1952

Biological assay of acetylcholine in the human placenta

Kenneth Dale Rodabaugh
University of Nebraska Medical Center

A BIOLOGICAL ASSAY OF ACETYLCHOLINE IN THE HUMAN PLACENTA

Kenneth Dale Rodabaugh

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine
College of Medicine, University of Nebraska
December 15, 1951
Omaha, Nebraska

It was first demonstrated in 1932 (1) that the human placenta contained a relatively large amount of acetylcholine. This was confirmed by others in the same year (2&3) and further stated that the placenta contains 0.000028 grams of acetylcholine per gram of tissue.

It was noted in 1934 (4) that acetylcholine had a strong oxytocic action on the uterus and since it was found in large quantities in the human placenta, it was inferred that it possibly had a role in labour. There was later introduced, by the same worker, evidence to show that the acetylcholine activity of the human placenta is directly related to the activity of the uterus as judged by the duration of labour and the intensity of the uterine contractions during labour, and further postulated that a high acetylcholine activity of the placenta was usually associated with such conditions as abortion and placenta praevia, whereas low acetylcholine values were to be found in uterine inertia. Other investigators found (5), in 1934, that the acetylcholine content of the placenta was higher in patients with a labour of under ten hours than in those with a labour over ten hours in duration. It was also noted that the highest concentration of acetylcholine occurred between the third and

sixth months of pregnancy and gradually diminished with the approach of term.

It was on the basis of this work that in 1937 acetylcholine was used in the treatment of twenty three cases of uterine inertia (6) and the conclusion reached that acetylcholine is of definate value in this regard; being, in the experience of the investigators, more successful than any of the other preparations used including oestrin, pitocin, pituitrin, pituchinol and quinine. These same investigators felt that acetylcholine should not be used until sedatives and minor stimulating measures have failed. They found that it was essential to give the full dosage of 0.2g intramuscularly every three hours for four doses and felt that some other preparation with acetylcholine activity which had longer action (such as Mecholyl) might give somewhat better results as the action of each dose of acetylcholine seemed somewhat transient.

The site of occurrence of acetylcholine in the placenta was believed to be the trophoblastic epithelium of the chorionic villi and it was possible in 1934, 1935, 1936 and 1938 (7,8,9 and 10) to demonstrate, with special techniques of fixation and staining, the presence of certain granules in the Langhans

and syncitial layers of the trophoblastic epithelium which were believed to represent choline groupings and possible precursors of acetylcholine. It was further noted that there were more granules present in early (abortive) placentae than were present in older placentae.

Further research in 1941 (11) showed a concentration of 0.000162 g. of acetylcholine per gram of placental tissue and further noted that the administration of the oxytocic principle of the posterior pituitary served to diminish the concentration of acetylcholine in the placenta.

It was also shown in 1942 (12) that intact placental tissues, when eserinized, shows an increase in the concentration of acetylcholine during the first four hours when incubated at 37 degrees C. This phenomenon seems to require the presence of cholinesterase but to be brought about by some factor other than cholinesterase. They also noted that grinding, freezing, or drying, etc., destroy this factor which they believe to be intracellular and require an intact cell to operate.

A further seemingly substantiating finding was reported (13) in 1948 showing that the concentration of acetylcholine in the blood and placenta in eleven healthy patients increased during labour and that the

blood level gradually decreased from the tenth post-partum day onward. The placental level of acetylcholine was found to be constantly lower than the blood level, and these workers considered acetylcholine to be one of the factors governing labour and felt that their observations as well as those of others, indicated that acetylcholine exerts an effect which is antagonistic to that of the posterior pituitary oxytocic hormone.

In looking through the literature it was noted that all determinations of the acetylcholine content of the placenta were carried out using the frog rectus abdominus reaction to placental extracts. It is a well known and generally accepted fact that the frog rectus is sensitive to histamine and various ion changes including hydrogen ion changes of relatively low magnitude.

In casting about for a method which would show a higher degree of sensitivity and specificity it was found that the use of the isolated ventricle of the Venus mercenaria fulfilled the requirements very satisfactorily. This preparation is insensitive to potassium, histamine, and adrenalin (14). It is insensitive to pH changes between pH 5 and 8.5 and is insensitive to anticholinesterases e. g. physostigmine,

prostigmine, and di-isopropyl flurophosphate. The threshold concentration for this preparation varies between 0.00000001 and 0.00000000025 grams percent.

This preparation requires the suspension of the isolated ventricle in sea water and maintainance at a temperature of between 10 and 15 degrees centigrade in addition to the usual standardization with standard series of solutions of acetylcholine in known concentrations. The determinations are then made in the usual manner. (15).

In this series the determinations were made on the basis of the amount of acetylcholine or the amount of placental extract necessary to produce complete inhibition of contraction of the "venus" ventricle.

