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EFFECTS OF ACTINOMYCIN D ON THE SALIVARY GLANDS OF THE RAT

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SINCE the isolation of actinomycin D from Streptomyces parvallus (1) in 1954 its potent cytostatic effects have been widely recognized and numerous reports have already appeared describing the effects of it and related compounds on embryonic tissues (2-5), various tumors (6-11) and other normal tissues (12-14) Meanwhile, the chemical mechanisms of actinomycin in inducing cytostatic effects have been clarified to a large extent and experiments have proven that the drug can be used as an excellent analytic tool for studies of the biosynthesis of proteins, especially of the role played by the messenger RNA (m-RNA) in the process (15-27). A survey of these articles and others illuminates the following: (1) m-RNA is produced in the nucleus by the DNA-dependent RNA polymerases; (2) m-RNA is important in the maintenance of the functional and structural integrity of polyribosomes which are primarily dependent upon the continued availability of m-RNA, the turnover of which is relatively fast and (3) the production of the m-RNA can be inhibited effectively by actinomycin D which specifically combines with DNA, resulting in the blockade of DNA-dependent RNA polymerase activity.

With respect to the digestive glands synthesizing enzymes for secretion; an insult imposed by the administration of actinomycin D would seriously effect the functioning of the gland which in turn would be reflected in its structure. The present study is aimed at describing the cytological changes observed in the parotid gland of the rat following a sublethal dose of actinomycin D.

Materials and Methods

Twenty-eight adult male Sprague-Dawley rats were used. Of these, twenty-

one each received an intraperitoneal injection of 75 μg actinomycin D dissolved in 0.5 ml of saline. The remaining seven rats served as controls and were injected with the same volume of saline alone. Animals were fed ad libiddum with regular laboratory rat chows. Following injection, three of the experimental and one of the control rats were sacrificed on days 1, 3, 5, 7, 10, 14 and 21. Each rat was weighed at the beginning of the experiment and at the time of sacrifice.

Upon sacrifice the right parotid and submandibular glands were dissected out and weighed. Pieces of the glands were fixed in Bouin's solution and in Zenker-formol, double-embedded in parlodion-paraffin and sectioned at 6μ. All sections were routinely stained with hematoxylin and eosin, while sections from blocs fixed with Zenker-formol were stained with PAS-azure II and methyl green-pyronin for studies on changes of the nucleic acid content of the cytoplasm and nucleoli.

Results and Observations

Weight Changes

Body Weights. Figure 1 records the changes in average body weight of rats receiving an intraperitoneal injection of 75 μg actinomycin D. A gradual and linear decrease was observed through the first two weeks followed by a partial recovery on day 21. This represented the actual diminution of body weights of the experimental group and, therefore, the degree of real insult would have been greater as the control rats kept growing at the rate indicated.

Glandular Weights. The changes in weight of the right parotid and submandibular glands are shown in Figure 2. Of the two glands the parotid suffered a greater and more rapid loss of weight. By 24 hours the average glandular weight was less than 66% of the control value. As was true with body weight the lowest point was obtained on day 14 when the glandular weight decreased to less than 50% of the control. Again by the end of the experiment the weight showed a significant recovery. The high value obtained on day 7 is not consistant with changes in body and other organ weights.

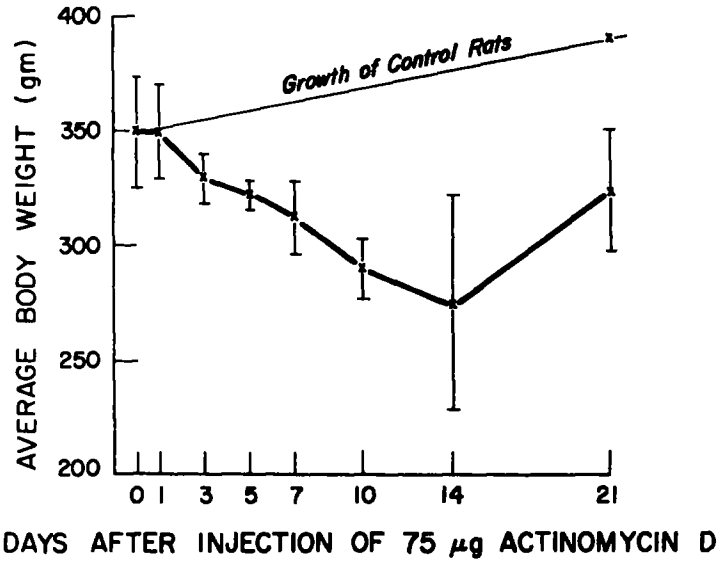

FIG. 1

The change in body weight of rats treated with actinomycin D as compared with the growth of control rats.

