodules in the left shoulder region and 7 skin nodules on the neck and back. These were similar in size and appearance to those previously removed. On 1/2/52 50 mg of cortisone (Cortone, Merck)* was administered intramuscularly twice daily and continued at this dose rate until 1/11/52. A nodule was excised on 1/5/52 and the remains of a nodule was excised on 1/11/52. Following each surgical procedure, the tissue was fixed in 10% formaldehyde solution and sections were stained with hematoxylin and eosin and Dominici's stain(4). Imprints were also made and stained with Wright-Giemsa stain.

Observations and results. The original skin tumors excised on 9/5/51 were a uniform pale tan color on section and not encapsulated. Microscopically the entire subepithelial tissue was completely replaced with dense diffuse masses of neoplastic mast cells and scattered eosinophils and lymphocytes. In sections the tumor cells exhibited moderate anaplasia and occasional mitotic figures were noted. The toluidine blue component of Dominici's stain revealed that the cellular cytoplasm contained relatively fine uniform sized metachromatic granules in ample numbers. The granules did not stain with hematoxylin as is not uncommon in more mature tumors with coarse granules. In the imprint preparations the fine discrete nature of the granules was more readily discernible and the cellular cytoplasm was seen to be intact and free from vacuolar and other degenerative changes (Fig. 1).

The gross appearance of all 15 recurrent nodules as observed on 1/5/52 after 3 days treatment with cortisone was identical with the same nodules before treatment except for a reduction of about 2/3 the pretreatment dimensions. This change occurred rather suddenly and was first noticeable on 1/5/52. Microscopic examination of sections of the excised nodule disclosed ample numbers of

* Dr. L. A. Michaud of Merck and Co. kindly supplied the cortisone.

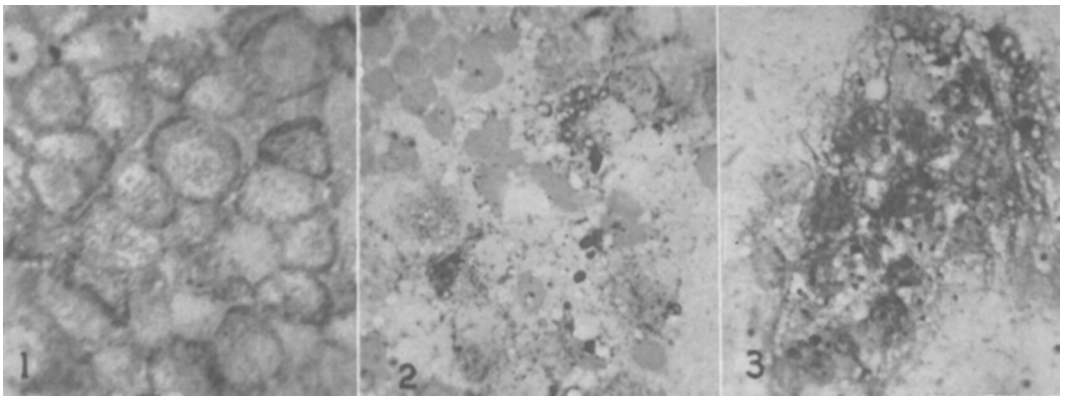

FIG. 1. Imprint of tumor mast cells prior to cortisone treatment showing intact cells with fine granules. Wright-Giemsa. × 600.

FIG. 2. Imprint of tumor mast cells after dog had received 300 mg of cortisone. The cells show vacuolization, granule clumping, granule scattering and disruption of the cellular mem brane. Wright-Giemsa. × 600.

