NUCLEIC ACID PHOSPHORUS OF MOUSE PANCREAS AFTER PILOCARPINE ADMINISTRATION*

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Ribonucleic acid has been related to protein synthesis in embryonic and adult tissues, including the secretory glands (1, 2). This conclusion was reached mainly through cytological methods of a type which have not yielded information on the amount of polynucleotides per cell. It should, however, be noted that changes in the intracellular distribution of polynucleotides need not, necessarily, be paralleled by changes in the amount of these substances per cell. Although some biochemical data have been obtained in growing tissues, which seem to suggest a relation between ribonucleic acid and protein synthesis (3, 4), few data were found supporting this contention in secreting glands in which no marked modification in the cell size occurs (the special case of the liver not considered). These data are referred to in the "Discussion."

Pancreas seems to be a good material for studying the biochemistry of secretion, as no considerable changes in the amount of basic cytoplasm occur during secretion (5) and protein synthesis can be judged after pilocarpine administration from the amount of proteic secretory granules (6-8) elaborated and from the restoration of the activity of the pancreatic digestive enzymes (9, 10). In this paper we present results of a study of ribonucleic acid phosphorus (RNAP) and desoxyribonucleic acid phosphorus (DNAP) levels of mouse pancreas in different secretory conditions produced by pilocarpine administration. Pilocarpine was chosen because of its known cytological (11, 12), cytochemical (7, 8, 13, 14), and chemical (9, 10) effects.

EXPERIMENTAL

Adult albino mice of both sexes were used. Differences due to sex were not observed.

1 mg. of pilocarpine hydrochloride ("poulenc") in aqueous solution (0.5 per cent) was injected into the tail vein of each animal, irrespective of body weight. Intense salivation and lacrimation were observed soon after the injection.

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injections. Autopsy was performed after 20 minutes and 1, 4, 7, and 12 hours. The study of the pancreas from four pilocarpine-treated animals for each experimental period, compared with sections from five control animals, disclosed cell vacuolation and reduction in the amount of secretory granules, maximal in the 1 hour group. Granule content was similar to that of the control group 7 hours after pilocarpine treatment and exceeded that of the control group 12 hours after the injection. No nuclear degenerative changes were observed in any of the animals studied. Five pilocarpine-treated animals died in the 4 hour group and three in the 12 hour group. In about 5 per cent of the animals, both control and pilocarpine-injected, the pancreas appeared grossly edematous. These animals yielded low values for nucleic acid phosphorus and were not included in the series reported.

Control mice were given injections of an equal volume of distilled water, which were started at 8 a.m. after a fasting period of 18 hours. Water ad libitum was allowed to the animals during this period and throughout the experiments. The mice were killed after different intervals by a blow on the head and weighed. The pancreas was removed and weighed on a torsion balance. 1 per cent homogenates were prepared with chilled distilled water and stored at $-12^\circ$ until the determinations were carried out. Animals from each experimental period of pilocarpine administration (including the controls) were injected and killed at approximately the same time, and nucleic acid phosphorus determinations were performed simultaneously. This was done in order to obtain the maximum of homogeneity in the experimental conditions.

**Determination of Nucleic Acid Phosphorus**—RNAP and DNAP were determined in 0.7 ml. of the homogenates by the method of Schmidt and Thannhauser (15) as modified by Davidson, Leslie, and Waymouth (16) with slight changes (17). Reproducibility of the method was tested in our material, and variation coefficients with values around 4 per cent for RNAP and 7 per cent for DNAP were found. Phosphoprotein P was not determined, as it constitutes only a very small fraction included in the RNAP (18).

Determinations of the dry weight–wet weight ratio were made in poolings from homogenates of at least twelve control and twelve pilocarpine-treated animals from each experimental series. As no difference over 1 per cent was observed, all our results are expressed in micrograms of P per 100 mg. of fresh tissue.

**Statistical Analysis**—Comparison between group means was performed by the t test (19). t values higher than those of the 0.01 level of probability were considered as indicating statistically significant differences between the means.
Results

Data obtained in different periods after pilocarpine administration are presented in Table I. *t* tests between means for each pilocarpine-treated group for RNAP and DNAP showed no statistically significant differences (*P > 0.01*). The constancy of DNAP can be considered as indicating that the cell population was the same in the samples of both groups of animals (20, 21). RNAP:DNAP ratios presented in Table I have not been analyzed statistically, owing to the non-conformance of quotients to normal distribution. Only small variations were observed.

### Table I

**Ribonucleic Acid Phosphorus, Desoxyribonucleic Acid Phosphorus, and Ribonucleic-Desoxyribonucleic Acid Phosphorus Ratio in Pancreas from Control and Pilocarpine-Treated Mice**

C. = controls; P. = pilocarpine-injected mice. RNAP and DNAP in micrograms per 100 mg. of fresh weight ± the standard error of the mean.

