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HUMAN PLACENTAL CHOLINERGIC SYSTEM

OCCURRENCE, DISTRIBUTION AND VARIATION WITH GESTATIONAL AGE OF ACETYLCHOLINE IN HUMAN PLACENTA*

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Abstract—Although acetylcholine (ACh)-like activity was demonstrated in human placental extracts by a number of investigators, substances responsible for this activity were not identified. We found by gas chromatographic techniques that the major component of the ACh-like activity of the term placenta was ACh (112 ± 7 nmoles/g of wet tissue). These results were confirmed by the separation of ACh from other quaternary ammonium compounds by column chromatography using Amberlite CG-50 resin. The placenta could be stored at 4° for a number of days without significant loss of ACh. Freezing and thawing of the placenta destroyed ACh. This indicates that ACh is bound within membranes. There were high concentrations of ACh in all segments of the placenta. The ACh concentrations in the concentric segments next to the periphery and the umbilical cord were lower than ACh concentrations in other segments. The concentrations of ACh in floating villi and the basal plate, which included the anchoring villi, were about 322 and 210% respectively, of that of the chorionic plate. There was variation in ACh content with gestational age; the highest concentration was found at about 22 weeks (wk) of gestation (nmoles/g: at 9–12 wk, 129; 13–16 wk, 342 ± 31; 17–20 wk, 317 ± 32; 21–24 wk, 723 ± 63; 25–28 wk, 231; 29–32 wk, 249; 33–36 wk, 153 ± 15; 37–40 wk, 105 ± 7; and 41–44 wk, 88 ± 5). These observations associate ACh with syncytiotrophoblast. Choline acetyltransferase (ChA) has a similar pattern of variation with gestational age. The placental cholinergic system (as indicated by ChA ACh) was fully formed at the early fetal period of histogenesis and functional maturation, during which the fetus exhibits the fastest rate of growth.

A number of investigators have reported the occurrence of acetylcholine (ACh)-like activity in the extracts of human placenta using bioassay preparations [1–7]. However, the components of the ACh-like activity in placental extracts were not identified.

It was shown by Comline [8] in 1946 that there is choline acetyltransferase (ChA) in human placenta. The placental homogenates of the guinea pig, dog, cat, mouse, horse and cow were shown to synthesize ACh in the presence of exogenous acetylcoenzyme A [9]. These homogenates synthesized other products besides ACh. Human placental homogenates required exogenous acetylcoenzyme A as well as choline for synthesizing ACh. A number of investigators have obtained partially purified preparations of ACh from human placenta [10–12]. Recently, it has been shown that brain ChA and placental ChA will synthesize ACh by the same enzyme mechanism, namely the Theorell–Chance mechanism [12–15].

The presence of cholinesterases in human placenta has been indicated by a number of investigations [16–18]. The human placental enzyme has been characterized as acetylcholinesterase (AChE) by Ord and Thompson [19] and by Koshakji *et al.* [20]. Acetyl-β-methyl-choline is a specific substrate for the placental AChE, which does not hydrolyze benzoylcholine to a significant degree.

The above observations indicate that the three components of the cholinergic system, ACh-like activity, ChA and AChE, are present in the human placenta. Since the placentae of man and animals lack innervation, a question arises as to whether the placental cholinergic system is in any way similar to that of the brain cholinergic system. Human placental ChA and AChE have been characterized. In the present study, we are interested in the characterization of the components of the ACh-like activity of the human placenta by gas chromatography, its distribution, the nature of its occurrence and its variation with gestational age. The present study indicated that the major component of the ACh-like activity of human term placenta is ACh (112 nmoles/g of wet tissue), which is localized mainly in the syncytiotrophoblast layer. The placental ACh levels during the second trimester of pregnancy were higher than the ACh levels during the first and the third trimesters of pregnancy.

MATERIALS AND METHODS

Collection of human placentae. Human placentae were collected after therapeutic abortions during the first trimester, after spontaneous and therapeutic abortions during the second trimester of pregnancy

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and after therapeutic deliveries during the third trimester of pregnancy. Each placenta was placed in a plastic bag immediately after delivery and cooled in an ice bath. It was processed immediately or kept in a cold room at 4° for processing 1 or 2 hr later. The ACh content remained constant even when the placenta was stored for several hr at 4°.

