Histological Changes in Adrenal Glands of Tumor-Bearing Mice Injected with *Serratia marcescens* Polysaccharide Alone and in Combination with Adrenal Cortical Extract

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As has been reported previously by Diller and Shear (6) and by Diller (4), tumor cells of mice injected with a polysaccharide derived by Shear (9) from *Serratia marcescens* culture filtrate show degenerative responses within 6 hours. At a dose level that produced rapid sloughing of sarcoma 37 in about 1 out of every 4 mice, an equal number of mice usually succumbed to treatment. When adrenal cortical extract was administered simultaneously, the toxic effect of the polysaccharide was measureably reduced (2). This did not interfere with tumor sloughing. Adrenal extracts alone are also capable of producing degenerative changes in sarcoma 37 and sloughing of the tumor, particularly in female mice (1, 5).

Injection of *S. marcescens* polysaccharide in tumor-necrotizing dosage into mice bearing sarcoma 37 was followed in 5 or 6 hours by hemorrhage, not only in the tumors but also in the adrenal glands. The adrenal hemorrhage was accompanied in these glands by destructive cellular changes which were most severe in the medullary region. Milder histological changes occurred in the adrenal glands of mice given polysaccharide plus adrenal cortical extract. The changes are described below.

**MATERIALS AND METHODS**

We selected as tumor hosts, young albino mice, 3 to 4 months of age, both males and females, reared in our laboratory from Carworth Farms stocks. Most of the studies were made on animals implanted with sarcoma 37, but some experiments were performed also on mice with methylcholanthrene-induced tumors and on C3H mice bearing spontaneous mammary tumors. In the experiments with sarcoma 37, mice bearing 7 day implants were divided into 3 groups of 20 animals each, one of which received polysaccharide (Sample P-3); the second, Upjohn adrenal cortical extract; and the third, the two substances in combination. The amount of polysaccharide administered (0.02 mgm. intraperitoneally) was sufficient to cause extensive destruction of tumor cells in 3 to 5 tumors of every 10, while causing the death of 2 or 3 of the mice within the first 24 hours. Mice of the group to be treated by adrenal hormone were given 0.5 cc. of Upjohn aqueous extract in 5 intraperitoneal doses of 0.1 cc. each 1 hour apart. The first dose was administered simultaneously with the polysaccharide injection. The control group received cortical extract only (0.5 cc. in 5 doses at hourly intervals). This experiment was later repeated with 15 animals in each of the 3 groups, using paired mice and female litter mates 3½ months of age. All of these animals were killed for histological study.

At the end of 6 hours, at which time polysaccharide alone produced gross hemorrhage in the tumor, 5 animals from each group were killed and the tumors as well as the adrenal glands were dissected for microscopic study. At 24 to 25 hours after initial treatment 5 more animals from each group were similarly sacrificed. The remainder of the animals were allowed to live for survival studies and tumor measurement (5).

The tumors were prepared for microscopic study by means of the acetic orcein technic; the adrenal glands by routine paraffin technics, stained with hemalum-eosin.

**OBSERVATIONS**

Six hours after injection of 0.02 mgm. of polysaccharide the adrenal glands of both male and female tumor-bearing mice showed definite cell changes which were most marked in the medullary region. Nuclei were greatly shrunken and the nuclear membranes crenulated (Figs. 1 and 2). The medullary tissue was disrupted and large open spaces sometimes appeared between the cells. There was infiltration of white blood cells in both cortex and medulla, particularly in the border region between the two primary zones, as well as along the radial septa of the cortex. Marked hemorrhage occurred
in some instances in the medulla. The extra-capsular connective tissue area showed destruction or degeneration, with some hemorrhage; the zona glomerulosa decreased in size and there was pyknosis and crenation of nuclei; the zona fasciculata increased peripherally at the expense of the glomerulosa; and the zona reticularis showed a tremendous increase in number of red blood cells at the line of demarcation between the cortex and the medulla. Nevertheless, there was less nuclear change in the cortex than in the medulla, where nuclear pyknosis and distortion, as well as cytoplasmic shrinkage and vacuolization, were common and accompanied by vascular degeneration. In some cases the entire tissue exhibited an unusual affinity for eosin.

