Placental Esterases.* (20671)

SUMNER I. ZACKS† AND GEORGE B. WISLOCKI. (Introduced by A. Baird Hastings.)
From the Department of Anatomy, Harvard Medical School, Boston, Mass.

Since the placentas of man and animals lack innervation(1), it is of considerable interest that acetylcholine(2-4) and a cholinesterase (5) have been identified in human placentas. Torda found that cholinesterase activity of human placentas at full term approximated one tenth of that of pig's pyloric mucosa. Ord and Thompson(6) reported that homogenates of fresh placentas hydrolysed acetylcholine, benzoylcholine and acetyl β-methylcholine, but that homogenates prepared from perfused placentas possessed much less activity toward benzoylcholine. According to the criteria of Mendel, Mundell and Rudney(7), these results indicate that both acetylcholinesterase and serum cholinesterase are present in the placenta. Further evidence of the occurrence of cholinesterases was the inhibition of the enzymes by di-isopropyl fluorophosphate in a concentration similar to that required to inhibit the cholinesterases of brain. Wen, Chang and Wong(8) devised a method, which they believed demonstrated the activity of acetylcholine in tissue sections, by means of which they identified large amounts of acetylcholine in the syncytial trophoblast of the chorionic villi and in the intervillous space of the human placenta. With the recent development of several histochemical methods for the demonstration of esterases, it seemed advantageous to explore the placenta by these means and with the use of inhibitors to differentiate between various enzymes of the esterase groups. From an examination of a normal human placenta of 10 weeks' gestation age (9,10), it was found that with the method for esterase of Barrnett and Seligman(11) on unfixed frozen sections there is an intense reaction in the trophoblast clothing the chorionic villi, involving mainly the syncytium. With the esterase method of Nachlas and Seligman (12), however, carried out on sections of acetone-fixed, paraffin-embedded material, the trophoblast showed no reaction. From these observations, the conclusion was reached that the combination of a positive Barrnett and Seligman reaction on unfixed frozen sections, and of a negative reaction by the method of Nachlas and Seligman upon acetone-fixed sections, indicated the presence of a cholinesterase and possibly a small amount of aliesterase in the trophoblast.

The present paper describes the results of histochemical experiments on a human and a cat placenta in an attempt to determine by the use of inhibitors which esterolytic enzymes are present.

Material and methods. Small blocks of tissue were obtained from a placenta of a cat, near the middle of gestation (fetal crown-rump length: 7.2 cm). Other blocks were obtained from a normal human placenta of 10 weeks of gestation (fetal crown-rump length: 3.2 cm). From the human specimen pieces of both labyrinth and decidua were taken. Several blocks were placed in cold acetone (4°C) and then embedded in paraffin and sectioned. Other blocks were frozen and sectioned in a cryostat without any previous fixation. Deparaffinized sections of the acetone-fixed material were stained by the methods of Gomori (13) and Nachlas and Seligman(12). The method of Barrnett and Seligman(11), which employs indoxyl acetate as the chromogenic substrate, was applied to fresh frozen sections. Frozen sections were also stained by the method of Ravin, Zacks and Seligman(14), which employs 6-bromo-2-naphthyl acetate and tetrazotized diorthoanisidine. The method of Ravin, Tsou and Seligman(15) was employed to detect serum cholinesterase activity by means of a specific substrate, carbonaphthoxycholine iodide and tetrazotized diorthoanisidine. Frozen sections were also pretreated for one hour in barbital buffer (.1 M at pH 7.5) containing physostigmine salicylate (3.1×10^{-5} M or 6.2×10^{-5} M)

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† Jeffries Wyman Scholar, Harvard University, 1952-53.

or sodium taurocholate 10^{-3} M, and staining was carried out in the presence of these inhibitors to distinguish cholinesterase from aliesterase and lipase. *Acetylcholinesterase* and serum *cholinesterase* are completely inhibited by low (10^{-5} M) concentrations of physostigmine(16,17), but aliesterase is somewhat inhibited when a concentration of 10^{-3} M is reached. The activity of both cholinesterases is completely destroyed by cold acetone, whereas the activity of lipase and aliesterase is at best 40-60% reduced by this treatment(12). The activity of serum cholinesterase can be distinguished from acetylcholinesterase, since only the former enzyme is able to hydrolyse the chromogenic substrate carbonaphthoxycholine iodide. Lipase is either activated or unaffected by sodium taurocholate 10^{-3} M (18), while aliesterase is inhibited to a variable but usually significant degree. Thus, this combination of reactions permits a differentiation of cholinesterases, on the one hand, from aliesterase and lipase on the other. An esterase which hydrolyses 6-bromo-2-naphthyl acetate and is inhibited by physostigmine (10^{-5} M) and cold acetone is regarded as a cholinesterase, but esterases which resist these inhibitors may be aliesterase, lipase or a mixture of both. Lipase can usually be distinguished by its activation or lack of inhibition by sodium taurocholate, and aliesterase, by its inhibition with this reagent.