Extraction and estimation of ACh and other quaternary ammonium compounds from placenta. One radial segment (unless otherwise specified) of the placenta was sliced, cleared of blood as much as possible and homogenized for 2 min with a solution of trichloroacetic acid (2%) in acetonitrile (1·0 ml/0·1 g of wet tissue), using a Sorvall Omni-Mixer. The homogenate (10%) was centrifuged at 2500 rev/min for 10 min. The sediment was discarded. The supernatant was diluted with an equal volume of distilled water. The aqueous supernatant was extracted twice with equal volumes of diethyl ether. The ether extracts containing the lipids were discarded. The residual ether in the aqueous supernatant was removed by bubbling a gentle stream of nitrogen gas through it. An aliquot of the aqueous supernatant (2 ml) was diluted with an equal volume of dilute HCl (2 ml, pH 4·3). To the diluted supernatant (4 ml), 0·05 ml tetramethylammonium iodide solution (12 mg/100 ml) and 0·15 ml potassium iodide (20%)–iodine (18%) reagent were added. The resulting mixture was stirred on a Vortex mixer and allowed to stand at 4° for 30 min. It was centrifuged, and the supernatant was removed by aspiration. The precipitate containing quaternary ammonium compounds (precipitate QC) was subjected to pyrolysis gas chromatography. ACh and related quaternary ammonium compounds were converted into their tertiary amines during the pyrolysis and the resulting tertiary amines were assayed by gas chromatography [21].

Pyrolysis gas chromatography of ACh and other quaternary ammonium compounds. ACh determinations were made according to the method described by Schmidt *et al.* [22] using a Hewlett–Packard model 5750 gas chromatograph and a Barber Coleman model 5180 pyrolyzer. The column of 20% Carbowax 6000 on Chromasorb W (HMDS) (Applied Scientific Co.) was prepared according to Schmidt *et al.* [23]. The column was run at 140° under a flow of nitrogen gas at 80 ml/min. Propionylcholine iodide was added as an internal standard to all tissue samples (20 nmoles/100 mg of wet tissue).

Separation of placental ACh from other quaternary ammonium compounds by column chromatography. Amberlite CG-50 resin (3·4 g) was washed with distilled water (3 × 30 ml) and the washed resin was soaked in distilled water (50 ml) overnight. The suspension of the resin was used to prepare a column (15 cm) in a 20-ml burette. The column was further washed with potassium phosphate buffer (100 ml, 0·5 M, pH 6·9). After the fluid was drained off the column, a solution (0·5 ml) of quaternary ammonium compounds (precipitate QC) from the placental extract was poured over the top of the column. After the sample was adsorbed by the column, the column was eluted with potassium phosphate buffer. During the elution period, about 2 ml of fluid was maintained over the column and 2-ml samples of the eluate were collected at a rate of 1 ml/min in an automatic fraction collector. The quaternary ammonium compounds in each fraction were extracted with acetonitrile, and ACh, choline and compound X were analyzed by pyrolysis gas chromatography.

In a separate experiment, [^{14}C]ACh (0·02 μCi) was subjected to separation by column chromatography. [^{14}C]ACh in the elution fractions was counted according to the procedure described elsewhere [12].

RESULTS

Occurrence of ACh and other quaternary ammonium compounds in placental extract. The pyrolysis gas chromatogram of a placental extract is shown in Fig. 1. During pyrolysis, the quaternary ammonium compounds are converted into their corresponding tertiary analogs. Peak B has been identified as 2-dimethylaminoethyl acetate, formed from exogenous ACh. The height of the peak could be increased quantitatively by the addition of known quantities of ACh to the placental extract. Peak B disappeared when the placental extracts were subjected to an alkaline hydrolysis (Fig. 2).

Peak A was due to 2-dimethylaminoethylpropionate, formed from propionylcholine which was added to the placental extract as an internal standard. Peak A was absent when placental extracts were analyzed without the addition of propionylcholine. Peak C was due to 2-dimethylaminoethanol, formed from choline in the placenta. Peak C was not affected by alkaline hydrolysis.

The compound responsible for peak X was not identified in our studies. It may be an amine present in placental extracts or formed during pyrolysis. Alkaline hydrolysis of the placental extracts had no effect on peak X. Peak X had a consistently longer retention time than peaks A, B and C.

Separation of quaternary ammonium compounds from the placental extract by column chromatography. It is possible to separate the quaternary ammonium compounds into two components using the cationic ion exchange resin Amberlite CG-50. In the elution chromatogram, the first component was collected in frac-

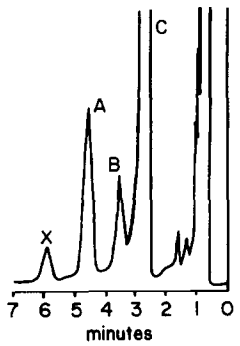

Fig. 1. Gas chromatogram of the quaternary ammonium compounds separated from the human placenta and subjected to pyrolysis. Peak A: 2-dimethylaminoethyl propionate formed from propionylcholine which was added as an internal standard. Peak B: 2-dimethylaminoethyl acetate formed from endogenous ACh. Peak C: 2-dimethylaminoethanol formed from endogenous choline. Peak X: an endogenous amine or a compound formed during pyrolysis which was not identified.