Twenty-four hours after initial injection of 0.02 mgm. of polysaccharide, nuclear changes were not much greater than those noted at 6 hours, though the medulla usually exhibited great loss of nuclear chromatin, and cytoplasmic detail was indefinite because of cloudy staining. Refractile crystalline bodies were often present.

When the amount of polysaccharide was increased to 0.1 mgm. which produced a higher percentage of deaths, the adrenal glands from animals with markedly hemorrhagic tumors usually showed still greater histological changes, as illustrated in Fig. 2, A to D. Fig. 2, C and D show the cortex and medulla, respectively, from the left adrenal gland of a moribund animal 25 hours after intraperitoneal injection of 0.1 mgm. of polysaccharide. Destruction of the medullary region (Fig. 2 D) is here practically complete, the nuclear substance degenerating and the entire medullary area karyorrhexic. The cortex also is damaged, as shown in Fig. 2, C.

Unexpectedly, striking changes were found also in the adrenal glands of the control group of tumor-bearing mice injected with Upjohn extract only, though they were not as extensive in most cases as those arising from polysaccharide damage. An example of the extreme effects obtained appears in Fig. 3, C and D, which is from an adrenal gland of a 3 month old female mouse injected with 0.5 cc. of Upjohn adrenal cortical extract over a 5 hour period. Here there is extensive hemorrhage in both medulla and cortex. The nuclei of the medulla were cloudy and reddish while those of the cortex were shrunken, distorted and apparently degenerated. The cytoplasm of the medullary cells was foamy and full of vacuoles. Connective tissue septa had broken down completely in the cortical region and nuclei were pycnotic and shrunken. The adrenal glands of female mice injected with cortical extract showed greater damage than those of males in most instances, as will be discussed later.

When polysaccharide and adrenal cortical extract were injected simultaneously, a balancing effect was apparently obtained. (See Fig. 4, A and B). Although there were some changes in both medulla and cortex, they were not as extensive as those which were produced by either substance alone. In the medulla the changes encountered were swelling of the cytoplasm, separation of cells, and nuclear distortion. Six hours after combined treatment, the zona glomerulosa and zona fasciculata were relatively normal but contained many polymorphonuclear leukocytes, while the zona reticularis was sometimes severely damaged and heavily infiltrated by white blood cells. Occasionally the latter zone was found to be greatly enlarged by crowding of red blood cells with which the entire region was usually engorged.

Twenty-five hours after simultaneous treatment with cortical extract and polysaccharide, degenerative changes in the adrenal glands had disappeared (Fig. 4, C and D). Whereas the capsule still showed vascular degenerative changes, the three zones of the cortex were practically normal (Fig. 5, D). The medulla here shows residual hemorrhage; however, the nuclear volumes appear to be not only reconstituted but even augmented, as if by uptake of fluid. Histological preparations stain sharply with nuclear dyes and the chromatin is concentrated within the medullary nuclei in definite, deeply stained granules. Therefore the histological pattern of adrenal gland damage agrees rather closely with the physiological results obtained (2). Though polysaccharide in tumor-necrotizing doses may cause notable destruction of adrenal tissue, toxic effects are somewhat lessened by simultaneous administration of adrenal cortical extract.

In an attempt to determine whether or not adrenal response to these toxic substances was part of a general alarm reaction in which the pituitary gland might conceivably participate, histological studies were made also of other endocrine glands, including the pituitary, the thymus and the thyroid. No distinct response could be detected in any of these glands.

In the case of the thymus and the thyroid, hemorrhage occurred in the sustentacular connective tissue supporting the glands following polysaccharide injection, but no effect whatever could be observed in either tumorous or non-tumorous mice receiving 0.5 cc. of adrenal extract only. Following polysaccharide
injection (0.02 mgm.) the pituitary showed alterations in staining of the chromophile cells. The eosinophilic cells became stained a vivid pink, while their granular character was lost in what appeared to be complete lysis of the cytoplasm. No changes were observed in these cells following injection of adrenal extract.

