admissible to the feeding apparatus of the organism concerned is usually satisfactory. The most important factor is, however, the medium. On account of the selective-permeability of the cell membrane, the constitution and changes of the medium have immediate and direct influence on the life processes of the organism.

The writer has for a long time devoted himself to the finding of inorganic physiological media for his investigations on various unicellular forms, and has been able to develop two media, one for fresh-water and the other for marine Protozoa. The formulae of these media are as follows:

MEDIUM A FOR FRESH-WATER FORMS

$CaCl_2$	0.0008 N	
$NaNO_3$	0.0003 N	
$MgSO_4$	0.0002 N	
K_2HPO_4	0.0001 N	
KH_2PO_4	0.0001 N	
NH_4NO_3	0.0008 N	(for green forms only)

MEDIUM B FOR MARINE FORMS

NaCl	0.1335 N	
$CaCl_2$	0.0112 N	
KCl	0.0084 N	
$NaNO_3$	0.0055 N	
$NaHCO_3$	0.0048 N	
$MgSO_4$	0.0040 N	
KH_2PO_4	0.0005 N	
Na_2SiO_3	trace	
NH_4NO_3	0.0125 N	(for green forms only)
$FeCl_3$	trace	(for green forms only)

In developing Medium A, extensive experimental work was done on Pleurotricha and Chlorogonium, which is also used as food for the former. In developing Medium B, experiments were performed with Kerenopsis and Dunaliella, which also serves as food for Kerenopsis. For other organisms, the total salt concentration and the hydrogen-ion concentration of both media may be slightly modified.

The working basis in formulating Medium A are: (1) The chemical analysis of fresh water; (2) the toxicity and antagonism of salts; (3) the buffering reaction of salts.

The formula of Medium B is based for anions on the chemical analysis of the salt content of the blood of certain marine organisms and for cations on the buffering properties of salts. In case of green forms, the chemical requirements for photosynthesis have also been taken into consideration.

These media are less toxic than natural fresh water or sea water and many other physiological media tested. They are satisfactory for most unicellular organisms the writer has tried to cultivate, but they are fatal to a few others. With frequent transferring to fresh media, organisms grow very well. By using these media for experimentation, surprisingly uniform results are obtained. In case of Pleurotricha and Kerenopsis, variation in size and fission rate are extremely small. Rhythmic variation in fission rate, such as that obtained by Jennings and others on Paramecium, has not been found.

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MICRO-METHODS FOR THE DETECTION OF PROTEASES AND AMYLASES

DURING the past two years the authors have been engaged in independent investigations of the digestive enzymes of amphibian embryos (F. D.) and of spiders (G. E. P.), respectively. The results of these investigations will be published separately elsewhere but we wish jointly to record two simple methods which proved convenient for the detection of proteases and amylases, respectively, when extremely small amounts of the extract or fluid to be tested were available. The method used for proteolytic enzymes is similar in principle to the more accurate quantitative method suggested by Gates;[1] the method used for amylases is based on that described by Bond,[2] but instead of using a thick layer of starch suspension in agar in the bacteriological manner a very thin dry film is substituted.

PROTEASES

A drop of the fluid or extract to be tested is mixed with several drops of an appropriate buffer solution; a drop of the resultant mixture is withdrawn and placed on the gelatin surface of an unexposed Eastman lantern slide plate which has been previously cleared in 20 per cent. sodium thiosulfate, thoroughly washed and dried. The slide should be placed in a moist chamber or otherwise protected from evaporation during the period of digestion. If enough of the original sample is available, it is convenient to run a series of drops at different pH values so that the approximate pH range of the enzyme is determined in the first experiment. The slide may be allowed to stand at room temperature if this does not exceed 20° C.; at temperatures of about 26° C. control drops of buffer solution dissolve the gelatin in the acid range, while at 30° C. the buffers dissolve the gelatin throughout the pH range used. In warm weather it is therefore necessary to perform the experiment in a thermostat. Gilman and Cowgill[3] used Gates's

[1] F. L. Gates, *Proc. Soc. Exp. Biol. Med.*, 24: 936, 1926–27.

[2] R. M. Bond, *Bull.* Bingham Oceanographic Coll., Yale Univ., 4: art. 4, p. 1, 1933.