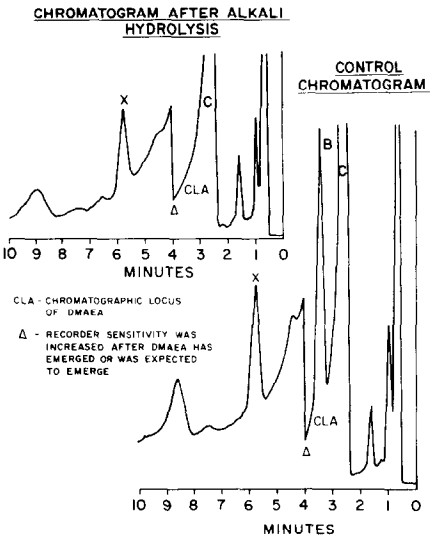

Fig. 2. Gas chromatogram of the quaternary ammonium compounds from the human placenta before and after alkaline hydrolysis. CLA: chromatographic locus of 2-dimethylaminoethyl acetate formed from endogenous ACh, which is indicated by peak B. Peak B was absent in the gas chromatogram of the endogenous quaternary ammonium compounds which were subjected to alkaline hydrolysis. Peak B: 2-dimethylaminoethyl acetate formed from endogenous acetylcholine of the placental extract during pyrolysis. Peak C: 2-dimethylaminoethanol formed from endogenous choline. Peak X: the compound which was not identified.

tions 6–12 and the largest amount was collected in fraction 9 (Fig. 3). When authentic [^{14}C]ACh was chromatographed under identical conditions, the largest amount appeared again in fraction 9 (Fig. 3). The pyrolysis gas chromatogram of fraction 9 exhibited a prominent peak, with a retention time of

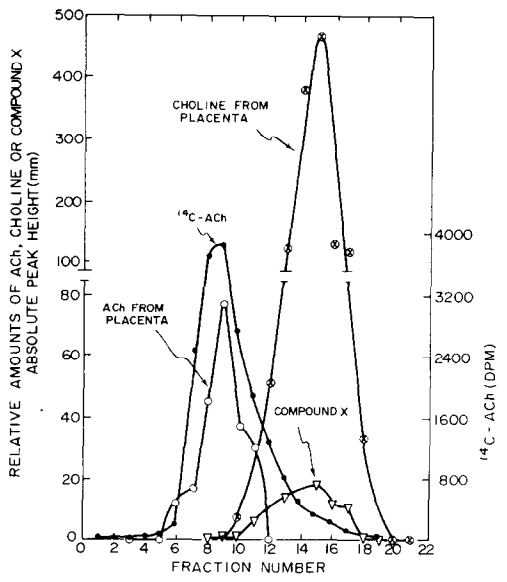

Fig. 3. Separation of the quaternary ammonium compounds from the placenta by chromatography on a column of Amberlite CG-50. The relative amounts of ACh, choline and compound X were analyzed by pyrolysis gas chromatography.

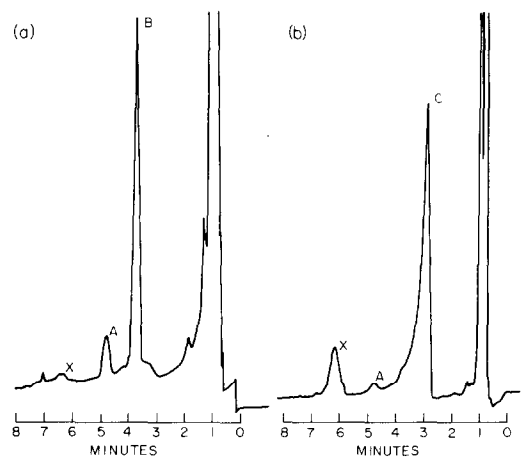

Fig. 4. Panel a: pyrolysis gas chromatogram of the quaternary ammonium compounds collected in fraction 9 of Fig. 3. Panel b: Pyrolysis gas chromatogram of the quaternary ammonium compounds collected in fraction 15 of Fig. 3. The two chromatograms were run at different electrometer settings. Peak A: 2-dimethylaminoethyl propionate formed from the internal standard, propionylcholine. Peak B: 2-dimethylaminoethyl acetate formed from endogenous ACh. Peak C: 2-dimethylaminoethanol formed from endogenous choline. Peak X: the compound which was not identified.