No mention has been made above of the effects of these substances on adrenal glands of mice which were not tumor-bearing. In some cases no damage at all could be detected in non-tumor-bearing mice, but where found the changes were, in every instance, less extensive. Compare, for instance, the difference between adrenal glands of a non-tumor-bearing mouse injected with 0.02 mgm. of polysaccharide (Fig. 1, A and B) with that produced in the glands of a tumor-bearing mouse of the same age and sex, injected with the same amount of polysaccharide, each excised at 6 hours after injection (Fig. 1, C and D). Even more striking is the difference between tumor-bearing and non-tumor-bearing mice injected with cortical extract alone (Fig. 3). In the case of the non-tumorous mouse there is pycnosis of the cortical cells and hemorrhage in the medulla; in the tumor-bearing mouse subjected to the same amount of cortical hormone for the same number of hours, extensive histological changes have taken place in both medulla and cortex. As noted above, these differences are particularly striking in female mice injected with cortical hormone, which agrees with our finding that a much higher degree of tumor destruction is attained in females than in males bearing sarcoma 37 following the injection of adrenal extract (5).

Further evidence in support of this interpretation is furnished by the responses of adrenal glands of C3H mice bearing spontaneous mammary carcinoma. Fig. 6 shows examples from a small series of such mice (8 in each group) injected with polysaccharide and with adrenal extract. This particular tumor does not respond with any degree of cellular destruction to either polysaccharide or cortical hormone treatment and the adrenal damage also appears to be negligible.

In Fig. 6, A and B, are the cortex and medulla of an untreated female bearing multiple mammary tumors of different sizes. C and D shows the cortex and medulla of another female mouse with one well developed mammary tumor, injected with 0.02 mgm. of polysaccharide. Shrinkage of cortical nuclei as well as of whole cells has occurred, and cell boundaries, so definite in the adrenal cortex of the healthy C3H mouse, are almost obliterated. The medulla also shows nuclear shrinkage and crenation as well as hemorrhage. These changes are much less severe than those appearing in albino mice with transplanted tumors, subjected to the same amount of polysaccharide (Fig. 1, A and B) but tumor-necrosis in the case of these primary carcinomas is also very slight, which lends further support to our assumption that polysaccharide alone is not responsible for all the adrenal damage, but that the reaction is greatly enhanced by tumor breakdown. E and F illustrate the cortex and medulla from a female C3H mouse with one large mammary tumor treated with cortical extract only, and in which there was little tumor cell destruction.

DISCUSSION

The changes described in the foregoing pages, at least as far as polysaccharide is concerned, may of course be merely one manifestation of the alarm reaction described by Selye (8). In fact, he has listed bacterial toxins as one class of substances which can elicit such responses. Olitski, Avinery, and Koch (7) conducted a series of experiments with guinea pigs injected with toxins from many different bacterial groups and found that 24 to 48 hours after injection, or when animals died as a consequence of injection, the adrenal glands became grossly enlarged and hemorrhagic. Microscopically, the deeper layers of the cortex were hyperemic. Such reactions were produced only by certain groups of microorganisms. S. dysenteriae and some other gram-negative bacteria produced in the guinea pig, within 24 hours post-injection, greatly enlarged adrenal glands, which were of a very dark red color. Usually these phenomena were accompanied by hypothermy, hemorrhage and leukopenia.

This agrees very well with what we have observed in tumor-bearing mice. Severe histological damage in the adrenals was encountered by us only in animals in which there was tumor necrosis and tissue breakdown.

When adrenal cortical extract is administered simultaneously with the polysaccharide, the adrenal picture, as previously noted, is much more nearly normal, despite the fact that tumors degenerate to about the same extent under the combined treatment. However, tumor cell destruction proceeds much more slowly, the maximum effect not being reached for 24 hours, instead of at 6 hours as with polysaccharide alone.

Apropos of the protective action of the adrenal extract, is Selye's observation (8, page 181) "that in animals overdosed with corticoids, the response
of the adrenal cortex to alarming stimuli is abnormal."

No explanation is proffered as to the uniformly more drastic response of the medulla than of the cortex, especially when adrenal cortical extract alone is injected. Why should even an overdose of adrenal corticoids cause degeneration of the medulla as severe as, for instance, that shown in Fig. 3, D?