Observations. Cat's placenta. After 20 minutes' incubation in 6-bromo-2-naphthyl acetate and tetrazotized diorthoanisidine(14) the epithelium of the subplacental uterine glands was filled with punctate, blue granules (Fig. 1), and a less intense reaction was present in the trophoblastic lamellae of the chorioallantoic placenta. Sodium taurocholate or physostigmine salicylate 3.1 x 10^{-5} M slightly inhibited the enzymatic activity in the trophoblast. The placenta and uterine glands failed to stain with carbonaphthoxycholine iodide and tetrazotized diorthoanisidine(15), a result which indicated the absence of serum cholinesterase. Acetone-fixed, deparaffinized sections stained by Gomori's procedure showed an intense reaction of the subplacental uterine glands, a milder reaction of the trophoblast facing the glands and no reaction of the lamellae of the placental labyrinth. The characteristic columnar cells of the brown border exhibited a variably strong reaction (Fig. 2), whereas the paraplacental uterine mucosa, including the surface epithelium and glands, was negative. The brown border was examined only in paraffin sections.

Human placenta. Frozen sections of the labyrinth of the human placenta, incubated for 20 minutes in a mixture of 6-bromo-2-naphthyl acetate, showed dense deposits of blue granules in the trophoblastic syncytium and lesser amounts in the Langhans cells (Fig. 3). The stroma of the chorionic villi, including the Hofbauer cells, did not react. The cells lining the uterine glands and the epithelium covering the decidua were somewhat less intensely reactive. Decidual cells of two types were observed; the larger of these were unreactive but the smaller contained some dye granules indicative of enzymatic activity. The large trophoblastic cells of the cell columns and basal plate were unreactive (Fig. 4). The uterine stroma and musculature appeared to be negative. Physostigmine salicylate 3.1 x 10^{-5} M slightly inhibited the enzymatic activity.

The method of Ravin, Tsou, and Seligman for serum cholinesterase was negative in all regions of the placenta. Acetone fixed, deparaffinized sections of placenta, stained by the esterase method of Nachlas and Seligman, were also completely negative.

Discussion. Since the enzymatic activity in the trophoblast and uterine glands of the cat's placenta is slightly inhibited by physostigmine and cold acetone and is not activated by sodium taurocholate, it is chiefly due to an aliesterase. The slight inhibition when physostigmine is used may be attributable to a small amount of acetylcholinesterase activity.

The esterase in human trophoblast and uterine glands behaves differently than acetylcholinesterase or aliesterase in other tissues. For example, the acetylcholinesterase activity of motor end plates is completely inhibited by cold acetone and 10^{-5} M physostigmine, whereas the activity of human placental esterase is abolished by cold acetone but only slightly inhibited by physostigmine. From the result of acetone fixation, it would appear that a