[3] A. Gilman and G. R. Cowgill, *Jour. Biol. Chem.*, 88: 3, 743, 1930.

method at a temperature of 25° C.; presumably this is just below the critical temperature for solution of the gelatin on the Eastman commercial ortho film.

After a period of about two hours the plate is gently rinsed, fixed in 4 per cent. formalin to prevent further action of the adsorbed enzymes, washed and stained in either acid fuchsin or Delafield's hematoxylin. The former is preferable for the acid and the latter for the alkaline range, since contact with the buffer solutions alters the staining properties of the gelatin unless washing is unnecessarily prolonged for practical purposes. When digestion is complete a clear spot appears; with partial digestion a clear ring is commonly obtained, since action proceeds most rapidly around the edges of the drop. The method may be adapted for approximate quantitative purposes by placing drops on the plate at ten- or fifteen-minute intervals and choosing an arbitrary end point, such as the first appearance of a clear ring. Thus with just over a fifth of a cc of the original sample, by taking 0.01 cc portions and diluting each with 0.09 cc of various buffers, twenty different pH mixtures may be tested and both the pH range and the approximate pH optimum determined, since with care at least eight or perhaps nine small drops may be recovered from each. This procedure was devised to demonstrate the presence of both a pepsin and a trypsin in the abdominal digestive glands of spiders, the whole experiment involving 160 or more test spots being performed on a single extract from a single specimen.

The approximate sensitivity of the method was tested with four different dilutions of Merck's pancreatin, the end point as determined by the gelatin plate method being compared with that as determined by the digestion of casein. In the first case one drop of the sample was mixed with five drops of diluted glycocoll buffer at pH 9.0 (1 part of buffer to 3 parts of distilled water); in the second case 0.5 cc of the sample was added to 2.5 cc of 0.1 per cent. casein in diluted buffer as before. The digestion period was two hours.

Final dilution of enzyme solution	Gelatin plate	0.1 per cent. casein, tested with 1 per cent. acetic acid	
	24° C.	24° C.	39° C.
1: 3000	Clear	Clear	Clear
1: 6000	Half digested	Clear	Clear
1: 30,000	No detectable action	Cloudy	Faintly cloudy
1: 60,000	No detectable action	Cloudy	Cloudy

From these preliminary data it appears that the method is sufficiently sensitive for biological purposes. The thickness of the gelatin film on the Eastman lantern slide plates is about 15 microns; no doubt the sensitivity of the method could be greatly increased by the preparation of much thinner gelatin films. It was also found that the sensitivity of the plates is greatly decreased if they are stored for some months after clearing.

Amylases

A very thin starch film is prepared by mixing equal parts of hot solutions of 2 per cent. starch and 4 per cent. agar, the resultant hot mixture being poured on a previously heated glass plate and allowed to drain first from one side, then from the other. When dry this plate is used to test for amylases in the same manner as the gelatin plate for proteases. The test may be performed at room temperature or in a thermostat at about 39° C. At the end of the digestion period the plate is rinsed and stained in a solution of dilute iodine; if digestion is complete a clear spot appears, with partial digestion the spot is only partially cleared and often reddish from the erythrodextrins produced. It is necessary to use a drop of the buffer solution as control, since contact with the buffer slightly changes the appearance on staining and this may otherwise be mistaken for weak digestion. On account of the difficulty of preparing a perfectly uniform film, this method can not be used for quantitative work; several attempts were made to pour a uniform film by carefully levelling the plate and allowing it to dry in the horizontal position, but because of the tendency of the starch granules to form irregular clumps the results were unsatisfactory.

The approximate sensitivity of this method, which is very high, was determined with different dilutions of Merck's pancreatin; the end point as determined by the starch-agar plate method being compared with that as determined by the digestion of soluble starch. In the first case one drop of the test solution was mixed with five drops of diluted phosphate buffer at pH 6.75 (1 part of buffer, 1 part of 1 per cent. NaCl, 2 parts of distilled water); in the second case

Final dilution of enzyme solution	Starch-agar film		Soluble starch solution tested with dilute iodine	
	24° C.	39° C.	24° C.	39° C.
1: 12,000	Clear	Clear	Clear	Clear
1: 18,000	"	"	Reddish	"
1: 24,000	"	"	Blue	Purplish
1: 30,000	"	"	"	Blue
1: 60,000	"	"	"	"
1: 120,000	Almost clear	"	—	—
1: 240,000	Half cleared	"	—	—
1: 480,000	Very slight action	Almost clear	—	—
1: 600,000	"	"	—	—

0.5 cc of the test solution was added to 2.5 cc of soluble starch in diluted buffer solution with NaCl. The digestion period was two hours.