As expected from the nature of secretory products, the weight of the submandibular gland was less affected than that of the parotid. The decrease was more gradual and did not go down as much as the latter. The low point was observed on day 10 and a moderate recovery was seen by the second week.

Histologicals

The most striking changes were observed in the parotid acinar cells (Figs. 3 and 4) and, therefore, only photographs from the parotid were used for the purpose of illustration. The first sign of degeneration was observed on day 1 as focalized areas of nuclear pyknosis which became progressively larger through the first 10 days (Fig. 3). The general staining of the cytoplasm was decreased to variable degrees. From day 14 the nuclei of serous cells appeared to undergo a reversal of the degenerative changes with evidence of recovery. Cells constituting the various ducts, including those of the salavary ducts of the submandibular gland, were not affected to any degree (Fig. 6). Cells of the interstitial connective tissue appeared essentially unaltered.

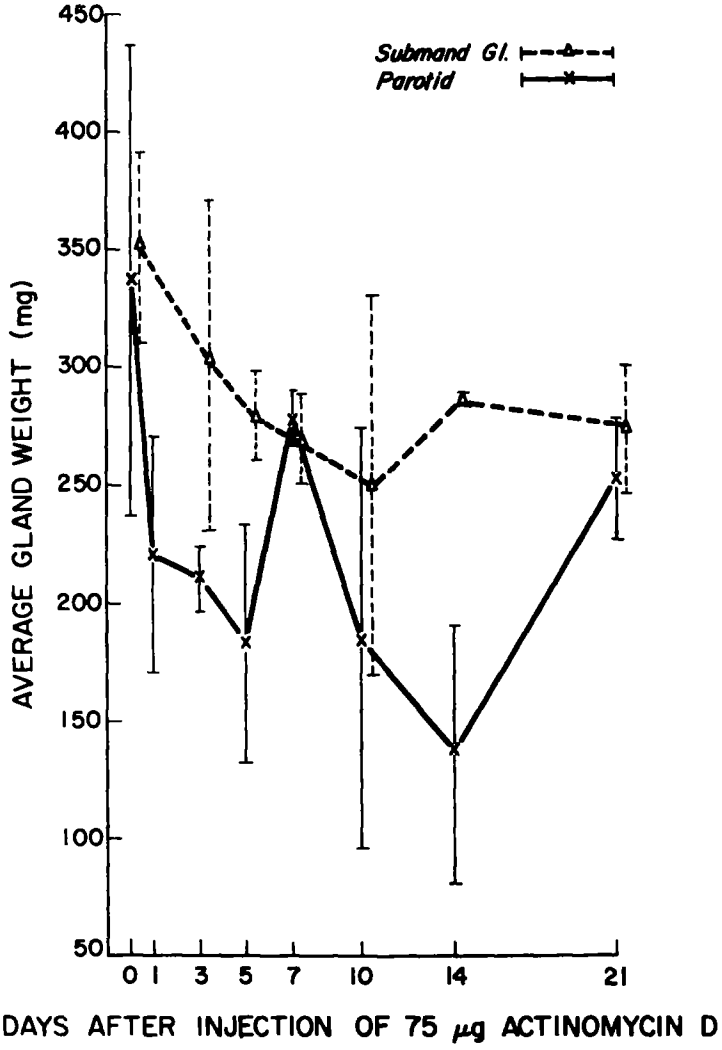

FIG. 2

The change in body weight of right parotid and submandibular glands in rats treated with actinomycin D. Note the precipitous drop of the parotid weights, whereas the submandibular weights appear to parallel the changes of total body weights.

In contrast to the normal acinar cell which contained a large cell body with pronounced basal ergastoplasm and a nucleus with smooth contour and prominent nucleoli (Fig. 5) the cells from experimental animals showed the following changes. The wrinkling of the nuclear membrane characterized the initial change and appeared to be related to the decrease in nuclear size (Figs. 7 and 8). Concomitantly the nucleous became smaller and often disappeared. Further

FIG. 3

A survey micrograph of the right parotid gland from a rat sacrificed 7 days after injection. Extensive nuclear pyknosis is observed. The cytoplasmic strain is also reduced.

