carbamates as herbicides indicate that some kinds of plants are more readily affected than others (*6–8*). Preliminary experiments to be reported elsewhere indicate that young fruits of apple are not as sensitive to 3-Cl-IPC as are those of peach.

Although extensive tests on animal toxicity have not been conducted, laboratory tests with small animals carried out by the Industrial Hygiene Foundation of America, Inc., Pittsburgh, Pa., for the Columbia-Southern Chemical Corporation, show that 3-Cl-IPC and the parent carbamate are relatively nontoxic.[1]

References

1. BATJER, L. P., and HOFFMAN, M. B. *U. S. Dept. Agr. Circ. No. 867* (1951).
2. HIBBARD, A. D., and MURNEEK, A. E. *Proc. Am. Soc. Hort. Sci.*, **56**, 65 (1950).
3. ANDERSON, W. P., LINDER, P. J., and MITCHELL, J. W. *Science*, **116**, 502 (1952).
4. MARTH, P. C., and SCHULTZ, E. S. *Am. Potato J.*, **29**, 268 (1952).
5. DOXEY, D. *Ann. Botany* (London), **51**, 229 (1949).
6. MITCHELL, J. W., and MARTH, P. C. *Science*, **106**, 15 (1947).
7. STEVENS, L. F., and CARLSON, R. F. *Proc. 6th Ann. Northeast. Weed Control Conf.*, 33 (1952).
8. WITMAN, E. D., and NEWTON, W. F. *Proc. 5th Ann. Northeast. Weed Control Conf.*, 45 (1951).

Manuscript received February 20, 1953.

[1] Appreciation is expressed to E. D. Witman of the Columbia-Southern Chemical Corporation, Pittsburgh, Pa., for supplying this information on animal toxicity and the 3-chloro-isopropyl-N-phenyl carbamate used in these studies.

Cells with Metachromatic Cytoplasmic Granules in the Stroma of Human Chorionic Villi

John S. Latta and Charles R. Beber[1]

Department of Anatomy, College of Medicine, University of Nebraska, Omaha

In the course of reinvestigating the histological characteristics of the human placenta with the use of various histochemical techniques, certain cells in mesodermal cores of chorionic villi were found to contain metachromatic, eosinophilic granules in their cytoplasm. These seemed to us to be of such interest as to warrant some discussion.

These granular cells were found in the villi of all of 14 placentas examined, ranging in age from 3.5 weeks to term. All material in which they were demonstrable was fixed either in 4% basic lead acetate, or 5% trichloracetic acid, after which paraffin-embedded sections were cut at 5 μ and stained with toluidine blue to demonstrate metachromasia, and with Wright-Giemsa stain to demonstrate eosinophilia. These specific granules were most abundantly demonstrated after basic lead acetate fixation. They were always found in villous stroma but rarely encountered in the basal chorion, placental septa, or amnion.

The granule-bearing cells were quite large, ranging from 12 to 18 μ in diameter. They were generally roughly spherical in shape (Fig. 1) but in many cases exhibited long processes (Fig. 2). The cell membranes quite indistinct, the limits of the cytoplasm being frequently demarcated by the contained granules (Figs. 1–4). The intergranular cytoplasm sometimes seemed to exhibit metachromasia, which was thought to be due to solubility of the granules. The nuclei were spherical or bean-shaped and homogeneously basophilic, on which background were superim-

[1] C. W. M. Poynter Fellow in the medical sciences.

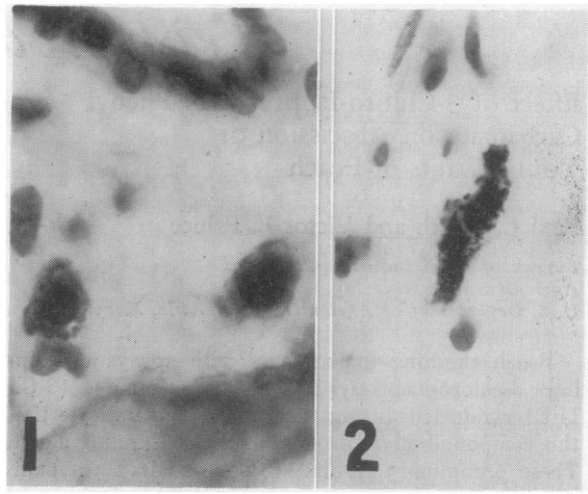

FIG. 1. Portion of a chorionic villus from a term placenta showing two large rounded cells with coarse, eosinophilic cytoplasmic granules in the stroma. Fixation in 4% basic lead acetate, Wright-Giemsa stain. × 1140.

FIG. 2. Villous stroma from a term placenta showing a single cell packed with coarse metachromatic granules. Fixation in 4% basic lead acetate; toluidine blue stain. × 1140.

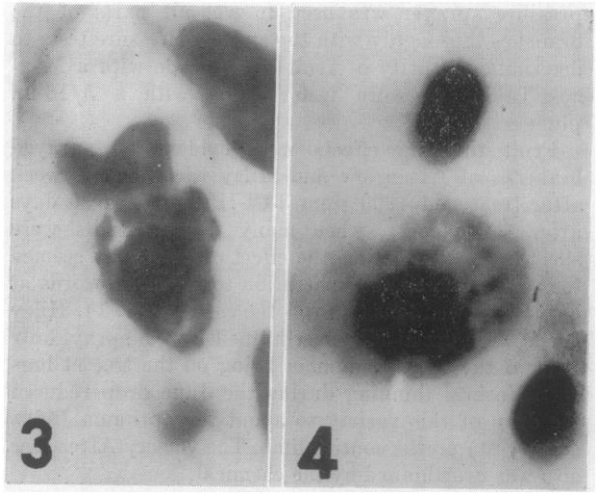

FIG. 3. The surface trophoblast and underlying stroma from a term placenta with a large cell containing very coarse eosinophilic cytoplasmic granules and a condensed diffusely stained nucleus. Fixation in 4% basic lead acetate; Wright-Giemsa stain. × 2190.

