COMMENTS AND COMMUNICATIONS

Comments relating to articles which have recently appeared in the Journal of Cellular and Comparative Physiology and brief descriptions of important observations will be published promptly in this Section. Preliminary announcements of material which will be presented later in more extensive form are not desired. Communications should not in general exceed 700 words.

THE USE OF MANGANESE IN THE HISTOCHEMICAL DEMONSTRATION OF ACID PHOSPHATASE[1]

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In presenting his valuable technique for the histochemical demonstration of acid phosphatase, Gomori ('41) remarked on the variable character of the pictures sometimes obtained. This variability is not a handicap in tissues which have a high phosphatase content, but I have found that it virtually precludes the achievement of consistent results in early embryonic tissues, in which the enzyme concentration is generally low.

A search for a suitable activator for the acid phosphatase of the chick embryo led to the discovery that manganous sulfate, added to the lead nitrate-sodium glycerophosphate incubating solution, has a marked and peculiar effect. Ordinarily the deposits that are visualized when the lead phosphate formed during the course of incubation is treated with sulfide range from pale golden-brown to black, and appear to be spread evenly through the cells in which the enzyme acts. After incubation with manganese however, even tissues which are very poor in acid phosphatase show deposits that are always intensely black, and are laid down in the form of fine discrete granules. These granules, even when few in number, appear with gratifying consistency in differ-

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ent samples of tissue. They are formed regularly, that is, in tissues in which the appearance of simple lead sulfide is sporadic, but they do not occur in tissues in which no deposits are ever found without the use of manganese. They have the further advantage of being easily visible, no matter how sparsely distributed, against almost any counterstain.

That this effect is not due primarily to activation of the enzyme, but rather to the formation of a lead-manganese complex, is indicated not only by the difference in appearance of the deposits, but also by the fact that the black granules are not soluble in xylol. The lead sulfide usually formed does dissolve in clearing agents, so that the deposits are easily lost in the course of a few minutes while the slides are being mounted; this solubility probably accounts in some part for the fugitive results noted by Gomori and also by the writer (Moog, '43). In comparative tests made on sections from a 6-day chick embryo, all the lead sulfide, including the heavy deposits in the liver, disappeared in 45 minutes' exposure to xylol, but the deposits formed during incubation with manganese did not dissolve out of any region in the course of 30 hours in xylol. The complex nature of the deposits is further shown by their failure to appear when lead is omitted from the incubating solution; manganese alone does not produce any visible result.

The method used is that of Gomori, with the addition of $MnSO_4$ to the incubating solution (prepared in these tests with mixed α and β glycerophosphate according to the formula given by Moog, '43). A final strength of 0.01 M for $MnSO_4$ was found to give most satisfactory results, but the black granular deposits appear in the range from 0.007 M to 0.05 M; at 0.001 M the manganese salt produced no effect, and at 0.01 M the slides became covered with a coarse precipitate which stained black with sulfide, while the phosphatase action itself was suppressed.

It is important in the use of this technique that the incubating solution, without manganese, be free of the fine precipitate it tends to throw down for some time after being mixed; if the solution is not perfectly clear, the precipitate, which stains with sulfide, becomes deposited on the slide and so confuses the results. The difficulty may be avoided by making up the solution 24 hours before using; after standing, the clear liquid may be poured off from the precipitate, which sticks to the container. The manganese is added just before use. A further precaution to be observed is that the incubation must not be allowed to continue too long, for the granules spread somewhat from points of heavy concentration. Four to 5 hours' incubation, at 38°, was found satisfactory for most tissues of the 6-day chick embryo.

The impossibility of comparing exactly the deposits formed in the presence and absence of manganese makes it difficult to say whether

any true activation of the phosphatase is also involved. Such an effect might be expected, since Bamann ('40) found that Mn^{++} activates alkaline liver phosphatase, while Massart and Vandendriessche ('40) reported a similar phenomenon with yeast phosphatase at pH 6.7. But acid phosphatase has long been considered not susceptible to activation by bivalent cations (cf. Folley and Kay, '36), and in any case Mn^{++} activation of enzymes generally occurs, as Nachmansohn ('40) has pointed out, at lower concentrations than those found necessary in this work. When the reaction reported here is run for a short time, and the deposits compared in water, without passage through xylol, it becomes clear that the complex deposits do not appear sooner than the simple lead sulfide deposits. Thus the advantage of using manganese lies not in speeding the hydrolytic action, but rather in producing deposits which are easily seen and are not attenuated in mounting. Yet for these reasons, of course, the incubation with manganese does not have to be continued as long as without, since no allowance need be made for masking of the deposits by the counterstain, or for loss in handling. With 6-day embryos 8 to 10 hours' incubation without manganese was in general required to achieve final results comparable to those obtained in 4 to 5 hours with manganese.

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