tralization (10,000 neutralizing doses) in the *in vitro* experiment is the same as that observed by animal inoculation. Apparently the scale of sensitivity for the detection of virus in both the titration and neutralization experiments has shifted one dilution higher than the *in vivo* tests. The possibility must also be considered that the shift in neutralization activity measured by the *in vitro* method may to some extent be a manifestation of reactivation of neutral mixtures by dilution.[2] Further study of *in vitro* neutralization technic should clarify this point.

Improvement of technical details is being attempted and will be further described.

Conclusions. Titration of potency of W.E.E. virus and quantitative estimation of neutralizing antibodies in hyperimmune horse serum have been demonstrated by use of tissue cultures.

The *in vitro* method of detecting the presence of virus appeared to be more sensitive than intracerebral mouse inoculation.

[2] Pierce, M. E., Kempf, J. E., and Soule, M. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 604.

13991

Choline Esterase Content of Tissues Without Innervation (the Placenta).

CLARA TORDA.* (Introduced by McKeen Cattell.)
From the Department of Physiology, Pharmacology and Biochemistry, University College, London.

The probable importance of the choline esterase-acetylcholine system to transmission in the nervous and neuromuscular system suggested that the choline esterase content of the placenta, an organ without nervous tissue (Schmitt[1]) should be investigated. Other tissues without innervation are the blood and the denervated muscle. The choline esterase content of denervated muscles decreases for some weeks after denervation but does not disappear completely as shown in the rat by Martini and Torda[2,3] and Meng[4], in the dog by Martini and Torda[5], in the frog by Feng and Ting[6], in the rabbit by Leibson[7] and in the guinea-pig by Couteaux and Nachmansohn.[8]

The human placenta has a rather high acetylcholine content (Chang and Gaddum,[9] Chang and Wong[10], Chang,[11,12] Reynolds and Foster,[13] Cattaneo[14]). Changes in acetylcholine sensitivity (Euler[15]) and acetylcholine content (Chang,[12,16], Chang, Lee, Meng and Wong[17]) of the placenta during different experimental conditions suggest that this tissue contains choline esterase.

Method. Some minutes after normal delivery the human placenta was perfused through the umbilical vein until all the blood was removed. The choline esterase content of 100 mg finely ground tissue was determined

* Now at Cornell University Medical College, New York.

[1] Schmitt, *Z. Biol.*, 1922, **75**, 19.

[2] Martini and Torda, *Klin. Wschr.*, 1937, **16**, 824.

[3] Martini and Torda, *Klin. Wschr.*, 1938, **17**, 97.

[4] Meng, *Chin. J. Physiol.*, 1940, **15**, 143.

[5] Martini and Torda, *Boll. Soc. Ital. Biol. Sper.*, 1938, **13**, 1056.

[6] Feng and Ting, *Chin. J. Physiol.*, 1938, **13**, 141.

[7] Leibson, *Bull. Biol. Med. Exp. U. R. S. S.*, 1939, **7**, 517.

[8] Couteaux and Nachmansohn, PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 177.

[9] Chang and Gaddum, *J. Physiol.*, 1933, **79**, 255.

[10] Chang and Wong, *Chin. J. Physiol.*, 1933, **7**, 151.

[11] Chang, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 665.

[12] Chang, *Chin. J. Physiol.*, 1938, **13**, 145.

[13] Reynolds and Foster, *Am. J. Physiol.*, 1939, **127**, 343.

[14] Cattaneo, *Arch. internat. Physiol.*, 1933, **37**, 58.

[15] Euler, *J. Physiol.*, 1938, **93**, 129.

[16] Chang, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1001.

[17] Chang, Lee, Meng and Wang, PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 380.

by Glick's method.[18] Four samples were taken from widely separated parts of each placenta. To 100 mg tissue 0.92 cc carbon dioxide-free distilled water and 1.6 cc veronal buffer solution containing 0.5% acetylcholine chloride were added. The mixture was kept at 35°C for 3 hours. Five cc of physostigmine-brom-thymol blue solution were then added and the mixture titrated with N/10 hydrochloric acid. Each mm of hydrochloric acid corresponds to 18 γ acetylcholine decomposed. Acetylcholine solutions without tissue and tissue without acetylcholine were treated in the same way. The difference between the hydrochloric acid used in the control experiments and that used with the samples containing tissue and acetylcholine corresponds to the acetylcholine which was decomposed by the tissue choline esterase. The choline esterase activity of placenta was calculated from the amount of hydrochloric acid used according to the formula given by Glick.[19]

Results. The choline esterase activity of 25 human placentæ was determined. The values are summarized in Table I.

TABLE I.
Choline Esterase Activity of Human Placentæ After Normal Delivery.
(The value is given in 1/10³ γ, per sec. per mg tissue.)

No. of exper.	Choline esterase activity	
	Mean	S.E.*
25 (4 samples each)	6.0	±0.27

$$*\text{S.E. of mean} = \sqrt{\frac{\Sigma(\Delta)^2}{N(N-1)}}$$

Σ = summation.
Δ = deviation of each sample from the mean.
N = 100.

Discussion. The results of this study show that 1 mg of placenta is able to decompose 6/10³ γ acetylcholine at 35°C in 1 sec. This activity is presumably due to the choline esterase fixed in the intracellular spaces because the prolonged perfusion would wash out much of the extracellular choline esterase. The data for the choline esterase content of the cells given above represent minimum values, since no correction has been made for the extracellular fluid and, further, prolonged perfusion produced an edema and therefore an increase of the weight of the tissue. The presence of choline esterase in the placenta is not due to the choline esterase activity of traces of blood left, a possibility that was excluded by examination of the tissue. Moreover, the samples taken from different parts of the placenta had the same choline esterase activity, even though the maternal and fetal blood differ significantly in choline esterase (Ammon and Voss,[20] Navratil,[21], Torda[22]).