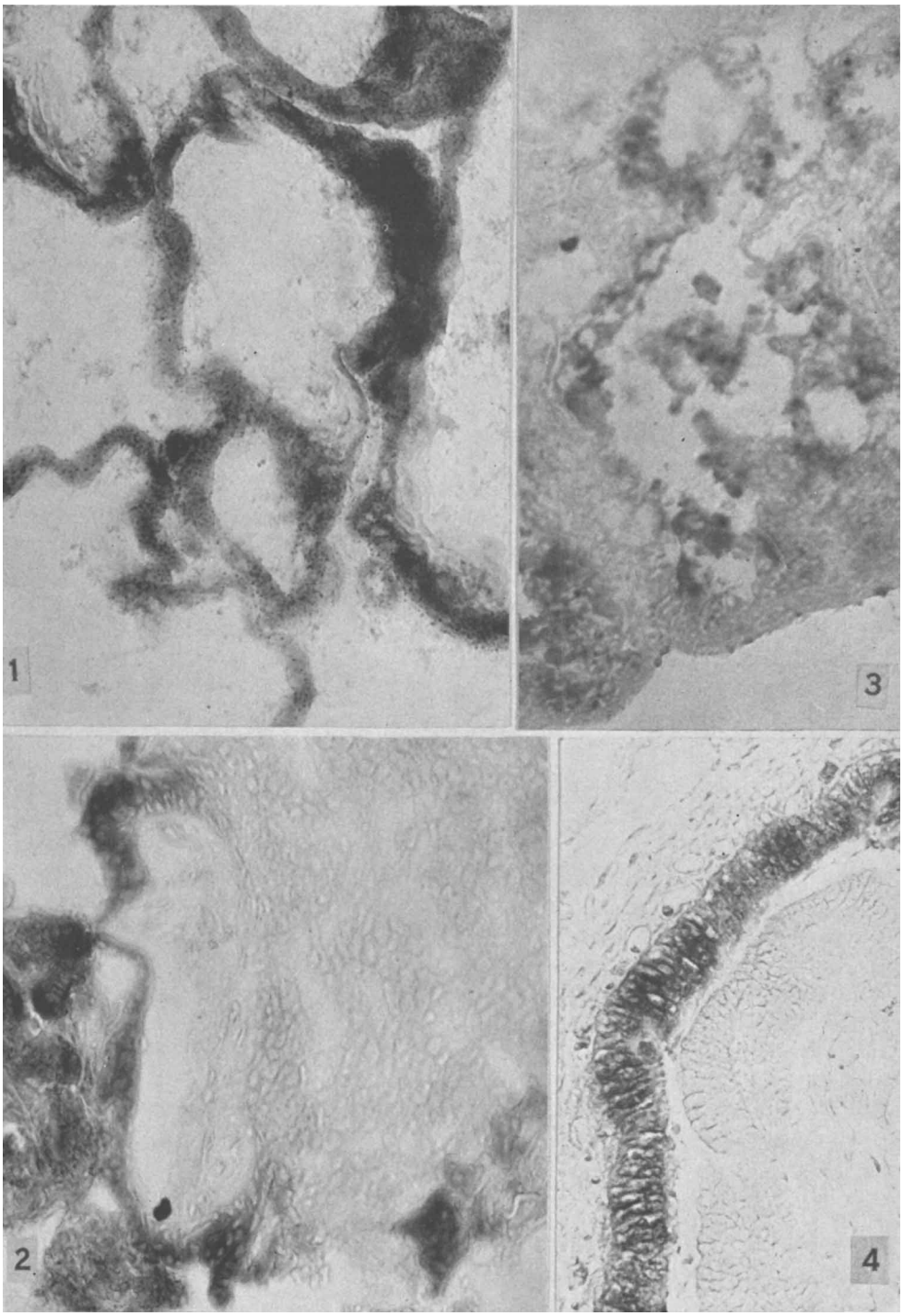

FIG. 1. Human placenta at 10 wk gestation showing intense esterase activity of chorionic villi by the method of Ravin, Zacks and Seligman. The granular reaction product is localized almost exclusively in the trophoblast (placental membrane) covering the villi. The chorionic stroma is negative. × 350.

FIG. 2. A portion of the same human placenta showing a secondary chorionic villus attached

to a trophoblastic cell column. The trophoblast of the secondary villus (left) reacts intensely for esterase, whereas the cell column is negative. × 350.

FIG. 3. Subplacental uterine gland of a cat, showing intense esterase activity (method of Ravin, Zacks and Seligman). × 250.

FIG. 4. The brown border of the membranous chorion of a cat showing a strong reaction for esterase in the columnar trophoblast (Gomori's method). The uterine epithelium opposite columnar cells is negative, as are also stroma and paraplacental glands of uterine mucosa. × 200.

cholinesterase is responsible for the esterase activity of human placenta, although the failure of physostigmine to inhibit this activity presents an unexpected difficulty. This apparent inconsistency may be explained either by the presence of low placental aliesterase activity which is inhibited completely by cold acetone, or by a cholinesterase which is resistant to low concentrations of physostigmine. The latter possibility gains added weight from the evidence of the presence of acetylcholinesterase and serum cholinesterase in the human placenta(6). Similar apparent inconsistencies have been noted in various tissues of the rat(19,20).

It is of interest that the esterase of human placenta differs from the esterase found in the cat's placenta. The cat's placental enzyme is presumably aliesterase, whereas the human placental enzyme is possibly a cholinesterase. The functions of esterases in the placenta are obscure. Since the organ lacks innervation, there is no apparent need for acetylcholinesterase which functions in synaptic transmission. One can only surmise that the esterases located in the trophoblast probably play a role in the transfer of substances across the placental barrier.

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