which the washed red blood cells were agglutinated by anti-dog serum no anemia or increase in reticulocytes or icterus index appeared. The osmotic fragility of the red blood cells remained normal. No cold or warm agglutinins were demonstrable. The complement titer of the serum (1:32) did not fall; and no serum complement-fixing antibody for the dog's own red blood cells was demonstrable. The mechanical fragility of the red blood cells ranged from 3.1 to 6.1%; values considered by Shen[17] to be normal for dog red blood cells.

Summary and Conclusions. Several attempts were made to sensitize 4 dogs to their own red blood cells. Three dogs showed no definite evidence of autosensitization. Although 2 of these 3 dogs showed occasional marked fluctuation in mechanical fragility unaccompanied by other changes during these studies this was considered to be due to technical difficulties. However, for a short period in one dog, 25 days after the injection of a modified Freund antigen, there was agglutination of washed red blood cells when they were suspended in anti-dog serum rabbit serum. During this period of apparent autosensitization there was no anemia, reticulocytosis or jaundice. The red blood cells showed no alteration in osmotic or mechanical fragility. There was no fall in complement titer. Cold and warm agglutinins did not appear. There was no evidence of a serum complement-fixing antibody for the dog's own red blood cells. Such apparent autosensitization supports the explanation that in certain instances of acquired hemolytic anemia in man serum autoagglutinins may be formed in response to an antigen in red blood cells.

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The Appearance of Acetylcholine During Normal Labor. (17451)

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In our earlier studies on the acetylcholine (Ach) of the human placenta,[1] blood from pregnant women was not available for investigation. In consideration of the potent hydrolytic action of the blood cholinesterase, it was inferred that Ach of human placenta does not appear to reach the maternal circulation in any noticeable quantity.

Opportunity to restudy this problem has not presented itself until lately. On the basis of an analysis of 288 blood samples of 152 cases during pregnancy and at different stages of labor, our previous impression has to be modified.

Four cc of blood were obtained each time and poured into 2 volumes of 95% pure alcohol. The mixture was thoroughly ground, diluted with 2 volumes of saline and centrifuged. The supernatant fluid was measured, and usually 0.5 cc or less was used for assay. Alcohol-free extract was also prepared by evaporating 5 cc to dryness in room (23-30°) and re-taking up with 5 cc of frog Ringer solution. The extracts were assayed on the toad's rectus test which depends on the principle of equal potentiation of Ach standard and the unknown by eserine. So if the matched ratio is identical before and after eserine, it is taken as Ach. Otherwise, Ach is considered to be nil.

Table I gives results showing a gradual appearance of Ach toward the second trimester of gestation, an apparent increase of Ach with the onset of labor, and a disappearance beginning 48 hours after labor.

The blood of 10 non-pregnant women and

[1] Chang, H. C., and Wong, A., *Chin. J. Physiol.*, 1933, **7**, 151.

TABLE I.
Increase in Acetylcholine of Blood During Pregnancy.

	During pregnancy			Before onset of labor	During labor		After labor	
	1st trimester	2nd trimester	3rd trimester		Early stage	Late stage	Before 48°	After 48°
Total No. of samples	26	30	23	26	44	66	47	26
Samples without Ach: No.	25	21	17	10	8	17	34	26
%	96.2	70.0	73.9	38.5	18.2	27.8	72.3	100
Samples with Ach: No.	1	9	6	16	36	49	13	0
%	3.8	30.0	26.1	61.5	81.8	72.2	27.7	0
Range of Ach in blood, γ/cc	1.61	0.69-3.06	0.71-1.94	0.73-4.70	1.02-7.59	0.71-6.81	0.67-6.21	—
Average	—	1.871 ± 0.220	1.332 ± 0.164	2.586 ± 0.324	3.089 ± 0.286	2.387 ± 0.199	2.929 ± 0.450	—

10 male individuals showed no evidence of Ach by the same test. Trichloracetic extract prepared according to Chang and Gaddum[2] gave results of the same order as that of alcohol extract.

Although alcohol has a potentiating action on Ach,[3,4] it did not appear to influence the results of our test under the routine precautions taken. In 25 trials to study this point, the alcoholic extracts gave an average of 1.355 ± 0.090 γ/cc, while the corresponding alcohol free extracts gave an average of 1.181 ± 0.105 γ/cc, the difference being 0.174 ± 0.139.

From the studies made on placental tissue and perfused placenta,[5] it was suggested that Ach may play a local role in the normal mechanism of labor. Any theoretical deduction made from studies on a tissue which has been discharged as being of no more use to the body is to be guarded against. By demonstrating an increased blood Ach toward term and with the onset of labor, our suggestion that Ach may play a role in the normal mechanism of labor is further strengthened.

In view of this finding of blood Ach, the suggestion of using eserine for induction of labor[5] deserves a trial which is in progress.

Summary. In a study of 288 samples of blood from 152 cases, a gradual appearance of Ach toward the 2nd trimester of pregnancy, an increase of Ach with the onset of labor, and a disappearance 48 hours or more after labor were demonstrated. Blood of 20 normal subjects of both sexes showed no evidence of Ach with the same test.

2 Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

3 Meng, C. W., *Chin. J. Physiol.*, 1941, **16**, 291.

4 Ettinger, G. H., Brown, A. B., and Megill, A. H., *J. Pharmacol.*, 1941, **73**, 119.

5 Chang, H. C., Lee, L. Y., and Meng, C. W., *Chin. J. Physiol.*, 1940, **15**, 343.

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