3·8 min (Fig. 4a). This peak was identified as that of 2-dimethylaminoethyl acetate, which was formed from ACh during pyrolysis. Therefore, the quaternary ammonium compound in fraction 9 was identified as ACh. No peak for the presence of 2-dimethylaminoethanol was observed. No significant peak for the presence of compound X was observed. Therefore, fraction 9 contains ACh, and is free from choline and compound X.

In the elution chromatogram, a second component was collected in fractions 10–18, and the largest amount was collected in fraction 15 (Fig. 4b). The pyrolysis gas chromatogram of fraction 15 indicated the presence of two prominent peaks, with retention times of 2·9 and 6·3 min. Pyrolysis of an authentic sample of 2-dimethylaminoethanol (or choline) gave a single peak with a retention time of 2·9 min on the gas chromatogram. The peak with the retention time of 6·3 min was due to the unidentified compound in the placental extracts.

According to the above experiment, fractions 6–9 contained ACh. Choline and the unidentified compound could not be detected in these fractions. Fractions 12–18 contained choline and the unidentified compound X. Fractions 11 and 12 contained all three compounds: ACh, choline and the unidentified compound X. Therefore, our experiments support the existence of ACh in human placenta.

Content of ACh in term human placenta. The ACh contents from ten term human placentae, collected after therapeutic deliveries, are shown in Table 1. There are significant differences in the concentrations of ACh from placenta to placenta. The reasons for these differences are not known. Some of these differences might be explained by the clinical histories of the mothers, the mode of delivery and the duration of labor, etc. According to our data from 34 deliveries,

Table 1. ACh in human term placenta*

Serial no. of placentae	ACh† (nmoles g wet tissue)	Unidentified compound in ACh equivalents‡ (nmoles g wet tissue)
1	61·51 ± 1·55 (5)§	20·98 ± 1·96 (3)§
2	116·90 ± 1·65 (7)	20·40 ± 1·35 (4)
3	141·43 ± 1·88 (3)	15·16 ± 0·37 (3)
4	131·77 ± 3·60 (3)	15·31 ± 0·26 (3)
5	77·20 ± 1·80 (4)	13·33 ± 1·10 (4)
6	91·37 ± 2·83 (3)	8·53 ± 1·02 (3)
7	51·68 ± 1·45 (4)	7·95 ± 0·44 (4)
8	93·84 ± 3·35 (3)	8·57 ± 0·15 (3)
9	129·97 ± 3·09 (3)	9·63 ± 0·37 (3)
10	91·10 ± 1·20 (3)	20·80 ± 2·12 (3)

* The whole placenta was extracted with acetonitrile and an aliquot of the extract was used for ACh analysis. Gestation period, 38–44 weeks.

† Mean (M_1) ± S. E. for the above first group of ten placentae: 98·66 ± 9·66 nmoles/g of wet tissue. A second group of 24 term placental samples (representative radial sections) was analyzed for ACh. Mean (M_2) ± S. E. for the second group: 117·63 ± 9·06. There was no significant difference ($P < 0·05$) between M_1 and M_2. The overall mean for 34 placentae of both groups was 112·05 ± 7·08 nmoles/g of wet tissue.

‡ Mean (M_3) ± S. E. for the above first group of placentae: 16·28 ± 2·84 nmoles ACh equivalents/g of wet tissue. Mean (M_4) ± S. E. for the second group of placentae: 14·07 ± 1·68. There was no significant difference ($P < 0·05$) between M_3 and M_4. The overall mean for compound X from both groups of placentae = 15·75 ± 1·83.

§ The values in parentheses indicate the number of aliquots analyzed from each placental extract.

human term placentae contained 112·05 ± 7·08 nmoles/g of wet tissue. The same group of placentae contained an unidentified compound X in small amounts (15·75 ± 1·83 nmoles ACh equivalents/g of wet tissue). This compound could be detected in 97–98 per cent of the placentae analyzed.

Distribution of ACh in the term human placenta. The three major regions (chorionic plate, villus region and basal plate) were dissected for ACh determinations as shown in Table 2. The relative distribution of ACh in various concentric segments of human placenta is shown in Fig. 5A. In order to determine this, each placenta was cut into six concentric segments and the segments were analyzed separately for ACh. The ACh concentration in the concentric segment next to the periphery or the umbilical cord was lower than the ACh concentration in the other segments. This suggests that there are higher concentrations of ACh where the placental villi are located. In order to verify this, we have dissected floating villi, the basal plate along with anchoring villi, the trophoblast layer and the chorionic plate (Fig. 5B). The total surface area of the trophoblast in the floating villi and the anchoring villi ought to be larger than the trophoblast layer of the chorionic plate. Therefore, one can associate ACh with the syncytiotrophoblast.

