Effects of Reserpine and Chlorpromazine upon Enterochromaffin Cell of the Rat. (23759)

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(Introduced by Chauncey D. Leake)
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As part of an effort to study the effects of drugs upon specific cell types, a program that we call "histopharmacology," we have been interested in examining certain drug-cell systems as models for future development of this field. One of these that has been studied is the reserpine-enterochromaffin cell interaction. Serotonin (5-hydroxytryptamine) is considered from histochemical evidence to be the storage and secretion product of the enterochromaffin cells, which are located in the gastrointestinal mucosa of all vertebrates except the cyclostomes and teleosts(4). Concentration of serotonin decreased in the intestine after administration of reserpine, one of the Rauwolfia alkaloids with tranquilizing action(7). The decrease was found to occur only with this group of alkaloids possessing a sedative action(2). Chlorpromazine, a synthetic, psychotherapeutic drug also exerting sedative action, was shown to be incapable of decreasing serotonin concentration and exhibits what is thought to be a different mechanism of action(3). Recently, Benditt and Wong(1) described an effect of reserpine upon enterochromaffin cells of the guinea pig, finding that these cells diminish in number following exposure to the drug. Feeling that the change in absolute number of cells per unit area is too crude an estimation of cytological response, we examined the effects of reserpine in producing changes in the most important cytological characteristic of these cells, namely the granulation which could be revealed by silver staining. Chlorpromazine was used as a pharmacological control.

Materials and methods. Observations were made upon microscopic sections of the duodenum of 5 series of drug-injected and saline-injected male albino rats, comprising a total of 30 animals. Eighteen of these were used in the reserpine study, divided into experimental and control series. The experimental and control animals were matched by weight. The experimentals were injected intraperitoneally with 5 mg/kg of reserpine, and the controls were simultaneously treated with the same volume per body weight of physiological saline as in the reserpine-injected series. Reserpine-injected and saline control animals were sacrificed at 8, 16 and 24 hours after treatment. In the chlorpromazine study injections were made in the same manner as in the reserpine-injected series, using 12 animals. Sections of duodenum 2-3 cm in length, adjacent to and including a small part of the pylorus, served as the source of the enterochromaffin cells whose responses were studied. Tissues were washed with physiological saline and fixed immediately in a 10% aqueous formalin solution. After 3 days of fixation tissues were infiltrated and embedded in a special embedding mass, M.P. 58-65°C. Longitudinal sections (1.0 cm) were cut at 6 μ. Of 4 different methods employed, the methenamine silver technic of Gomori(5) was found to be the most reliable for specifically differentiating enterochromaffin cells, and revealing cellular detail. All sections used for analysis were reacted under the same conditions at the same time (60°C for 3-3½ hours, buffered at pH 7.8 with Holmes' alkaline borate buffer prior to impregnation). In attempting to establish a quantitative method for arriving at total granulation in the duodenum an arbitrary numerical assay or *weighted granulation count* was established. Cells were classified into 4 groups according to the density or apparent number of granules, from 1 plus (+), the least dense, to 4 plus (++++), the most dense (Fig. 1). The enterochromaffin cells in 4 random sections, each 1.0 cm in length, were counted for each experimental and control animal. The *weighted granulation count* was achieved by multiplying the total number of cells in each granulation stage by the numbers 1, 2, 3 and 4, corresponding to the differences in total granulation, as es-

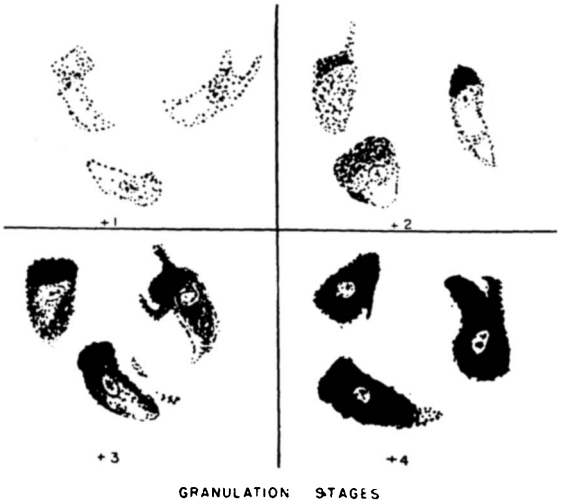

FIG. 1. Density classification of enterochromaffin cells of rat duodenum. Methenamine-silver stain.

tablished previously. Thus, the number of plus 1 was multiplied by 1, the number of plus 2 was multiplied by 2, etc. This is a conservative adaptation of a weighting device used by the Hartrofts in their study of the renal juxtaglomerular granulation(6). They used the geometric series 1, 2, 4 and 8 to weight the granulation index. It is felt that the arithmetic series used in this study more accurately represents the quantitative differences in the granule content of the cells studied. These *weighted granulation counts* were totaled, and the average count was calculated for a 1.0 cm section of duodenum. This figure was expressed as the *Weighted Granulation Index*. In presenting experimental results the *Weighted Granulation Index* of each animal was expressed as percentage difference of control minus the experimental. Fig. 2 shows the percentage differences in weighted granulation indices for the reserpine and chlorpromazine series, sacrificed at the different time intervals.