FIG. 4. Another large cell in a villus from a placenta of 6 weeks, containing numerous coarse cytoplasmic granules, which stained metachromatically with toluidine blue. Some cytoplasmic metachromasia is apparent. Fixation with 4% basic lead acetate. × 2190.

posed clumped chromatin granules. No nucleoli were observed. These nuclear and cytoplasmic features made the cells easily distinguishable from Hofbauer or phagocytic cells.

The specific granules were quite soluble with routine fixation, although some granules were seen streaming out into the villous stroma and apparently dissolving. With the special fixatives the number per cell varied from 10 to 12 to huge numbers densely packed in the cytoplasm, obscuring the nucleus (Fig. 2). These variations may be real or the result of difficulties of fixation. In all material fixed in basic lead acetate the granules were eosinophilic after Wright-Giemsa or azur II-eosin staining and metachromatic after toluidine blue, but remained unstained after methyl green-pyronin.

The constancy of their presence in chorionic villi of placentae of such widely varied stages and their consistent staining qualities after certain fixatives led to the conclusion that these granule-bearing cells may represent an important feature of the histological structure of the chorionic villus. The eosinophilic staining quality and constancy in size and shape of the granules make it highly improbable that they represent phagocytized material. Cells with such metachromatic granules have been reported in the endometrium and myometrium of the bat uterus by Wimsatt (*1*) and by Asplund and Holmgren (*2*) in human uterine mucosa. The latter authors found them only during the fertile period and varying with the stage of the menstrual cycle. They found them rarely during estrogenic stimulation, but in increasing numbers during progestational stimulation, with a premenstrual peak. They apparently disappeared with the menses.

A recent contribution by Pescetto (*3*) describes cells with metachromatic and basophilic granules in placental tissue after 4% basic lead acetate. His figures indicate a marked resemblance to the cells we describe except for the basophilic reaction of the granules he reported and the age range in which he found them.

Although further chemical assay of these granules has not been attempted, it may be pointed out that Sylvén (*4*) in studies of mast cells has demonstrated that heparin is manufactured as a lipoprotein complex in such cells, and that heparin is metachromatic and eosinophilic. It might be suggested that the cellular granules we have described represent heparin or heparinoid substances to be associated with incoagulable, free-flowing blood in such locations where one might assume it would clot. Such granule-bearing cells have been reported only in uterine mucosa (*1, 2*).

The granules we have studied fail to stain with pyronin in Taft's (*5*) method for nuclear protein and are not destroyed by ribonuclease. The special fixers required for their preservation indicate that they are not a mucopolysaccharide.

It is interesting to note that current therapy as used by Rumbolz, Moon, and Novelli (*6*) in certain types of abnormal uterine bleeding involves intravenous introduction of toluidine blue or protamine sulfate, both of which are known to inactivate heparin. The most striking feature of the cytoplasmic granules we have described is the intensity with which they stain with toluidine blue.

We would suggest then that the metachromatic eosinophilic granules consist of heparin or heparinoid substances that normally are related to the function of maintenance of incoagulable blood in the intervillous spaces of the placenta.

References

1. WIMSATT, W. A. *Am. J. Anat.*, **84**, 63 (1949).
2. ASPLUND, V., and HOLMGREN, H. *Acta Anat.*, **3**, 312 (1947).
3. PESCETTO, G. *Biol. Latina*, **2**, 744 (1950).
4. SYLVÉN, B. *Exptl. Cell Research*, **2**, 252 (1951).
5. TAFT, E. B. *Ibid.*, 312.
6. RUMBOLZ, W. L., MOON, C. F., and NOVELLI, J. C. *Am. J. Obstet. Gynecol.*, **63**, 1029 (1952).

Manuscript received September 17, 1952.

Effect of Salicylic Acid on the Hypoprothrombinemia Induced by Dicumarol in the Dog and the Rat[1]

John B. Field[2]

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, and ***Department of Medicine, University of Southern California School of Medicine, Los Angeles***

Salicylic acid readily induces a hypoprothrombinemia in the rat (*1*) and augments the hypoprothrombinemia induced by Dicumarol[3] (*2*) in the dog. The purpose of this report is to show that under specified experimental conditions, salicylic acid may actually *reduce* the hypoprothrombinemic response of Dicumarol in the dog as well as in the rat, and prolong the survival time of rats receiving the anticoagulant continuously.

The details of the animal experiments and of the modified one-stage prothrombin assay have already been given in detail (*1–4*). In control studies not included here, the prothrombin time of whole plasma (100%) and of the 50, 25, and 6.25% dilutions was measured. As before, the data will be restricted to the prothrombin time of 12.5% plasma (1 part oxalated plasma, 7 parts saline solution) for reasons emphasized in (*5*).

Acetylsalicylic acid[4] will increase and prolong the

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Abstracted from a thesis submitted by John B. Field to the Graduate Faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree, May 1944. This work was supported through special grants from the Graduate Research Committee of the university.

[2] Present address: Department of Medicine, University of Southern California, Los Angeles 33.

[3] Dicumarol is the trademark for the anticoagulant 3,3′-methylenebis (4-hydroxycoumarin).

[4] Acetylsalicylic acid (aspirin) caused the most drastic hypoprothrombinemia of a large number of salicylate compounds tested in the rat (*6*).