mention should be made of the author's discussions of dielectrics, electrokinetics, viscosity, diffusion and membranes, since these topics have been neglected in many elementary text-books.

In general the treatment is clear and accurate, but there are occasional lapses. The definition of the erg (p. 15) is grossly incorrect. The author implies (p. 108) that Harned and Ehlers did not use buffered solutions, while as a matter of fact their precise values for dissociation constants were obtained only by the use of buffer mixtures. A method for extrapolating electromotive force data (p. 110) is presented in a sadly garbled form. The name of the man who formulated the law of diffusion (p. 272) should not be identical with that of an American steel magnate. The method of obtaining partial specific volumes would be more intelligible if the words (p. 292, line 4) agreed with the symbols. However, most of the errors will be caught by a careful reader.

The lectures on which this book was based were probably a pretty stiff dose for the medical and biological students who heard them. The book should be particularly useful to future research workers who are willing to supplement it, as the author suggests, by a generous amount of outside reading.

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SPECIAL ARTICLES

SUPPRESSION OF GROWTH OF THE BROWN-PEARCE TUMOR BY A SPECIFIC ANTIBODY

THE cells of the Brown-Pearce carcinoma possess a distinctive constituent which can be identified *in vitro* through its reaction with an antibody that appears in the blood of certain rabbits implanted with the growth, as previous studies have shown.[1] This constituent is regularly present in large amounts in cell-free, saline extracts of the Brown-Pearce tumor, but it has not been detectable in similar extracts of other rabbit tissues, normal or neoplastic; it is readily sedimentable in the high-speed centrifuge, and certain of its properties suggest that it may be a protein.[1,2] Inquiry has now shown that the antibody which reacts specifically with the distinctive constituent has an influence on living Brown-Pearce tumor cells.

For *in vitro* experiments, serum specimens known from trial complement fixation tests[1] to contain the specific antibody in high titer were procured from "blue-cross" rabbits[4] in which the Brown-Pearce tumor had recently regressed. The sera were mixed with suspensions of living tumor cells, prepared by pressing "healthy" tumor tissue through a 40-mesh monel metal sieve into Locke's solution and allowing the clumps to settle out in a cylinder, the final preparations containing some 20 to 40 individually suspended tumor cells per microscopic field (×400). The mixtures were incubated 2 to 3 hours at 37° C. and then injected into the leg muscles of three or four normal rabbits. Control injections were made at corresponding situations in the same hosts with an equal quantity of incubated mixtures containing tumor cell suspensions and sera from normal rabbits or from rabbits carrying tumors of other kinds (V2 carcinoma;[5] Sarcoma I of Andrewes and Ahlström[6]). The control mixtures gave rise almost always to tumors that reached 2.0 to 3.5 cm in diameter within two to four weeks, whereas the tumor cells incubated with the antibody-containing sera usually failed to grow, though occasionally they formed small nodules.[7]

The effect was specific in that the antibody-containing sera had no influence on V2 carcinoma cells or those of Sarcoma I in concurrent tests. Yet the antisera did not lyse, agglutinate or alter the appearance of the Brown-Pearce tumor cells during 3 hours at 37° C.; and furthermore the proportion of tumor cells stainable with trypan blue (final concentration

[1] J. G. Kidd, *Proc. Soc. Exp. Biol. and Med.*, 38: 292, 1938; *Jour. Exp. Med.*, 71: 335, 351, 1940; *Jour. Bact.*, 39: 349, 1940. See also J. G. Kidd and W. F. Friedewald, *Jour. Exp. Med.*, 76: 543, 557, 1942.

[2] As bearing further on the nature of the distinctive constituent, recent experiments have shown that it is acted upon *in vitro* by purified proteolytic enzymes (chymotrypsin and trypsin), which rapidly render it unable to react with its specific antibody. In addition, Claude and I have found that the distinctive constituent seems to be associated with the "small particles" or cytoplasmic microsomes of the Brown-Pearce carcinoma cells—the finding having a greater interest since the filtrable agent responsible for Chicken Tumor I appears to be associated with the microsomes of fowl sarcoma cells.[3]

[3] A. Claude, *Proc. Soc. Exp. Biol. and Med.*, 39: 398, 1938; SCIENCE, 91: 77, 1940; J. Furth and E. A. Kabat, *Jour. Exp. Med.*, 74: 247, 257, 1941. See also L. Foulds, *Am. Jour. Cancer*, 31: 404, 1937; J. G. Kidd and W. F. Friedewald, *Jour. Exp. Med.*, 76: 543, 557, 1942; A. Claude, *Biological Symposia*, 10: 111, 1943.

[4] English × Lilac—Rockefeller Institute strain, inbred from fertile hybrids.

[5] J. G. Kidd and P. Rous, *Jour. Exp. Med.*, 71: 813, 1940.

[6] C. H. Andrewes and C. G. Ahlström, *Jour. Path. and Bact.*, 47: 87, 1938.

[7] It may be significant that the Brown-Pearce tumor cells do not "protect" the distinctive constituent from the action of the specific antibody, whereas many living cells, notably certain neoplastic ones, provide such protection for viruses.[8]

[8] P. Rous, P. D. McMaster and S. S. Hudack, *Jour. Exp. Med.*, 61: 657, 1935; J. G. Kidd, *Jour. Exp. Med.*, 75: 7, 1942.

1:300)[9] was no greater in incubated mixtures containing specific antisera than in control mixtures with normal sera. The pH of incubated mixtures ranged between 7.95 and 8.08 as determined with the glass electrode, those containing the specific antisera being no more alkaline than the controls.

