rabbit neoplasms, including virus papillomas of the type from which the V2 carcinoma originally derived.

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A CONFIRMATION OF THE PRESENCE OF PANCREOZYMIN IN THE DUODENAL MUCOSA*

HARPER and Raper[1] have recently demonstrated that extracts of duodenal mucosa contain two hormonal agencies affecting the external secretory activity of the cat's pancreas. One of these is the familiar secretin; the other, heretofore not characterized, they have termed pancreozymin; their findings indicate secretin to stimulate the production of fluid and bicarbonate by the pancreatic acini, whereas pancreozymin does not affect the output of the inorganic constituents and causes a marked increase in the production of pancreatic amylase. By implication, all the pancreatic enzymes are similarly affected, since a parallelism in their appearance in pancreatic juice or pancreatic extracts has been repeatedly demonstrated. The obvious academic importance and practical implications of these studies by Harper and Raper clearly indicated that a re-examination be made of various fractions obtained in the isolation of secretin as performed in this laboratory,[2] with a view to substantiation of their findings in the dog and extension of their studies to the three chief pancreatic enzymes.

METHODS

Fasted mongrel dogs were anesthetized with sodium pentobarbital, the femoral vein exposed for intravenous injections, and the chief pancreatic duct (Santorini) cannulated. Three of the secretin concentrates previously described[2] served as the stimulating agents employed, including the crude preparation designated SI; the fraction precipitated by aniline treatment of a solution of SI in 80 per cent. acetone, designated AP in the present discussion; and an aqueous solution of the material purified by treatment of SI with aniline, followed by butyl alcohol extraction, which on treatment with picrolonic acid yields a crystalline picrolonate. All materials were free of vasodilator activity; they were injected intravenously, and the first sample following a given injection was discarded, as it served to flush the ducts and cannula of pancreatic juice resulting from a previous stimulation. Enzymes determined included amylase, trypsin and lipase; they were estimated by the methods previously employed.[3] The volume and enzyme responses were noted following injections of pure secretin and of pure secretin plus the AP fraction before and after a six hour incubation with dog serum. The latter procedure was designed to determine whether pancreozymin is inactivated by serum in a manner analogous to secretin.[4]

RESULTS

It was found in every case that purified secretin evoked a secretion poor in enzymes. Either the SI fraction or purified secretin combined with the AP fraction evoked a secretion with a markedly increased enzyme content, all enzymes being similarly affected. Incubation of the AP fraction with serum vitiated its enzyme-stimulating properties. The averaged results are listed in Table 1.

TABLE 1

Stimulus	No. of dogs	Volume	Trypsin		Amylase		Lipase	
			Units per cc	Units total	Units per cc	Units total	Units per cc	Units total
Secretin ..	10	3.0	367	1,101	0.148	0.444	172	516
Secretin + AP	10	3.2	1,403	4,500	0.412	1.320	865	2,760
Secretin + serum treated AP	5	4.1	314	1,285	0.152	0.624	166	680

DISCUSSION

On the basis of the results submitted it is evident that the AP fraction contains a principle which stimulates production of enzymes by the pancreas; and these are washed out in the secretion stimulated by secretin. It is therefore evident that the findings of Harper and Raper are in all respects confirmed by the results of these experiments and that all pancreatic enzymes are equally affected. Thus separation of secretin and pancreozymin was effected by us some years ago, without a recognition on our part that this had been done. The failure of the pancreozymin fraction to stimulate enzyme production after incubation with serum signifies that there is a substance in the blood, probably an enzyme, which with time inactivates pancreozymin.

SUMMARY

Confirmatory evidence has been secured for the existence of pancreozymin, a hormone present in extracts of the duodenal mucosa the effect of which is to stimulate enzyme production by the pancreas. It is separated from secretin by precipitation with aniline, and stimulates equally the formation of the three chief

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[1] A. A. Harper and H. S. Raper, *Jour. Physiol.*, 102: 115, 1943.

[2] H. Greengard and A. C. Ivy, *Am. Jour. Physiol.*, 124: 427, 1938.

[3] M. I. Grossman, H. Greengard and A. C. Ivy, *Am. J. Physiol.*, 138: 676, 1943.

[4] H. Greengard, I. F. Stein, Jr., and A. C. Ivy, *Am. Jour. Physiol.*, 133: 121, 1941.

pancreatic enzymes; it is inactivated by incubation with serum, probably on an enzyme basis.