Influence of cold storage (4°) *and freezing on placental ACh content.* When a human placenta was frozen for 48 hr and then warmed to laboratory temperature, the entire amount of ACh had decomposed (Figs. 6 and 7). There was no change in the concentration of the unidentified compound X, even after freezing a placenta for 168 hr (Fig. 7). When a placenta was kept for 77 days at 4°, about 30 per cent of the ACh remained in the tissues. These observations suggest that ACh was bound within membranes. Freezing breaks the membranes and liberates ACh, which is subsequently hydrolyzed by AChE. The unidentified compound X might not contain an ester bond, which would explain why it was not affected by freezing or AChE hydrolysis or alkaline hydrolysis.

Variation of the concentration of human placental ACh as a function of the gestation period. Human placentae collected from 82 cases of therapeutic abortions, spontaneous abortions and therapeutic deliveries were analyzed for ACh content (Table 3). ACh values for the first 8 weeks of pregnancy could not be quoted, because the placenta is not clearly defined until the third month. The gestation period could be assessed within a range of ±2 weeks. Therefore, the ACh values for 4 consecutive weeks of pregnancy were pooled. The values in Table 3 indicate that the ACh concentration was lower during the first and third trimesters of pregnancy than it was during the second trimester of pregnancy. The peak values for ACh concentration were found during weeks 21–24 of pregnancy.

DISCUSSION

According to Chang [25], the ACh-like activity of term placental extracts is equivalent to about

Table 2. Distribution of ACh in specific placental tissue

Placenta no.	Gestation period (weeks)	ACh in tissue* (nmoles g wet tissue) Whole tissue	Villi†	Basal plate	Chorionic plate
1	32	269·50 ± 10·17	334·13 ± 9·28	252·50 ± 1·53	100·37 ± 5·37
2	38	109·10 ± 0·25	166·22 ± 1·18	84·10 ± 2·20	73·45 ± 0·05
3	40	141·40 ± 1·31	223·67 ± 4·81	190·00 ± 2·804	91·37 ± 3·98
4	40	53·45 ± 1·81	65·82 ± 1·55	54·35 ± 1·46	16·29 ± 0·57
5	41	77·38 ± 8·03	103·30 ± 5·50	72·85 ± 0·35	57·95 ± 2·45
6	41	92·65 ± 2·95	117·08 ± 7·51	76·85 ± 2·65	40·00 ± 0·60
7	41	85·01 ± 7·64	184·65 ± 4·45	99·98 ± 7·73	37·75 ± 2·26
8	41	50·40 ± 3·27	68·33 ± 1·08	36·49 ± 0·75	17·82 ± 1·07
9	41	66·20 ± 2·16	123·37 ± 1·87	68·58 ± 5·04	24·40 ± 0·23
10	42	246·43 ± 3·82	307·27 ± 6·87	241·40 ± 28·94	191·23 ± 6·07

* Mean ± S.E. from a minimum of three determinations for each tissue sample.

† The villi contained about 3·22 ± 0·39 times higher concentration of ACh than that in the chorionic plate; the basal plate contained about 2·10 ± 0·23 times higher concentration than that in the chorionic plate.