FIG. 3. Imprint of tumor mast cells after dog had received 900 mg of cortisone. Advanced degenerative changes, manifested by marked vacuolization, conglomeration of granules, disappearance of many fine granules and loss of cellular structure, are apparent. Wright-Giemsa. × 600.

mast cells with decrease in the number of eosinophils. Although many cells in the Dominici stained slides showed no alteration in the number, shape, size, and tinctorial characteristics of the granules, a greater number of cells exhibited specific cytoplasmic and granule aberrations. In these altered cells the cytoplasm sometimes contained small and large vacuoles, and the granules were frequently conglomerated to form irregular clumps several or more times larger than the original granules. The conglomerated masses often stained a deep purple-blue in contrast to the reddish-purple coloration of the fine granules. These cytoplasmic changes were especially conspicuous in the imprint preparations, and all gradations between intact tumor cells without degenerative alterations to those showing vacuolization, granule clumping, granule scattering and fraying of the cytoplasmic border were readily apparent (Fig. 2). In both imprints and sections the nuclei were well preserved.

From 1/5 to 1/11/52 the recurrent 14 nodules (one had been excised on 1/5/52) rapidly decreased in size so that by the latter date they had completely disappeared. All that remained was a pale tan coloration of the skin over the previous site of the nodules, which contrasted with the pale pink color of the surrounding normal skin. Microscopic examination of sections of the excised tissue revealed that the mast cells had almost completely disappeared. Practically no cells of normal structure were noted, and those present exhibited various degrees of degenerative change. The cells showed a ballooning of the cytoplasm caused by extensive vacuole formation. The intervacuolar cytoplasm consisted of an irregular fine tracery of sparse numbers of minute and conglomerated aberrant staining metachromatic granules that indistinctly outlined and occasionally occurred within the vacuoles. The cytoplasmic borders were indistinct and frayed. Very few nuclei appeared normal; many had disappeared and others were pyknotic. The eosinophils were also fewer in numbers. The lymphocytes, on the other hand, appeared to be increased; however, this increase was relative inasmuch as the other cellular elements were numerically reduced. The degree of degenerative alterations of the mast cells was particularly noticeable in the imprints (Fig. 3). In these, solitary or groups of mast cells were hardly recognizable as such. Instead, the cytoplasm consisted of a mass of vacuoles in and between which were irregularly distributed varying sized conglomerated granules with few of fine structure. The cellular borders were indis-

tinct, frayed and hazy. The nuclei were likewise difficult to outline, although they exhibited a better state of preservation than the cytoplasm.

Examination of the animal on 2/21/52 indicated no recurrence of the tumor nodules that had disappeared following cortisone therapy. However, a new nodule 1½ by 0.6 cm had appeared in the right lower lip and the regional lymph nodes were enlarged. Up to the present day, no additional nodules or recurrence of the previous nodules has appeared.

During the course of cortisone therapy the animal showed no unusual symptoms and appeared to be normal. No abnormal bleeding tendency occurred during or after surgical excision of the nodules.

Discussion. Of especial interest is the complete macroscopic regression of the mast cell tumors following cortisone treatment. This observation was confirmed microscopically inasmuch as the mast cells had almost completely disappeared and that the relatively few remaining cells were all in various stages of degeneration. Such involution has never been noted to occur spontaneously in personal observation of over 100 canine mast cell tumors; instead, the usual sequence in malignant types is a progressive intensification of the neoplastic process with terminal death. Although it would be premature to suggest that the disappearance of the tumors is permanent, 61 days have thus far elapsed up to the present writing without recurrence. The new nodule, presumably a mast cell tumor, that appeared after cessation of cortisone indicates that the neoplastic process has not been arrested. Further observations are being conducted and if additional tumors occur further cortisone treatment will be instituted.

The sequence of changes noted in the tumor mast cells are similar to those seen in normal mast cells following cortisone therapy. The cytoplasmic vacuolization, conglomeration and altered staining properties of the metachromatic granules, disappearance of many fine granules, fraying and disruption of the cellular membrane, cellular destruction and granule scattering seen in sections and imprints are essentially the same as that observed in aging tumor mast cells cultivated *in vitro*(5). The degenerative changes of the tumor mast cells that eventually lead to their death, whether due to aging or hormonal substances, are thus alike in type and degree. Duplications of these cellular alterations have also been noted in normal tissue mast cells *in situ* without cortisone therapy, but have usually been thought of as artifacts(6).