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CHLORELLIN, AN ANTIBACTERIAL SUBSTANCE FROM CHLORELLA

CULTURES of *Chlorella,* in inorganic nutrient solutions, produce and accumulate a substance that tends to inhibit further multiplication of the cells.[1] Recently extracts containing this growth-inhibiting substance have been prepared in larger quantities than heretofore and have been tested for antibiotic activity against other organisms. These extracts have been found to possess antibacterial properties against both Gram-positive and Gram-negative organisms: *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Bacterium coli* and *Pseudomonas pyocyanea* (*Ps. aeruginosa*).

Both *Chlorella vulgaris* and *Chlorella pyrenoidosa,* commonly considered to be different species, were used in these experiments. The cells were cultured in batteries of 5-gallon bottles containing solutions of the conventional mineral nutrients for pure algal cultures. Various proportions and concentrations of these salts were tested. The more dilute solutions, and those containing ammonium nitrogen, were all sterilized before inoculation. With more concentrated solutions,[1] sterilization was not essential when the solutions were continuously illuminated. A mixture of 5 per cent. carbon dioxide in air was bubbled continuously through the cultures. Some cultures were grown under continuous illumination from white fluorescent lamps for about two weeks. Others were grown in a greenhouse under natural illumination for approximately one month, finally being subjected to continuous illumination from fluorescent lamps for two to three days. Best yields of antibacterial substance were obtained from mature cultures which were harvested immediately after the above mentioned period of illumination.

Various methods have been used to obtain extracts containing the active principle. Crude extracts, suitable for determining the biological activity of the inhibitory substance, were prepared by extraction of the cell-freed culture solutions. For these extractions chloroform, 1,2-dichloroethane or benzene have proven superior to other solvents tried. The separated organic solvent, used for extraction, was removed *in vacuo* and the crude extract was obtained as a brown mass, in yields of 1 to 8 mg per liter of cell-free solution. The consistency of the crude extracts varied from a viscous, tacky liquid to a brittle solid. In some instances, extraction of the cell-freed culture solution was preceded by concentration to one half volume by distillation *in vacuo* at bath temperatures of 50–55° C. Thus far adsorption and elution techniques have been inferior to the extraction procedure. Active material has also been obtained from the cell mass. Apparently moderate heat does not greatly affect the yields of the active material.

Biological tests were carried out on clear aqueous extracts of the crude material. To this end the crude material was thoroughly shaken with a small amount of water. Such a procedure has commonly yielded solutions containing 0.03 to 0.1 mg solids per cc. This solution was adjusted to pH 7.0. When tested against *Staphylococcus aureus,* strain 209, in standard cup assay (18 hours at 37° C., diameter of cup 8 mm) 0.2 cc portions of these solutions commonly produced zones of inhibition 18 to 35 mm in diameter. A zone of 45 mm was obtained from extracts of one lot of crude material. Attention should be directed to the fact that only a very small portion of the active principle is extracted in a single treatment by this procedure, for repeated extractions of a given lot of crude material continue to yield solutions showing a relatively high order of antibacterial activity. The same order of activity was observed when strains of *Str. pyogenes* (on blood agar) or of *B. coli* were substituted as the test organism. The strain of *Ps. pyocyanea* that was used is only slightly less sensitive to the action of this antibacterial agent, while the strain of *Bac. subtilis* is more sensitive than the staphylococcus.

Other tests indicate that the active principle may be bactericidal. One tenth of a cubic centimeter portions of 24-hour broth cultures of *Staph. aureus* were added to 5 cc portions of solutions having the same concentration of active principle as were used in the cup tests. Less than 10 minutes contact with the active principle prevented subsequent multiplication of the organisms when transferred to nutrient broth (incubated at 37° C. for 48 hours). Contact of about 20 minutes was required in the case of *B. coli* to achieve the same result. Similar tests in which the bacterial cells were exposed to the solution of the active principle in fresh rabbit serum showed that the bactericidal activity of the active principle is but slightly inhibited in the presence of serum proteins.

The results of numerous experiments carried out over a period of a year and a half show that an antibacterial substance accumulates in uncontaminated cultures of *Chlorella* and that the activity of this substance can be tested by standard bacteriological methods. For convenience of reference it is proposed to designate this substance by the name *chlorellin.* It is recognized that the products thus far obtained repre-

[1] R. Pratt, *Am. Jour. Bot.,* 27: 52 and 431, 1940; 29: 142, 1942.