A comparison of the choline esterase content of placenta with the data in the literature for other tissues is difficult because the different methods of determination do not give comparable values. An evaluation may be attempted by comparing the choline esterase content of tissues determined with Glick's method and also with other methods. The choline esterase content of the placenta is about one-tenth that of the choline esterase content of the mucosa of pig pylorus (Glick[23]). By comparing the choline esterase content of the pylorus and serum of pig (Glick, Lewin and Antropol[24]) and that of pig and human serum (Stedman and Stedman,[25] Hall and Lucas[26]) it appears that the choline esterase activity of the maternal blood is about twice as great as that of placenta. If this is the case it is reasonable to assume that the choline esterase content of the placenta originated from the blood by an exchange.

The choline esterase may be distributed equally or may be concentrated in the epithelium of the chorionic villous tissue where, according to Wen, Chang and Wong,[27] the acetylcholine is liberated. In the latter case

18 Glick, *J. Gen. Physiol.*, 1938, **21**, 289.
19 Glick, *J. Gen. Physiol.*, 1938, **21**, 439.
20 Ammon and Voss, *Pflüger's Archiv*, 1935, **235**, 393.
21 Navratil, *Geburtshilfe*, 1937, **114**, 146.
22 Torda, *Biochemica e Terapia Sper.*, 1938, **25**, No. 12.
23 Glick, *J. Gen. Physiol.*, 1938, **21**, 297.
24 Glick, Lewin and Antropol, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 28.
25 Stedman and Stedman, *Biochem. J.*, 1935, **29**, 2107.
26 Hall and Lucas, *J. Pharm. Exp. Therap.*, 1936, **61**, 10.
27 Wen, Chang and Wong, *Chin. J. Physiol.*, 1937, **10**, 559.

the choline esterase concentration of the chorionic villous tissue would be much greater than that of the blood.

The results of this study suggest that the presence of choline esterase is a generalized phenomenon and is not necessarily related to the presence of nerve elements.

Summary. 1. The choline esterase activity of 25 perfused human placentæ was determined by the method of Glick. 2. The choline esterase contained in 1 mg tissue is able to decompose at 35°C in the average $6/10^3$ γ acetylcholine per sec.

13992 P

Mechanism of the Colloidal Gold Reaction of Blood Serum in Liver Disease.

SEYMOUR J. GRAY. (Introduced by G. F. Dick.)

From the Department of Medicine, University of Chicago.

When a colloidal gold suspension is added to certain dilutions of blood serum from patients with liver disease flocculation of the colloidal gold occurs in one or more of the first serial dilutions.[1,2] The colloidal gold reaction of blood serum in liver disease has proven a sensitive method of detecting early liver involvement according to Loew and Noth,[3] Mateer *et al.*,[4] and Sweet, Gray and Allen.[5]

Flocculation of the colloidal gold by serum from patients with liver disease does not depend primarily upon a quantitative increase in globulin or upon an inverted albumin-globulin ratio.[1,2] It was suggested by the author[1] that the mechanism of this reaction in liver disease might depend upon a qualitative variation within the serum globulins rather than upon a quantitative change in the total serum globulin.

Electrophoretic studies of the serum proteins in all types of liver disease, both acute and chronic, revealed that the most characteristic and consistent alteration of the serum proteins was an increase in the gamma globulin associated with a decrease in serum albumin.[6]

Since globulin promotes colloidal gold flocculation and albumin protects the colloidal suspension from flocculation[7] these studies were undertaken to investigate the effect of the purified protein fractions, prepared by Dr. Edwin J. Cohn,[8] upon the serum colloidal gold reaction.

Method. Increasing amounts of electrophoretically pure human gamma globulin, albumin, and a purified fraction containing alpha and beta globulins were added to normal blood serum. The serum was then diluted 1:350 with 0.9% sodium chloride and the blood serum colloidal gold reaction was performed in the usual manner. Five cc of properly acidified colloidal gold were added to each of 3 tubes containing 1 cc of serum in final dilutions of 1:3500, 1:7000 and 1:14,000. The tubes were allowed to stand at room temperature and were read after 24 hours. Color changes were expressed by the following numbers: 0—red orange, 1—reddish blue, 2—orchid, 3—blue, 4—light blue, and 5—colorless. Complete flocculation in one or more of the 3 tubes designates a positive test. The usual negative reaction is 222 or 332, and the positive test is 532, 553, or 555.

1 Gray, S. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 470.

2 Gray, S. J., *Arch. Int. Med.*, 1940, **65**, 524.

3 Loew, E. R., and Noth, P., *Proc. Am. Physiol. Soc.*, 1941, 176.

4 Mateer, J. G., Baltz, J. I., Marion, D. F., Hollands, R. A., and Yagle, E. M., *Am. J. Digest. Dis. and Nutrition*, 1942, **9**, 13.

5 Sweet, W. H., Gray, S. J., and Allen, J. G., *J. A. M. A.*, 1941, **117**, 1613.

6 Gray, S. J., and Barron, E. S. G., *J. Clin. Invest.*, in press.

7 Reznikoff, P., *J. Lab. and Clin. Med.*, 1922, **8**, 92.

8 Cohen, E. J., *Chem. Rev.*, 1941, **28**, 395.