With a view to testing for an effect of the specific antibody *in vivo,* attempts were made to stimulate its formation in normal rabbits by repeated intraperitoneal injections of watery, cell-free extracts of the tumor. The results were negative when agouti, chinchilla and Dutch hybrid rabbits were employed, though animals of these breeds may manifest the antibody following the growth of Brown-Pearce tumors.[10] In several experiments, however, blue-cross rabbits have developed the specific antibody following three or four intraperitoneal injections with cell-free extracts of the tumor, and all of the animals in which this happened (16 out of a total of 44) proved resistant, generally completely so, to a small "dose" of Brown-Pearce tumor cells implanted intramuscularly 7 to 10 days after the final intraperitoneal injection.[11] By contrast, the blue-cross rabbits that had not developed detectable titers of the antibody as result of the intraperitoneal injections proved quite as susceptible as normal rabbits, the implantations resulting in large growths in most instances. Again the effect was specific in that the rabbits that had developed the antibody and were resistant to the Brown-Pearce tumor cells proved as susceptible as normal controls to implantation with tumor cells of other types (V2 carcinoma, Sarcoma I).

It is common knowledge that the Brown-Pearce carcinoma, like other cancers transplanted in hybrid hosts, is frequently resorbed after having attained considerable size. Yet many animals have overcome it in our experiments without manifesting the specific antibody in detectable titer at any of repeated bleedings, and sera procured from them have failed to influence the later proliferation of Brown-Pearce tumor cells when incubated therewith *in vitro.* Hence it seems plain that regression of the growth, at least as it occurs in many instances, is probably not brought about by the specific antibody.[12] Recent observations have shown, however, that the antibody may influence the course of events when it develops following the implantation of Brown-Pearce tumor cells. For in the rabbits that have developed the antibody in high titer under such circumstances the growths have almost always regressed abruptly within 3 or 4 weeks after the implantations, while, conversely, the animals in which the tumor has grown progressively and metastasized have usually had little or none of the specific antibody in their blood.[15]

The findings as a whole would seem to indicate that the specific antibody is capable of preventing the proliferation of living Brown-Pearce tumor cells,[16] and they are consistent with the view that the distinctive sedimentable constituent with which the antibody reacts may play some part in the proliferative activities of the tumor cells. In further studies to determine whether the cells of other tumors possess distinctive constituents of similar sort, recent observations[21] have indicated that the V2 carcinoma regularly yields a sedimentable substance that is not detectable in extracts of normal rabbit tissues or in those of other

[9] A. M. Pappenheimer, *Jour. Exp. Med.,* 25: 633, 1917; M. N. Richter and E. C. MacDowell, *Jour. Exp. Med.,* 57: 1, 1933.

[10] I. MacKenzie and J. G. Kidd, *Jour. Exp. Med.,* in press.

[11] In this relation, MacDowell, Claude *et al.* observed in two experiments that 5 per cent. and 35 per cent., respectively, of the C58 mice injected intraperitoneally with sedimented materials procured from Line I leukemia cells later survive implantation with tumor cells that overcame the control animals (Carnegie Institution of Washington, Year Book No. 40: 248, 1940–41).

[12] It seems probable that isoantibodies such as those encountered by Gorer and by Lumsden in tumor-resistant mice and rats[13] might be responsible for regression of the Brown-Pearce carcinoma as this usually occurs. However this may be, experiments have shown that the specific Brown-Pearce antibody does not react with extracts of the normal tissues of tumor-bearing, tumor-regressed or normal rabbits, and it does not lyse or agglutinate their erythrocytes—findings which render it unlikely that the Brown-Pearce antibody is an isoantibody of this kind. Nevertheless, it is obvious that genetic or constitutional factors influence the incidence of the specific antibody, though the nature of these factors remains obscure.

[13] P. A. Gorer, *Jour. Path. and Bact.,* 44: 691, 1937; *ibid.,* 47: 231, 1938; *ibid.,* 54: 51, 1942; T. Lumsden, *Am. Jour. Cancer,* 32: 395, 1938. See also reference 14.

[14] M. J. Eisen and W. H. Woglom, *Cancer Research,* 1: 629, 1941.

[15] In an exceptional instance fulminant growth of the tumor brought about death of the host with widespread metastases on the 34th day after implantation, despite the development of a moderately high titer of the antibody between the 18th and 28th days.

[16] The principle is not new in immunology that antibodies may render cells unable to proliferate without altering appreciably their form or other functions, in this respect resembling certain chemotherapeutic agents.[17] Ascoli[18] and Dochez and Avery[19] have studied the "antiblastic" effects of certain antibacterial antibodies, and Taliaferro has described "ablastic" antibodies which prevent reproduction of *Trypanosoma lewisi* in the rat and *T. duttoni* in the mouse, an interesting fact being that the parasites remain alive, motile and capable of infecting new hosts after a sojourn of months in the blood of animals having effective titers of the ablastic antibodies.[20]

[17] H. Dale, *Lancet,* 2: 761, 1941; *Brit. Med. Jour.,* 2: 411, 1943; H. McIlwain, *Nature,* 151: 270, 1943. See also A. J. Clark, in Heffter's Handbuch der experimentellen Pharmakologie, Berlin, Springer, Ergänzungswerk. 4, 1937.

[18] A. Ascoli, *Centr. Bakt. u. Parasit.,* 1, Abt., Orig., 46: 178, 1908.

[19] A. R. Dochez and O. T. Avery, *Jour. Exp. Med.,* 23: 61, 1916.

[20] W. H. Taliaferro, *Jour. Exp. Med.,* 39: 171, 1924; *Am. Jour. Hyg.,* 16: 32, 1932; *Jour. Immunol.,* 35: 303, 1938.

[21] J. G. Kidd and W. F. Friedewald, unpublished experiments.

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.