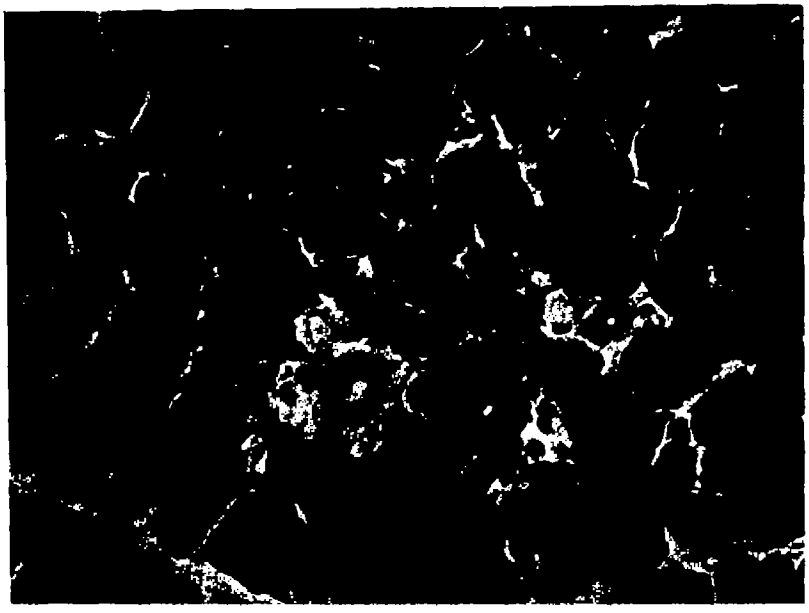

FIG. 4

A survey micrograph of the right parotid gland from a control rat.

changes brought about the aggregation of chromatin masses which often adheared to the basal plasma membrane (Figs. 6 and 8). By day 3 the cell appeared to swell somewhat resulting in the rarification of cytoplasm which appeared finely granular. Small vacuoles which grew progressively in number and size were seen elsewhere in the cytoplasm. They were most numerous on day 7 and were often located adjacent to the nucleus (Fig. 8). Sometimes their increase in size appeared to be facilitated by the coalescence of smaller vacuoles. The basophilia of the basal cytoplasm became somewhat reduced.

FIG. 5

A portion of the parotid acinus from a control animal. Photographs of this and following figures were from parotid glands fixed in Zenker-formol and stained with azure II-PAS.

FIG. 6

A portion of the intercalated duct of the parotid from a rat 7 days after injection. Note the intactness of duct cell nuclei as compared to the phyknotic one which belonges to an acinus below.

Discussions

It is significant that an early precipitous drop in weight was observed only in the parotid gland, which produces a purely proteineous secretion. The average rate of turnover of m-RNA in parotid gland cells is not known. However, the rapidity of its turnover has been shown by various experiments using actinomycin (20, 27). For instance 50 to 80 percent of the ribosome-associated m-RNA breaks down within 4 to 8 hours after injection of actinomycin D, which specifically inhibits the nucleus dependent RNA polymerase activity. Since it has

FIG. 7

An acinus from the parotid gland of a rat sacrificed 3 days after injection. Note wrinkling of nuclear membrane and decreased size of nucleoli.

FIG. 8

An acinus from the parotid gland of a rat sacrificed 7 days after injection. The pyknosis is advanced and extensive vacuolization is present in the cytoplasm

been established that the polyribosomal activities are dependent on the continued availability of the nucleus-produced m-RNA (28, 29) the early and specific reduction of parotid weights might be taken as indicative of the arrested synthesis of digestive enzymes by the gland due to blockade of m-RNA.

Cytologically the early changes in appearance of the nuclei support the concept that the cells indeed have suffered an initial insult to the nucleus with resulting impairment of enzyme production. The dramatic changes in nucleolar morphology following actinomycin D administration has been recently demonstrated by Schoefl (30) in his electron microscopic studies of cultured kidney cells.