Since it has been determined(7) that the canine mast cell tumor may contain almost 50 times as much heparin as has been obtained from the richest normal source of the most active form of heparin, one might expect that the treated animal should exhibit interference with the coagulation of its blood. Although the circulating heparin-like substances, which rapidly rise in man after the administration of cortisone(8,9), were not determined, no unusual bleeding tendency existed as the surgical procedures were not accompanied by excessive hemorrhage. The explanation for the normal clotting time in the treated animal might be that insufficient amounts of heparin were liberated to have an anticoagulant effect or that inactivation or destruction of heparin followed its release from the cells. This anomaly, the presence of large numbers of mast cells and a normal state of blood coagulation, is not peculiar to these experiments where the animal was treated with cortisone; similar normal clotting time of the blood has been repeatedly observed during surgical manipulation and removal of mast cell tumors in dogs who have not received cortisone.

Although the therapeutic effectiveness of cortisone in all malignant mast cell tumors may be speculative, the results obtained in the cited animal are encouraging and warrant further investigation.

Summary. The effect of cortisone was studied in a dog with malignant multiple mast cell tumors (mastocytoma). A total of 900 mg was given intramuscularly at the rate of 100 mg daily. The tumors rapidly regressed in size so that by the 9th day of treatment they had completely disappeared macroscopically. Microscopically the neoplastic mast cells showed cytoplasmic vacuolization, conglomeration and altered staining reaction of the metachromatic basophilic granules, disap-

pearance of many fine granules, fraying and disruption of the cellular membrane, granule scattering, and, finally, cellular destruction and disappearance. The encouraging results obtained in the treated animal warrant further investigation of cortisone in the treatment of dogs with malignant mast cell tumors.

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Urinary Excretion of Hydrazine Derivatives of Isonicotinic Acid in Normal Humans.* (19476)

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Recent reports have shown that isonicotinyl hydrazide (Rimifon) and 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) are effective antitubercular agents in mice(1,2), guinea pigs and rabbits(3), monkeys(4), and humans(5-8). We have described an assay method and determined levels of Rimifon and Marsilid in blood plasma after oral dosage to humans, dogs and mice(9). In this method, the hydrazides are converted by permanganate treatment to isonicotinic acid, which is determined colorimetrically by the reaction with 10% cyanogen bromide and ammonia used by Mueller and Fox(10) for nicotinic acid assay. Preformed isonicotinic acid is determined by assay *before* permanganate oxidation. In the experiments described below, the cyanogen bromide-ammonia reaction has been applied to untreated and permanganate-treated urines to study the products and rate of excretion by normal humans of oral doses of Rimifon and Marsilid.

Experimental. Nine normal subjects (laboratory personnel), 2 females and 7 males ranging in age from 26 to 43 and in weight from 56 to 82 kg, participated in the urine excretion tests. To determine the color produced by normal urines in the cyanogen bromide reaction, pre-dosage control urines were collected on each of 2 successive days. Collections were started at 9 A.M. and made at intervals ending after 1, 2, 3, 8 and 24 hours. On the test days, a single oral dose of Rimifon or Marsilid was given at 9 A.M. and urine collections made at the same intervals for 24 hours. A pooled sample was collected by each subject for the second 24 hours after dose. Diets were not controlled, but the subjects were instructed to follow as nearly as possible the same diet on each of the collection days. In the first test, the subjects were divided into 2 groups and given 250 mg of Marsilid or 125 mg of Rimifon. These doses were reversed in all subjects in the second test; in the third test, all subjects received 192 mg of Rimifon, which is equivalent on a molar basis to 250 mg of Marsilid. At least one week was allowed between successive doses. Both control and test urines were assayed directly and also after permanganate treatment, using the reaction with 10% cyanogen bromide in ammonia buffer as previously described(9). For direct assay, 1 ml of urine plus 5 ml of buffered ammonia reagent were

* Roche Publication No. 306.