The above tests indicate that the method is at least twenty times as sensitive as the modification of Wolgemuth's method with which it was compared; it is capable of detecting at least one part in half a million of Merck's pancreatin.

EFFECT OF PYREX GLASS

In connection with these experiments we should like to record an apparent inhibition of enzyme action obtained by extracting in pyrex glass. At a stage when the pancreas of *Amblystoma* embryos normally gives a strong amylase reaction, it was found that negative results were obtained if the extract was ground and kept in a pyrex mortar for some hours; similar effects were not obtained with ordinary soft glass nor with quartz. At the same stage the pancreas gives a strong trypsin reaction whether pyrex is used or not, but at an earlier stage when the presence of trypsin can first be demonstrated, negative results were obtained when pyrex was used in the preparation of the extract. It thus appears that pyrex glass has a definitely inhibitory effect on both trypsin and amylase.

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SPECIAL ARTICLES

OBSERVATIONS ON FUNCTIONAL INTERRELATIONSHIP BETWEEN THE ADRENAL AND PARATHYROID GLANDS

EARLIER concepts of functional interrelations between the endocrine organs were based largely upon hypothetical assumptions. More recently, however, reliable experimental evidence has been accumulating to prove specific, more or less complex, functional interdependence of certain endocrine glands. While in no instance does the evidence warrant final conclusion or definite functional interpretation, in many cases it points the way to further investigation and to a better understanding of some phenomena observed in experimental animals.

In the case of the adrenal gland so little is known at present, in spite of the voluminous literature, that functional interpretations from experimental evidences should be made with caution. It is not the purpose of this communication to attempt to prove a definite correlation of function between the adrenal and parathyroid glands. Nevertheless, there are some experimental results that suggest very strongly that such a correlation exists. These have opened avenues for further investigation which is in progress.

Rogoff and Stewart[1] observed that in animals deprived of their adrenal glands symptoms commonly develop which are also seen associated with disturbances in calcium metabolism. Muscular twitching, spasms and tetanic convulsions often occur. This observation led us to study the calcium content of the blood in adrenalectomized dogs, with the very interesting results showing a decided hypercalcemia in most of the animals.[2]

In our previous studies on adrenalectomized dogs[1] a striking picture in the alimentary canal was revealed at autopsy. Profound congestion of the mucosa of the entire gastro-intestinal tract and severe hemorrhage into the intestine was commonly found. It is significant, in view of the present subject, that a similar picture was described by Collip and co-workers[3] as occurring in dogs following administration of excess of parathyroid hormone.

In the course of our investigations I have observed that animals deprived of both adrenal glands and those subjected to sub-acute or chronic adrenal cortical insufficiency (by sub-total ligations of adrenal blood vessels) show, at autopsy, a relatively high incidence of parathyroid enlargement. While this is quite obvious grossly it has not been possible, thus far, to detect any significant microscopic changes in the gland.

Recently, Schour and collaborators[4] have found very interesting disturbances in calcification of dentin in the rat incisor, following administration of parathyroid hormone. At Dr. Schour's suggestion we undertook to study the influence of removal of the adrenal glands. This investigation is still in progress, but the results thus far obtained show a remarkable resemblance between the changes which occur in teeth of white rats following administration of an excess of parathyroid hormone and those found after excision of the adrenal glands.

These significant observations indicate an accumulation of experimental evidence which supports the view that a functional correlation exists between the adrenal and parathyroid glands. Whether the cortex

[1] Rogoff and Stewart, *Am. Jour. Physiol.*, 78: 683, 1926.

[2] Rogoff and Stewart, *Am. Jour. Physiol.*, 86: 25, 1928.

[3] Collip, *Am. Jour. Physiol.*, 76: 472, 1926; Collip, Clark and Scott, *Jour. Biol. Chem.*, 63: 439, 1925.

[4] Schour, Tweedy and McJunkin, *Am. Jour. Path.*, 10: 321, 1934.