A more detailed paper on this investigation will be submitted for publication in a later number of this journal*.

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THE ROLE OF RIBONUCLEIC ACIDS IN AMYLASE SECRETION BY PANCREAS SLICES

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CASPERSSON AND BRACHET and collaborators[1,2] observed that cells which synthesize digestive enzymes (*e.g.*, acinar cells of pancreas and chief cells of stomach) contain high concentrations of ribonucleic acids. They regarded these findings as evidence that ribonucleic acids are concerned in the synthesis of cytoplasmic proteins. GUBERNIEV AND IL'INA[3] were led to similar conclusions by their observation that the *in vivo* stimulation of enzyme secretion in digestive glands resulted in increases in the rate of incorporation of ^{32}P into the nucleoproteins (400% in parotid, 500% in liver and 1200% in pancreas).

An alternative explanation for the above observations is the assumption that ribonucleic acids are concerned with enzyme secretion (by which is meant the active extrusion of enzymes) rather than enzyme synthesis. Pancreas slices *in vitro* represent a system in which the synthesis and secretion of enzymes can be studied separately, since either can be stimulated without effect on the other (HOKIN[4]). The experiments reported below were designed to test whether there is a correlation between either of these processes and ribonucleic acid metabolism.

Amylase synthesis and secretion by pancreas slices were measured as described earlier[4]. 10–20 μC of ^{32}P was added as H_2PO_4 to each vessel. The specific activities of the ribonucleic acids were determined after their isolation (as a mixture of nucleotides) by the method of SCHMIDT AND THANNHAUSER[5] followed by paper chromatography (MARKHAM[6]).

An approximate doubling of the rate of amylase synthesis by the addition of an appropriate amino acid mixture did not result in any appreciable increase in the rate of uptake of ^{32}P into ribonucleic acids. This suggests that protein synthesis is not linked to ribonucleic acid metabolism. On the other hand, a 50–100% stimulation of amylase secretion by the addition of carbamylcholine was accompanied by a corresponding increase in the rate of uptake of ^{32}P into ribonucleic acids. The phosphorus of the ribonucleic acids in unstimulated slices reached equilibrium with inorganic phosphorus after about 80 minutes, when about 0.5% of the phosphorus in the ribonucleic acids had exchanged. On the other hand, in stimulated slices the phosphorus of the ribonucleic acids did not reach equilibrium with inorganic phosphorus until about 1–1.5% of the phosphorus had exchanged. Thus in stimulated slices more phosphate groups in the ribonucleic acids seem to be labile. Since neither respiration nor the turnover of acid-soluble organic phosphorus was increased when

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enzyme secretion was stimulated, the increase in the uptake of ^{32}P into ribonucleic acids is fairly specific.

The above observations can best be interpreted in the light of cytological studies on the resting and secreting pancreas (COVELL[7], BABKIN *et al.*[8]). In the resting gland the acidophilic enzyme granules are surrounded by basophilic material which has been shown to be ribonucleic acid[2]. During secretion the acidophilic enzymes are accumulated into vacuoles in preparation for their extrusion. The increased number of labile phosphoric acid groups in the ribonucleic acids of the secreting pancreas is consistent with the view that during secretion these phosphoric acid groups are set free and made available for combination with the basic groups of the enzymes. By such a combination the enzymes existing on the granules could be concentrated into vacuoles. This mechanism is further suggested by the observation that reticulocytes can accumulate basic vital dyes (analagous to the basic reacting enzymes above) into vacuoles, whilst erythrocytes, which in contrast to reticulocytes do not contain ribonucleic acids, are incapable of vacuole formation (DUSTIN[9]). The mechanism by which phosphoric acid groups in ribonucleic acids could be set free during secretion is not known, but it could result from a dissociation of a basic histone-like protein from the phosphoric acid groups of the ribonucleic acid or a possible alteration in the ribonucleic acid structure, such as depolymerization.

The view that ribonucleic acids function in the rearrangement and movement of enzymes during the secretory process by the formation of salt-like linkages between nucleic acid and protein can be extended to other phenomena: (1) A participation of nucleic acids in the movement and rearrangement of proteins during cell division could explain the high content of nucleic acids in rapidly dividing cells[1,2]. (2) By acting as a specific framework onto which enzyme systems could be organized a nucleic acid (or nucleoprotein) could direct the synthesis of more of the same nucleic acid (or nucleoprotein). This may be a mechanism by which viruses, genes, the pneumococcus transforming factor, and possibly cytoplasmic particles, may replicate themselves. In this connection it is of interest that viruses utilize the enzymes of their host for their own reproduction (COHEN[10]). Furthermore, virus reproduction is inhibited by lysine polypeptides which combine with the virus by salt-like linkages (BURGER AND STAHMANN[11]). In the light of the above suggestions, this inhibition could be accounted for by a competition between lysine polypeptide and host enzyme for the virus nucleoprotein framework.

A structural basis for the above hypothesis is provided by the observation that the internucleotide distance in the nucleic acids is the same as the distance between the side groups of an extended polypeptide (ASTBURY[12]).

Full details will be published shortly.

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