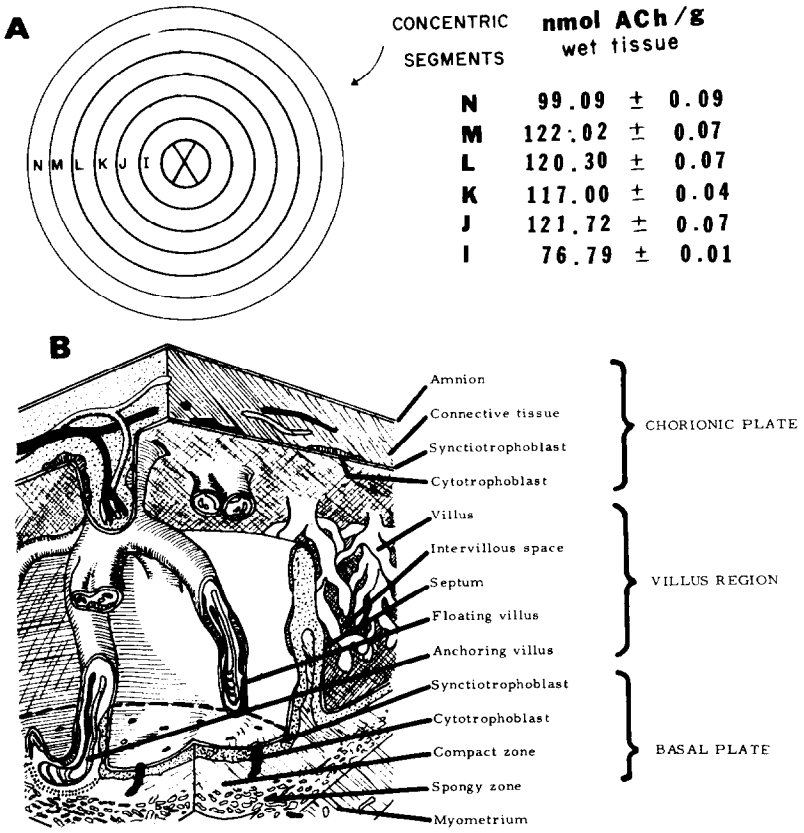

Fig. 5. Panel A: Concentrations of ACh (nmoles/g of wet tissue) in various concentric segments of placenta. Each value is the mean ± S. E. from three determinations. Panel B: a modified schematic diagram [24] of the term placenta.

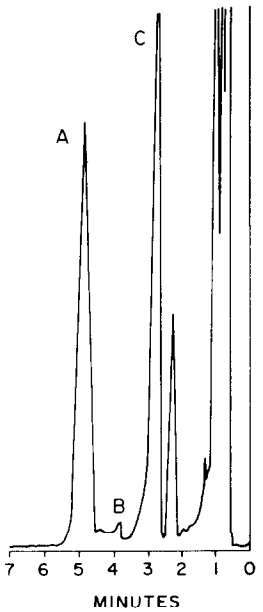

Fig. 6. Gas chromatogram of the quaternary ammonium compounds which were separated from the placenta, after freezing it for 48 h, and subjected to pyrolysis. Peak A: 2-dimethylaminoethyl propionate formed from propionylcholine which was added as an internal standard. Peak B: 2-dimethylaminoethyl acetate formed from ACh. The height of this peak was not significant. Peak C: 2-dimethylaminoethanol formed from choline.

180–230 nmoles ACh/g of wet tissue. Our results indicate that term placenta contains about 112 nmoles ACh/g of wet tissue. Therefore, about 55 per cent of the ACh-like activity of the placental extracts could be identified as ACh.

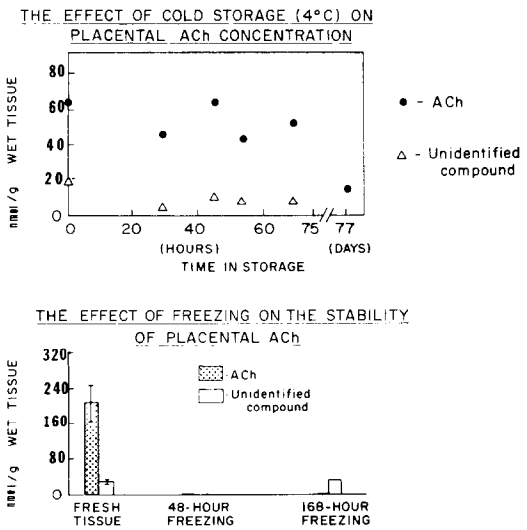

Fig. 7. Influence of cold storage, and freezing and thawing on the stability of placental ACh. Each point is the mean ± S. E. from three determinations. When the S. E. is too small to indicate on figures, it is omitted.

Table 3. Variation of placental ACh as a function of gestational age

Gestational ages* (weeks)	No. of placentae	ACh† (nmoles g wet tissue) Mean ± S.E.	Range
9–12	1	129·45	
13–16	11	342·21 ± 30·57	218·73–531·00
17–20	12	317·08 ± 31·92	181·70–542·95
21–24	4	722·90 ± 63·33	566·00–843·45
25–28	2	231·00 ± 42·67	188·33–273·67
29–32	4	249·18 ± 28·32	173·13–307·67
33–36	11	152·92 ± 14·68	103·70–285·00
37–40	25	104·75 ± 6·70	51·68–202·35
41–44	12	87·77 ± 5·43	50·40–122·75

* Gestational age could be assessed within ±2 weeks.
† A representative radial section of the placenta was analyzed for ACh.

The unidentified compound in the term placenta was not hydrolyzed by alkali. It could not be separated from choline by ion-exchange chromatography using the ion exchange resin Amberlite CG-50. The source of this unidentified compound is not known, that is, whether it exists in placental extracts or whether it is formed by dealkylation from another compound.

Our investigations indicate that placental ACh is bound within membranes. When human placenta was frozen for 48 hr and warmed to laboratory temperature, the entire amount of ACh decomposed. When the placenta was kept for 77 days at 4°, about 30 per cent of it remained in the tissues. The formation of ice crystals in the frozen tissue destroys the membrane structure and ACh is released, which is then hydrolyzed by AChE. Similar results with brain tissue have been reported in the literature [26, 27]. Freezing and thawing of brain tissue bring about the release of tissue-bound ACh, which is destroyed by AChE. According to some estimations [12, 25], a major portion of ACh-like activity (95 per cent) in human placenta is in a bound form.