Placentas were obtained immediately upon delivery and a small section of approximately 10 grams of tissue were taken near the margin of the placenta (incorporating both the maternal and foetal sides of the placenta) and quickly placed into a speciman bottle and then into a mixture of dry ice and ether. Any specimen requiring longer than two minutes from the time of delivery to be placed in the freezing mixture was discarded. The specimens were kept at a low temperature (below 0 degrees C) until such time as the determinations could be made. The specimens were later weighed and a suitable portion macerated in a solution con-

taining trichloroacetic acid and adjusted to pH below 3.0. This was allowed to stand for two hours with occasional stirring to get the maximum yield of acetylcholine with a minimum of destruction (16). The suspension was then filtered and the filtered solution adjusted to a pH between 6.0 and 6.5 with $NaHCO_3$. This pH is considered high enough to prevent any effect on the "venus" preparation and low enough to prevent excessive destruction of the acetylcholine by alkaline hydrolysis. The determinations were then made as described and controls, consisting of portions of the prepared placental extracts which had been alkalinized and heated to boiling and held there for 15 minutes to hydrolyze the acetylcholine, were frequently run.

The results of the determinations may be seen in the table below:

Case	Length of Labour	Concentration of Acetylcholine (g/g of tissue)
1	16	0.0 to less than 0.000000365
2	4	0.0 to less than 0.000000216
3	26	0.0 to less than 0.000000464
4	21	0.0 to less than 0.000000256
5	18	0.0 to less than 0.000000209
6	9	0.0 to less than 0.000000196
7	9	0.0 to less than 0.000000429
8	3	0.0 to less than 0.000000240
9	8	0.0 to less than 0.000000517
10	11	0.0 to less than 0.000000249
11	14	0.0 to less than 0.000000458

In addition to the eleven quantitative determinations, three qualitative determinations were done with varying amounts of tissue and dilution to require between 0.0000000705 grams of acetylcholine per gram of placental tissue and 0.000000142 grams of acetylcholine per gram of placental tissue. The lengths of labour in these cases were: two hours, three hours and thirty-three hours. In none of these qualitative determinations was any acetylcholine detected.

In all material upon which determinations were made, the onset of labour was spontaneous, delivery was uncomplicated, and material had, at the time of collection, been frozen within two minutes of the estimated time of separation of the placenta.

Plate No. 1 demonstrates the typical response of the "venus" heart preparation to the addition of the amount of acetylcholine which is the least amount capable of producing the standard inhibition of the preparation. Plate No. 2 demonstrates the lack of response of the preparation to the addition of the placental extract. Plate No. 3 demonstrates the lack of response of the preparation to the control soultion used.

Summary & Conclusions

1- Eleven quantitative determinations on the human placental tissue were carried out and three additional qualitative determinations were recorded.

2- In these determinations a concentration of 0.000000196 grams of acetylcholine to 0.000000517 grams per gram of placental tissue would have been required in order to have produced inhibition of the"venus" heart preparation.

3- In no case was any inhibition of the "venus" preparation noted.

4- It was demonstrated in determinations not included here, that acetylcholine was not destroyed in the extraction process as determinations were carried out in which acetylcholine was added to the placental tissue and then the extraction carried out in the usual manner and in all such cases inhibition of the preparation noted.

5- It was impossible, with this method, to demonstrate the presence of any acetylcholine in the placenta.

6- It is postulated that either the previous determinations recorded in the literature are, in fact, the determination of some other substance since the preparations used were much less specific for acetylcholine; or that in the time required to freeze the specimens and so halt the action of cholinesterases, all or nearly all of the acetylcholine present was destroyed.

PLATE I

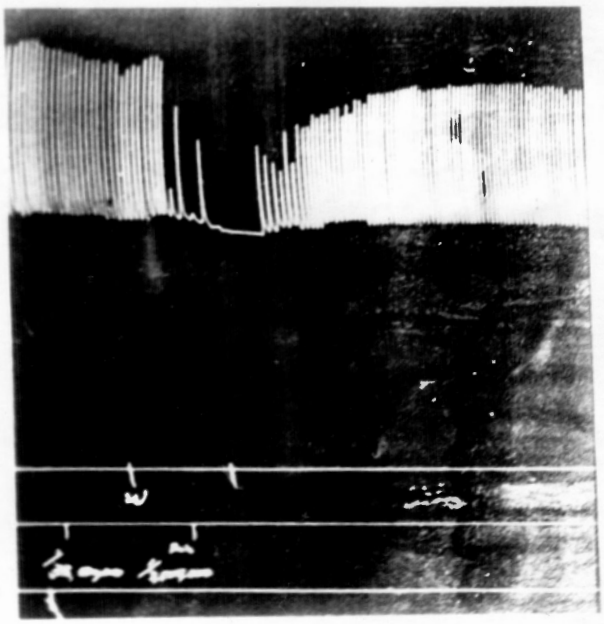
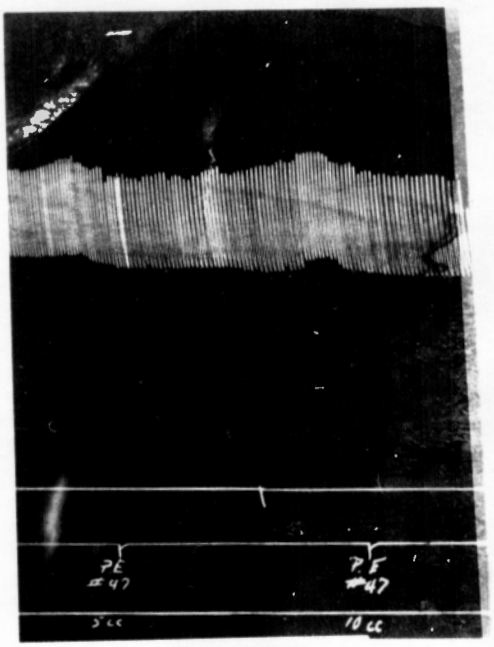


