Whether differences between the iodine content of the water and of the "accessory" foods (yeast, alfalfa, cod liver oil) used in the two laboratories might explain the small differences in outcome is not apparent. One feature already emphasized by Hanzlik, Talbot and Gibson is particularly noteworthy, namely, the apparent harmlessness of very prolonged administration of iodide.

Mr. G. B. Moment of the Osborn Zoology Laboratory, Yale University, has kindly made histological examinations of the thyroids of all of the rats observed. He reported the absence of detectable differences in structure; on the other hand rats raised in our laboratory on diets without any additions of iodine other than that present in the natural foods and drinking water used showed somewhat defective thyroids.

4980

Vital Staining With Methyl Red.

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A generally accepted hypothesis advanced to explain the permeability of protoplasm to certain substances is that these substances penetrate because of the solubility of their associated molecules in the plasma-membrane of the living cell.

This hypothesis would account satisfactorily for the vital staining of the basic dye, neutral red, which is a chloride or iodide of a colored organic base. At the pH of the normal medium of living cells, viz., a pH greater than 7.0, this dye is presumably in its least dissociated state and it readily penetrates and accumulates within the cells. When the stained cells, e.g., marine ova, are placed in a medium having a pH of about 6.0 which is lower than that of the protoplasm[1, 2] the color quickly washes out. Moreover, vital staining with this dye does not occur when the outside medium is more acid than the protoplasm. Apparently, therefore, the dye passes readily through the plasma-membrane from a medium of a higher to that of a lower alkalinity.

An interesting case which is explicable on the same hypothesis of

[1] Needham, J., and D. M., *Proc. Roy. Soc., London*, 1926, xcix, 173.

[2] Chambers, R., and Pollack, H., *J. Gen. Physiol.*, 1927, x, 739.

permeability is the vital staining with methyl red. This dye which has not yet been recorded as a vital stain, except in the special instances which are noted below, is an amphoteric electrolyte possessing both a carboxyl and an amino group (carboxylic benzene azo dimethyl aniline).

Because of this it exists in a dissociated state both on the alkaline and acid side of its neutral pH range which lies between pH 5.0 and 5.5. In its alkaline range there is a low dissociation of the basic group while the acid group is highly dissociated. The dissociated state of the acid group would account for the fact that methyl red is not a vital stain at the pH of fluids which surround lving cells under normal conditions, their pH being well on the alkaline side of 6.0.

The following experiments show that when the environment of living cells is brought to a pH of about 5.5 methyl red acts as a vital stain.

Samples of sea-water the normal pH of which is about 8.4 were brought to a pH of 5.0 and 6.0 by the addition of sodium acid phosphate. Several drops of Clark and Lub's standard 0.4% aqueous solution of methyl red were added to the samples and also to normal sea-water. Mature starfish eggs were then immersed in the several samples. After several minutes to half an hour the eggs were removed and washed with sea-water at the same pH. The eggs from the sea-water having a pH of 8.4 and of 6.0 were unstained, while those in sea-water at pH 5.0 were brilliantly and diffusely colored yellow and contained red granules. The colored eggs were then transferred to sea-water having a pH above 6.0 whereupon the color quickly passed out of the eggs.

Similar experiments with the fresh water *Amoeba dubia* gave analogous results, viz., they were vitally stained with methyl red only when the pH of the medium was definitely below 6.0.

The above results may be explained on the basis of penetrability of associated molecules since methyl red is largely associated in an aqueous medium in the neighborhood of pH 5.0. At a more alkaline pH the acid group dissociates and the dye behaves as a dissociated acid salt.

Schaede[3] describes the staining yellow of the protoplasm of onion-skin cells immersed in a carmine-red solution of acidified methyl red, with various organic and inorganic acids. He also found that no protoplasmic staining occurs when the medium is alkaline.

[3] Schaede, R., *Ber. d. deutsch. bot. Ges.*, 1924, xlii, 219.

In this respect, therefore, the permeability of plant protoplasm appears to be similar to that of the material described in this paper.

Since methyl red is amphoteric and, by virtue of its basic group, should become dissociated also in the pH ranges below 5.0 attempts were made to ascertain if, in spite of this, vital staining would still occur. This was found not to be feasible. At a pH of 4.0 and below both the marine ova and the amebae become moribund. In addition the methyl red becomes increasingly insoluble.

4981

Anaphylactic Reactions Produced by Azodyes in Animals Sensitized With Azoproteins.

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In the course of investigations on inhibition effects[1] in animals sensitized with azoproteins, anaphylactic reactions were observed following the injection of azodyes containing the same specific group as the antigen. These reactions did not occur regularly, but in a considerable number of animals; in some batches of animals the results were not well pronounced.

In such an experiment guinea pigs were sensitized with an azoprotein prepared from dextro-paraaminotartranilic acid and horse serum[2] and were found to be sensitive to 0.35-0.7 mg. of an antigen made from dextro-paraaminotartranilic acid and chicken serum. To such animals were given intravenous injections of an azodye made by coupling diazotized dextro-paraaminotartranilic acid and resorcinol. Two guinea pigs injected with 5 mgs. each did not show any symptoms; of 8 which received doses from 0.5 to 2.5 mgs. all but one showed reactions which consisted, in 5 animals, in a temperature drop from 1.3° to 2.4°C. and slight anaphylactic symptoms or general weakness and paresis of the hind legs. The 3 remaining animals showed typical severe anaphylactic shock and died, one after night, one after an hour and the third after 4 minutes. Eight other animals of this batch were injected in the same way with an azodye prepared from levo instead of dextro-paraaminotartranilic acid. These showed no anaphylactic symptoms and there

[1] Landsteiner, K., *J. Exp. Med.*, 1924, xxxix, 631.

[2] Landsteiner K., and van der Scheer, J., *J. Exp. Med.*, 1929, l, 407.