The available evidence indicates that ACh is localized in the villus tissue of the human placenta. There are 20–30 large villus trunks which correspond to the cotyledons (lobes) arranged in a circle around the umbilical cord [24]. The concentric segments next to the umbilical cord and the peripheral segment have lower concentrations of ACh than the central concentric segments. This suggests that high ACh concentrations are found at positions where villi are localized. The ACh concentrations in the various sections of the placenta could be arranged in the following order (Fig. 5B): villus tissue > basal plate (containing parts of anchoring villi) > chorionic plate. The surface areas of the trophoblast in the above sections could be arranged in the same order.

The syncytiotrophoblast layer is fully formed by about 4 months and the cytotrophoblast disappears [24]. At about this time, high concentrations of ACh are found in the placenta. The fully developed villus contains only three layers, syncytiotrophoblast, connective tissue and vascular fetal endothelium [24]. In histological studies, certain granules can be localized in the syncytiotrophoblast by a special fixative (1% ammonium reineckate in 10% formaldehyde or 80% alcohol) which precipitates ACh and many other amines [28]. These granules were localized along the free border of the syncytiotrophoblast. Electron microscopic studies have demonstrated that there are vesicles at the base of the microvilli of the syncytiotrophoblast [29, 30]. The surface areas of the syncytiotrophoblast could be arranged in the following order: villus tissue > basal plate which contains anchoring villi > chorionic plate [24]. The ACh concentrations in these tissues could be arranged in the same order. These observations indicate that ACh is possibly localized in the syncytiotrophoblast. However, it has yet to be demonstrated that ACh is contained in vesicles.

There was a variation in the ACh concentration with gestational age of the placenta. The highest values for ACh concentration were found during weeks 21–24 of pregnancy. The variation in ChA activity shows a pattern similar to that seen for ACh [31, 32]. This pattern remained the same when ChA activity was expressed per unit weight of the tissue or protein [31]. There was peak ChA activity at about 16–20 weeks of gestation and a 4-fold decrease in activity at parturition as well as at 8–13 weeks of development [32]. The development of the placental cholinergic system, as indicated by ACh–ChA, follows the development of the syncytiotrophoblast [24] during the first 6 months of pregnancy. The reasons for the decrease in ChA–ACh concentrations in the term placenta are not known.

There are two principal stages in the development, an embryonic period (1–2 months) and a fetal period (2–9 months) [24]. Neither the syncytiotrophoblast nor the placental cholinergic system (ChA–ACh) is fully developed during the embryonic period. Many substances [33, 34] (e.g. α-aminoisobutyric acid [32] and diphenylhydantoin [35]) cross the placental barrier with relative ease. The degree of the teratogenic sensitivity of the human fetus to chemicals reaches the highest levels during this period [34]. During the first part of the fetal period (2–6 months), the organs undergo little more than maturation (histogenesis) at the histological level. The size of the human fetus increases by about 250 times during this period. Chemicals do not cross the placental barrier as easily as they did during the embryonic period; their transport does seem to be regulated [32, 34]. The placental cholinergic system is fully developed during this period. During the latter part of the fetal period, functional maturation of the fetus is achieved. The placental levels of ChA–ACh have decreased. Chemicals do seem to cross the placental barrier more easily than they did during the mid-gestation period [33, 34, 36]. These observations indicate that the placental cholinergic system may play a significant role in the regulation of the transport of nutrients and chemicals across the syncytiotrophoblast and thereby regulates the fetal growth. Studies on the inter-relationships between placental transport and fetal growth during the manipulation of the placental cholinergic system using pharmacological agents (e.g. inhibitors of AChE and ChA) may provide an answer for the role of ACh in the placenta.

Besides placenta, several other tissues, in which the involvement of the nervous system is either remote or absent, are known to contain one or more components of the cholinergic system. These include red

blood cells [37], spermatozoa [38], cornea [39, 40], and many other structures [41]. Just like in the placenta, relationships between some activities of these tissues and the occurrence of the components of a cholinergic system in them have been described in the published literature [37–41]. However, concrete evidence is not available about the role of the cholinergic system in placenta or these tissues.