<table>
<thead>
<tr>
<th>Time after pilocarpine administration (min.)</th>
<th>Animal weight (gm.)</th>
<th>RNAP (µg)</th>
<th>DNAP (µg)</th>
<th>RNAP:DNAP</th>
<th>Significance p, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 C.</td>
<td>26 ± 0.8 (20-33)</td>
<td>263 ± 5.0 (214-300)</td>
<td>32 ± 1.1 (25-45)</td>
<td>&gt;0.5</td>
<td>21 8.4 (5.6-10.4)</td>
</tr>
<tr>
<td></td>
<td>23 ± 0.6 (17-29)</td>
<td>263 ± 8.2 (204-356)</td>
<td>33 ± 1.0 (22-41)</td>
<td>&gt;0.5</td>
<td>20 8.1 (4.6-12.0)</td>
</tr>
<tr>
<td></td>
<td>28 ± 0.7 (22-34)</td>
<td>264 ± 8.0 (180-380)</td>
<td>&gt;0.7</td>
<td>24 34 ± 1.2 (25-46)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>29 ± 0.8 (24-30)</td>
<td>267 ± 8.3 (160-304)</td>
<td>33 ± 1.3 (22-45)</td>
<td>&gt;0.5</td>
<td>25 8.2 (4.0-12.7)</td>
</tr>
<tr>
<td></td>
<td>29 ± 0.9 (20-35)</td>
<td>264 ± 7.4 (206-328)</td>
<td>&gt;0.6</td>
<td>21 35 ± 1.2 (28-46)</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td></td>
<td>27 ± 0.9 (19-31)</td>
<td>260 ± 4.8 (222-294)</td>
<td>&gt;0.6</td>
<td>18 37 ± 1.6 (30-52)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>24 ± 0.8 (20-30)</td>
<td>259 ± 6.8 (192-310)</td>
<td>&gt;0.3</td>
<td>20 36 ± 1.1 (23-44)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>21 ± 0.6 (18-25)</td>
<td>267 ± 4.9 (229-300)</td>
<td>&gt;0.3</td>
<td>16 33 ± 1.2 (26-42)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>7 C.</td>
<td>24 ± 0.8 (19-28)</td>
<td>273 ± 6.8 (210-313)</td>
<td>&gt;0.02</td>
<td>19 35 ± 1.6 (15-45)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>23 ± 0.9 (15.5-30)</td>
<td>253 ± 7.5 (185-301)</td>
<td>&gt;0.02</td>
<td>20 35 ± 1.0 (25-42)</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>
In regard to evidence suggesting protein synthesis after pilocarpine administration, it is of interest to refer to the paper by van Weel and Engel (9) which related the extrusion and reconstitution of secretory granules to pancreatic carboxypeptidase activity. Results from this laboratory, obtained with the animals used in the present report, showed that 20 minutes after pilocarpine administration amylase activity was reduced to around 50 per cent of the control values and then increased to levels higher than those of the control animals 12 hours later. These results, allied to our histological data, indicate that pilocarpine administration in our material was followed by processes of protein synthesis. In this connection we may mention that amylase activity was also used by Hokin (22) as an index of protein synthesis by pancreatic tissue in vitro.

Only a few papers could be found in the literature concerning variations in the nucleic acid content of pancreas as related to secretion. Guberniev and Kovyrev (23), studying the nucleic acid content of dog pancreas after secretin administration, reported an increase of both RNA and DNA after 3 to 4 hours. Guberniev and Il’ina (24) later reported an increase in the turnover rate of isotopic nucleic acid phosphorus in the same material.

In a recent paper Hokin (25) reported that RNAP turnover in vitro in slices of pigeon pancreas could not be related to amylase synthesis. On the other hand, in secretion promoted by carbamylcholine the turnover was accelerated and this was thought to depend upon excretion phenomena and not on the protein synthesis per se. We are not in position to compare our results with theirs (22-24) in view of the different methods used.

As a result of the utilization of the cytochemical ribonuclease method in studies on pancreas, it was shown that the cytoplasmic basophilic zone of its acinar cells (also known as chromidial bodies, ergastoplasm, etc., in the cytological literature) contains ribonucleic acid (25, 8, 13, 14). The utilization of microspectrophotographic methods led to similar conclusions (7, 8).

Pilocarpine administration causes a rapid extrusion of secretory granules in pancreatic acinar cells (11, 12). In the literature their reconstitution has been related to variations in the amount and distribution of basophilic and ultraviolet- (maximum at 2.600 A) absorbing substance (12, 7, 8, 13, 14). No comparison of our results with certain of those of the above authors (7, 8, 14) can be made, as no reference is made in their papers to dose and route of administration of pilocarpine, number of animals, timing.

1 A. Sesso, N. Sawaya, V. Valeri, Layla Nahas, and M. Rabinovitch (1951), to be published.
of experiments, number of cells measured, and variability of the results. It is to be noted that the conclusions obtained by the investigators who utilized Brachet’s method are based solely on the aspects of cytological preparations, without any quantitative results.

Our findings demonstrate no variation in the RNAP and DNAP contents of pancreas in different secretory stages after pilocarpine administration. It is suggested that the data obtained by histochemical methods might be due to an intracellular redistribution of ribonucleotides during secretion, the total amount per cell remaining constant.

SUMMARY

No statistically significant variations were observed in either ribonucleic or desoxyribonucleic acid phosphorus levels in mouse pancreas 20 minutes and 1, 4, 7, and 12 hours after intravenous pilocarpine administration, compared with the controls. No variation in RNAP content could therefore be related to the protein synthesis that follows pilocarpine administration. The bearing of these results on previous histochemical data is discussed.

BIBLIOGRAPHY

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