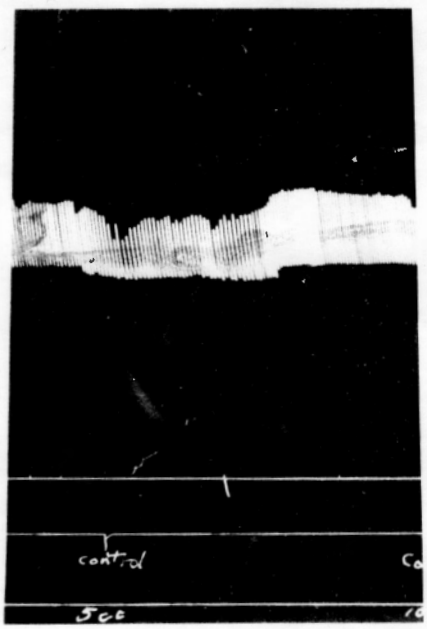
Plate I
Typical Acetylcholine
Response

PLATE II



~~Plate~~ II
Lack of Response to
Placental Extract

PLATE III



~~Plate~~ III
Lack of Response to
the Control

BIBLIOGRAPHY

1. Bischoff, G., Grab, W., and Kapfhammer, J., Hoppe-Seyl. Z., 207:57, 1932, as cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire 56: 1051, 1949.

2. Hauptstein, P., Arch. Gynak., 151: 262, 1932. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire, 56: 1051, 1949.

3. Chang, H. C., and Gaddum, J. H., Choline Esters In Tissue Extracts, J. Physiol, 74: 255, 1933.

4. Chang, H. C., Proc. 7th Annual Meeting of Chinese Physiological Society, Nanking. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire, 56: 1051, 1949.

5. Walker, F., and Henderson, D. N., Choline As Related To Labour, Canadian Medical Association Journal 30: 158, 1934.

6. Bell, A. C., and Playfair, P., The Use Of Acetylcholine In Uterine Inertia, J. Obstet. & Gyn., 44:470, 1937.

7. Chang, H. C., Proc. 7th Annual Meeting of Chinese Physiological Society, Nanking., 1934. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire, 56: 1051, 1949.

8. Chang, H. C., Wen, I. C., and Wong, A., On The Site Of Occurrence, Formation, And Significance Of Acetylcholine In The Human Placenta. Proc. Int. Physiol. Congr., U. S. S. R. 21:208, 1935. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit.Empire, 56: 1051, 1949.

9. Chang, H. C., Liberation of Acetylcholine From The Perfused Human Placenta, Proc. Soc. Biol. a. Med. 24: 665, 1936. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire, 56: 1051, 1949.

10. Chang, H. C., Chinese J. Physiol. 13: 145, 1938. As cited by: Loraine, J. A., Placental Extracts, J. Obst. Gyn. Brit. Empire, 56: 1051, 1949.

11. Heirman, P., L'Acetylcholine Placentaire, Arch. Internat. De Physiol. 51: 85, 1941.

12. Chang, H. C., Lee, L. Y., Meng, C. W., and Wang, Y. K., Biologic Synthesis of Acetylcholine, Proc. Soc. Exp. Biol. and Med. 49: 380, 1942.

13. Stefanelli, S., and Petronic, G., Changes In The Blood Level Of Acetylcholine In Pregnancy At Term, During Labour, And In The Puerperium. Relationship to Placental Acetylcholine, Folio Gynaecologia, 43: 323, 1948.

14. Tower, D. B., and McEachern, D., Experiences With The "Venus" Heart Method For Acetylcholine Determination, Canadian J. Research, Sect. E, 26: 183, 1948.

15. Downing, F. M., Personal Communication.

16. Perry, W. L. M., The Time Course Of Events In The Extraction Of Acetylcholine With Trichloroacetic Acid., J. Physiol. Lond. 110: No. 1-2 Proc. 20, 1949.