According to Selye the chromaffin system "has been shown to play an important rôle only during the first few minutes of the alarm reaction—the discharge of the chromaffin granules which occurs during the shock phase may be secondary to dehydration."

The photographs in Fig. 3 indicate that in the non-tumor-bearing mouse 0.5 cc. of adrenal cortical extract is not an effective agent in eliciting the histological changes in the adrenals which are characteristic of the alarm reaction (Fig. 3, A and B, in which the histological appearance is normal). On the other hand, histological changes characteristic of the alarm reaction do occur in the adrenal glands following the injection of this amount of extract into mice bearing sarcoma 37 (Fig. 3, C and D). Since this particular tumor shows marked cellular degeneration following administration of this amount (0.5 cc.) of adrenal extract, it would appear likely that the changes in histological appearance occurring in the adrenals of sarcoma 37 mice following administration of adrenal extract are due to the presence in these mice of tumor tissue disintegrating under the action of the extract rather than (a) to a direct action of the extract on the adrenals of these mice, or (b) to a more generalized change in susceptibility of tumor-bearing mice. Further evidence for this point of view is provided by the fact that female mice bearing sarcoma 37 show more pronounced histological damage in both tumor and adrenals, following administration of adrenal extract, than do male mice bearing sarcoma 37.

SUMMARY

1. The intraperitoneal injection of Serratia marcescens polysaccharide causes enlargement of and hemorrhage in the adrenal glands of tumor-bearing mice. Histological changes of a degenerative nature also occur and these increase in severity in proportion to the dose.

2. Changes of the same nature are produced in the adrenal glands of host mice when adrenal cortical extract is used to produce necrotization of transplanted tumors.

3. When adrenal cortical extract is employed in combination with polysaccharide, tumor breakdown is delayed and the effect on the adrenal glands is minimized. At 24 hours after simultaneous injection of the two substances, both cortex and medulla appear relatively normal, except for residual hemorrhage.

4. In non-tumor-bearing animals, or in animals with primary tumors which do not respond to agents through tumor degeneration, adrenal damage following polysaccharide, adrenal cortical extract, or the two in combination, is negligible.

REFERENCES


FIG. 1
DESCRIPTION OF FIGURE 2

Fig. 2.—Sections of adrenal glands from tumor-bearing male albino mice injected with 0.1 mgm. of S. marcescens polysaccharide. A and B—cortex and medulla, respectively, 6 hours after injection; C and D—cortex and medulla, respectively, 24 hours after injection. Mag. × 440.
DESCRIPTION OF FIGURE 3

Fig. 3.—Sections of adrenal glands from mice injected with Upjohn adrenal extract. A and B—cortex and medulla, respectively, of a nontumorous mouse. C and D—cortex and medulla of a female tumor-bearing mouse, injected in each case with 5 doses of 0.1 cc. each of Upjohn beef adrenal extract, 6 hours after initial injections. Mag. × 440.
Fig. 3
DESCRIPTION OF FIGURE 4

Fig. 4.—Sections of adrenal glands from albino female mice implanted with sarcoma 37. A and B—cortex and medulla, respectively, of mouse treated simultaneously with S. marcescens polysaccharide and Upjohn beef adrenal extract, 6 hours after initial injection; C and D—cortex and medulla from mouse similarly injected, 24 hours after initial injection. Mag. X 440.
Fig. 4

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DESCRIPTION OF FIGURE 5

FIG. 5.—Sections of adrenal glands, showing response of entire adrenal to polysaccharide, adrenal cortical extract, and the two in combination. A—untreated control. B—6 hours after injection with 0.02 mgm. of polysaccharide. C—6 hours after injection with 0.5 cc. adrenal cortical extract. D—25 hours after combined treatment. Mag. × 80.
DESCRIPTION OF FIGURE 6

Fig. 6.—Adrenal glands from female mice of C3H strain bearing spontaneous mammary tumors. A and B—cortex and medulla from untreated tumor bearing mouse; C and D—cortex and medulla from tumor bearing mouse injected with *S. marcescens* polysaccharide. E and F—cortex and medulla from tumor bearing C3H mouse injected with adrenal cortical extract only. Mag. × 440.
Fig. 6
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