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REFERENCES

1. C. Bischoff, W. Grab and J. Kapfhammer, *Hoppe-Seyler's Z. physiol. Chem.* **207,** 57 (1932).
2. P. Haupstein, *Arch. Gynaek.* **151,** 262 (1932).
3. H. C. Chang and J. H. Gaddum, *J. Physiol., Lond.* **79,** 255 (1933).
4. H. C. Chang and A. Wong, *Chin. J. Physiol.* **7,** 151 (1933).
5. E. Strack, H. Geissendorfer and E. Neubaur, *Hoppe-Seyler's Z. physiol. Chem.* **229,** 25 (1934).
6. H. C. Chang, I. C. Wen and A. Wong, *Proc. Fifteenth Int. Physiol. Cong.* (Leningrad) 208 (1935).
7. P. Heirman, *Archs int. Physiol.* **51,** 85 (1941).
8. R. S. Comline, *J. Physiol., Lond.* **105,** 6P (1946).
9. F. Welsch, *Am. J. Obstet. Gynec.* **118,** 849 (1974).
10. D. Morris, *Biochem. J.* **98,** 754 (1966).
11. J. Schuberth, *Biochim. biophys. Acta* **122,** 470 (1966).
12. B. V. R. Sastry and G. I. Henderson, *Biochem. Pharmac.* **21,** 787 (1972).
13. G. I. Henderson and B. V. R. Sastry, *Fedn Proc.* **31,** 561A (1972).
14. D. Morris and D. S. Grewaal, *Biochem. J.* **114,** 85 (1969).
15. H. L. White and C. J. Cavallito, *Biochim. biophys. Acta* **206,** 343 (1970).
16. C. Torda, *Proc. Soc. exp. Biol. Med.* **51,** 398 (1942).
17. S. I. Zacks and G. G. Wislocki, *Proc. Soc. exp. Biol. Med.* **84,** 438 (1953).
18. O. Bodansky, *Ann. N.Y. Acad. Sci.* **47,** 521 (1946).
19. M. G. Ord and R. H. S. Thompson, *Nature, Lond.* **165,** 927 (1950).
20. R. P. Koshakji, B. V. R. Sastry and R. D. Harbison, *Res. Commun. Chem. Path. Pharmac.* **9,** 181 (1974).
21. P. I. A. Szilagyi, D. Schmidt and J. P. Green, *Analyt. Chem.* **40,** 2009 (1968).
22. D. E. Schmidt, R. C. Speth, F. Welsch and M. E. Schmidt, *Brain Res.* **38,** 377 (1972).
23. D. E. Schmidt, P. I. A. Szilagyi, D. L. Alkon and J. P. Green, *J. Pharmac. exp. Ther.* **174,** 337 (1970).
24. H. Tuchmann-Duplessis, G. David and P. Haegel, *Illustrated Human Embryology*, Vol. 1, pp. 66–82. Springer, New York (1972).
25. H. C. Chang, *Chin. J. Physiol.* **13,** 145 (1938).
26. W. E. Stone, *Archs Biochem. Biophys.* **59,** 181 (1955).
27. W. E. Stone, *Archs Biochem. Biophys.* **59,** 193 (1955).
28. I. C. Wen, H. C. Chang and A. Wong, *Chin. J. Physiol.* **10,** 559 (1936).
29. A. C. Barnes, *Intra-uterine Development*, pp. 35–67. Lea & Febiger, Philadelphia, Pennsylvania (1968).
30. B. J. Martin and S. S. Spicer, *Anat. Rec.* **175,** 15 (1973).
31. G. Bull, C. Hebb and D. Ratkovic, *Nature, Lond.* **190,** 1202 (1961).
32. R. D. Harbison, C. Dwivedi, L. Lafrenaye and B. V. R. Sastry, *Fedn Proc.* **33,** 565 (1974).
33. J. Dancis, J. Lind, M. Oratz, J. Smoleus and P. Vera, *Am. J. Obstet. Gynec.* **101,** 820 (1968).
34. A. Goldstein, L. Aronow and S. M. Kalman, *Principles of Drug Action*, pp. 711–34. Harper & Row, New York (1969).
35. J. Olubadewo, M. W. Stevens, B. V. R. Sastry and R. D. Harbison, *Pharmacologist* **15,** 199 (1973).
36. B. E. Smith, *Adv. Teratol.* **3,** 127 (1968).
37. F. Herz and E. Kaplan, *Pediat. Res.* **7,** 204 (1973).
38. R. D. Harbison, C. Dwivedi, M. C. Orgebin-Crist and B. V. R. Sastry, *Proc. Int. Union of Physiol Sci.* (abstr.) **11,** 325 (1974).
39. G. G. Fitzgerald and J. R. Cooper, *Biochem. Pharmac.* **20,** 2741 (1971).
40. R. W. Stevenson and W. S. Wilson, *Biochem. Pharmac.* **23,** 3449 (1974).
41. G. B. Koelle, in *Handbuch der Experimentellen Pharmacologie* (Eds. O. Eichler and A. Farah), Vol. XV, pp. 187–298. Springer, Berlin (1963).