Although the nature of vacuolization and rarification of the cytoplasm can not be visualized with clarity in light microscope observations, it has been pointed out that the ultrastructural sequence of cytoplasmic vacuolization may be similar in cells affected by different toxic agents (31). The ultrastructural aspects of the cytoplasmic vacuolization in glandular cells with impaired protein synthesis have been described by Herman and Fitzgerald (32) who, in a study of degenerating pancreas after ethinoine administration, described the

widening of endoplasmic reticulum and development of fine and coarse vacuoles containing granular disoriented membranes along with other changes such as decrease in ribosomes associated with the endoplasmic reticulum. Further electron microscopic studies on salivary glands treated with actinomycin D are being initiated in our laboratory.

References

1. R.A. MANAKER, F.J. GREGORY, L.C. VINING and S.A. WAKSMAN, Antibiot. Ann. 853 (1954-55).
2. H. TUCHMANN-DUPLESSIS and L MERCIER-PAROTT, C. R. Acad. Sci. Paris 247, 2200 (1958).
3. H. TUCHMANN-DUPLESSIS and L. MERCIER-PAROTT, C. R. Soc. Biol. 153, 386 (1959).
4. H. TUCHMANN-DUPLESSIS and L. MERCIER-PAROTT, C. R. Soc. Biol. 154, 914 (1960).
5. H. DENIS, Exp. Cell Res. 30, 613 (1963).
6. F.J. GREGORY, L.H. PUGH, T. HATA and R. THIELEN, Cancer Res. 16, 985 (1956).
7. F. BORIC, S. IVANKOVIC and N. SEEMAYER, Z. Krebsforsch. 62, 239 (1957).
8. F. BORIC, N. SEEMAYER and S. IVANKOVIC, Z. Krebsforsch. 62, 242 (1957).
9. K. OOTA, A. NISHIBORI and N. TAKAHASHI, J. Anatibiot. 10, 46 (1957).
10. J. PLUMMER and D.G. WALKER, J. Nat. Cancer Inst. 21, 263 (1958).
11. A. AWA, Gann 52, 49 (1961).
12. C. HACKMANN, Z. Krebsforsch. 60, 250 (1954).
13. L. FRIEDERICI, Z. Krebsforsch. 60, 553 (1955).
14. R. BIERLING, Z. Krebsforsch. 63, 519 (1960).
15. J.M. KIRK, Biochem. Biophys. Acta 42, 167 (1960).
16. E. REICH, R.M. FRANKLIN, A.J. SHATKIN and E.L. TATUM, Science 134, 556 (1961).
17. I.H. GOLDBERG and M. RABINOWITZ, Science 136, 315 (1962).
18. E. HARBERS and W. MUELLER, Biochem. Biophys. Res. Commun. 7, 107

19. J. HURWITZ, J.J. FURTH, M. MALAMY and M. ALEXANDER, Proc. Nat. Acad. Sci. U. S. 48, 1222 (1962).

20. C. LEVINTHAL, A. KEYNAN and A. HIGA, Proc. Nat. Acad. Sci. U. S. 48, 1631 (1962).

21. E. REICH, R.M. FRANKLIN, A.J. SHATKIN and E.L. TATUM, Proc. Nat. Acad. Sci. U. S. 48, 1238 (1962).

22. T. TAMOKI and G.C. MUELLER, Biochem. Biophys. Res. Commun. 9, 451 (1962).

23. E. KAHAN, F.M. KAHN and J. HURWITZ, J. Biol. Chem. 238, 2491 (1963).

24. I. MERITZ, Biochem. Biophys. Res. Commun. 10, 254 (1963).

25. J. PAUL and M.G. STRUTHERS, Biochem. Bophys. Res. Commun. 11, 135 (1963).

26. R.P. PERRY, Exp. Cell Res. 29, 400 (1963).

27. T. STAEHELIN, F.O. WETTSTEIN and H. NOLL, Science 140, 180 (1963).

28. M.W. NIRENBERG and H. MATTHAEI, Proc. Nat. Acad. Sci. U. S. 47, 1588 (1961).

29. A. RICH, J.R. WARNER and H.M. GOODMAN, Cold Spring Harbor Symp. Quart. Biol. 28, 269 (1963).

30. G.I. SCHOEFL, J. Ultrastr. Res. 10, 224 (1964).

31. J.G. LAFONTAINE and C. ALLARD, J. Cell Biol. 22, 143 (1964).

32. E. HERMAN and P.J. FITZGERALD, J. Cell Biol. 12, 277 (1962).