Results. It was found that an average 1.0 cm section of rat duodenum contained 183 enterochromaffin cells, predominantly of the moderately granulated types. Weighted granulation indices averaging 457 were obtained for controls. There was a variation of cell count with the weight of the animal, so that it was important to match the weights of the controls and the experimentals quite closely. Intraperitoneal injection of reserpine caused a decrease in the total enterochromaffin cell granulation at 8 and 16 hours, and a marked increase at 24 hours to an apparently significant degree in the rat. The changes were primarily due to changes in the degree of granulation rather than a change in the absolute number of cells. Chlorpromazine was found to have no significant change upon enterochromaffin cell granulation at 8, 16 and 24 hours. Pharmacological and physiological effects of both drugs were noted and were found to be in agreement with observations reported by other authors.

Discussion. The pharmacologic relationship of reserpine action to serotonin release, as developed by Brodie's laboratory, has been discussed(8,9). These results have been based upon chemical estimation of serotonin in tissue or serotonin metabolite in urine. Our findings on the changes in the enterochromaffin cell granulation parallel intestinal serotonin assay closely, in that both show a minimum at 16 hours. However, where the chemical values do not return to normal for several days, the cellular pattern has been restored at

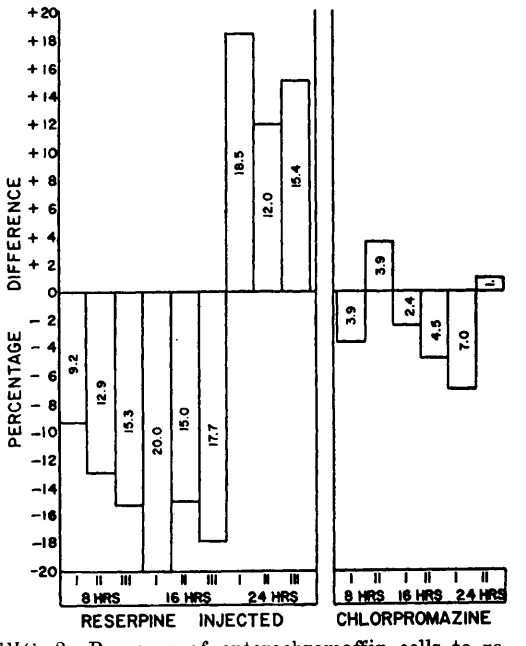

FIG. 2. Response of enterochromaffin cells to reserpine and chlorpromazine. Percentage difference in weighted granulation indices of treated and control rats.

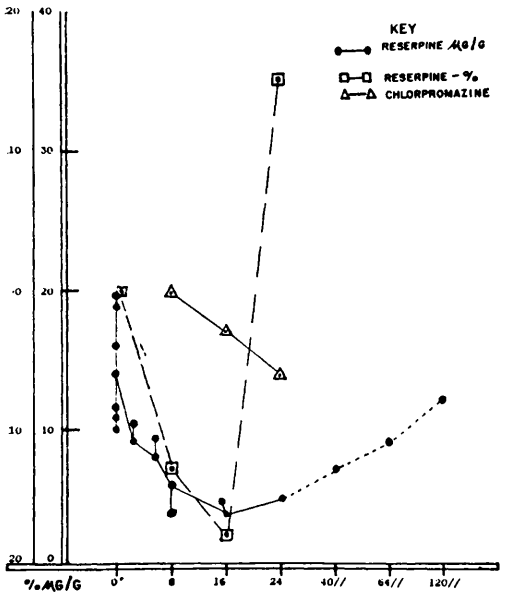

FIG. 3. Comparison between histological and chemical estimate of reserpine effect upon the enterochromaffin cell. The 2 ordinates are percent change of weighted granulation indices and µg of serotonin of intestine. The latter data are from Pletscher(7).

24 hours (Fig. 3). It is believed that this represents a period of resynthesis and reaccumulation of serotonin, or a precursor, without release. Cytological data are not sufficient to confirm this. Benditt and Wong(1), describe a similar effect upon enterochromaffin cells to that noted here. We feel that their method. that of counting cells per unit area and ignoring the intensity of granulation, does not offer the degree of precision required for pharmacological evaluation. On the other hand, weighting each cell according to its degree of granulation does give a better estimate of graded change along with a dynamic picture of cytological response to drug action. Also we feel it important that the experimental and control animals be matched by weight as our data indicate that the cell count and the weight or age may be correlated, perhaps due to a change in thickness of the mucous membrane with age.

Summary. Cytological evidence has been obtained, using a semiquantitative technic, for the action of reserpine upon enterochromaffin cells. Concomitant with release of serotonin, there is a loss of granulation in these cells. Chlorpromazine, as a pharmacological control, was found to be without significant cytological effect. The method of the *Weighted Granulation Index* offers one approach to the quantitative study of drug effects upon specific cell types.

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