support the findings published by Gruber and Brundage[1] and by Slaughter and Gross.[2] 2. Apomorphine when injected intravenously may either increase or decrease the general tonus of the intact intestine, depending upon the animal and the condition of the animal at the time of the injection. Some animals respond to apomorphine only by increased tonus, others by decreased tonus, and still others by either or both responses. 3. The peristaltic contractions may be augmented by apomorphine especially when the general tonus is diminished. 4. Borborygmi are commonly noted following the intravenous injection of apomorphine.

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Hydrolysis of Acetycholine by Turtle Blood.*

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In connection with studies being conducted in this laboratory on the physiology of the turtle heart, it was of interest to know the choline-esterase activity of turtle blood. Work already done on the blood of other forms has revealed marked differences in the hydrolysis of acetylcholine between whole blood and serum and further variations depending upon the species. Galehr and Plattner[1, 2] found that human whole blood, shadow cells and washed corpuscles have a greater hydrolytic action on acetylcholine than the serum has. The filtrate from an acetylcholine solution treated with animal charcoal was from 78-100% physiologically inactive, but the total activity could usually be restored by acetylation. By analogy to the charcoal effects they concluded that the hydrolysis of acetylcholine by blood was due to surface catalysis. On the other hand, Engelhart and Loewi[3] repeated the charcoal experiments of Galehr and Plattner and found that partial inhibition of the destruction of acetylcholine could be obtained with a 1:5,000 dilution of physostigmine, which had been shown to be effective in preventing acetylcholine hydrolysis by extracts of frog hearts, and complete inhibition

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1 Galehr, O., and Plattner, F., *Pfluger's Arch.*, 1927, **218**, 488.

2 *Ibid.*, 1927, **218**, 506.

3 Engelhart, E., and Loewi, O., *Arch. exp. Path. u. Pharm.*, 1930, **150**, 1.

with equimolar concentrations of strychnine. They showed that physostigmine also completely prevented this hydrolysis by defibrinated beef blood in much greater dilutions (1:30,000) and by human serum in dilutions as great as 1:400,000. Strychnine did not have this effect on blood. When beef blood was heated at 58°C its hydrolytic activity was lost while human blood retained its activity when treated in the same manner. However, it was shown that although dried human blood retained its hydrolytic activity, it was lost after heating at 58°C. On the basis of these results, they concluded that the acetylcholine-splitting activity of human and beef sera, plasma, erythrocytes and stromata is due to the presence of an enzyme rather than to surface catalysis, and that this enzyme is not necessarily different in beef and human blood since drying of the blood might inactivate a protective agent. Ammon and Voss[4] found that human, rabbit and beef sera had less activity than whole and laked blood, while dog and horse sera had more activity than whole and laked blood. There was complete inhibition with physostigmine in all these preparations. Their findings on dog and horse sera do not conform to the view of Galehr and Plattner. Stedman and Stedman[5] investigated defibrinated blood, laked and unlaked, sera and corpuscles in a variety of species. They found that human, horse, guinea pig, pig and rabbit sera and corpuscles showed activity; in the cat, fowl and duck only the sera showed activity; while in the ox, sheep and goat, corpuscles alone showed activity. There was no difference in laked and unlaked blood. Physostigmine inhibition was not tried.

Method. In this work *Pseudemys elegans* was used. Blood was obtained by decapitation and was oxalated or allowed to clot as desired. Determinations of the hydrolysis of acetylcholine were made of 0.5 cc samples, using a modification of the Glick[6] method. The sample was added to 5 cc of water containing 20 mg of acetylcholine bromide with or without 0.10 mg of Prostigmin, the volume being made to 10 cc with distilled water (Prostigmin dilution of 1:100,000). When unlaked blood was examined, Ringer's solution replaced the water. The solution was adjusted to pH 8.0 and maintained there for 20 minutes by the addition of 0.01N NaOH from a microburette, using a glass electrode and gently stirring with a stream of air bubbles. All determinations were made at room temperature.

4 Ammon, R., and Voss, G., *Pfluger's Arch.*, 1934, **235**, 393.

5 Stedman, Edgar, and Stedman, Ellen, *Biochem. J.*, 1935, **29**, 2107.

6 Glick, D., *Biochem. J.*, 1937, **31**, 521.

Results. The results are expressed in Table I in cc of 0.01N NaOH neutralized by the acetic acid liberated upon hydrolysis of acetylcholine by 1.0 cc of sample during 20 minutes. Detectable spontaneous hydrolysis of the ester did not occur in distilled water, while in Ringer's solution this amounted to 0.12 cc of 0.01N NaOH; the tabulated results have been corrected for this. It will be seen that laked whole blood has considerable choline-esterolytic activity, while serum and plasma show none in some cases and only traces in others, which may be due to slight hemolysis. Prostigmin completely inhibits the activity of laked blood and plasma. Unlaked whole blood has from 18-59% more activity than laked whole blood and this difference is not inhibited by Prostigmin. Since there is no difference in activity between unoxalated serum and oxalated plasma, the oxalate had no effect on the choline-esterolytic activity. Several experiments with unlaked whole blood alone showed that there was no glycolysis under these conditions and that the acid developed was due solely to the hydrolysis of acetylcholine.

The hydrolytic activity of turtle blood towards acetylcholine was found to be quantitatively as variable as that of other forms and apparently this activity is related solely to the corpuscular elements. It is of interest to note that of the 3 forms investigated which have nucleated erythrocytes the fowl and duck have activity only in serum, while the turtle blood activity is due to the corpuscles. A part of the hydrolysis may be ascribed definitely to the presence of choline-esterase since Prostigmin has some inhibitory effect, but there remains a

TABLE I.
Results Expressed in cc of 0.01N NaOH Neutralized by the Hydrolysis of Acetylcholine.

		Whole Blood				
			Unlaked			
No.	Plasma, Normal	Laked, Normal	Normal	With Prostigmin	Hæmatocrit, % cells	Cells
1	.30*					
2	.34*	1.27				
3	.06*	0.31				
4	.00	0.33			25.3	1.31
5	.00	1.84†			23.1	7.97
6	.10†	1.99†			34.1	5.82
7	.07	1.06†			23.5	4.59
8	.22†	0.81†	0.96	0.12	14.5	5.54
9	.06	1.76†	2.16	0.45	22.2	7.93
10		2.57	3.72	1.18		
11		1.60	2.54	0.92		

* Serum.
† Showed no hydrolysis with added prostigmin.

considerable portion of the activity which is not due to esterase since it is not inhibited by Prostigmin. Furthermore, this fraction of the activity is dependent upon the integrity of the corpuscles since it is not present after laking the blood. The greater activity of unlaked blood is not due to the presence of Ringer's solution since it is not a constant value as would be expected if this were true. These results indicate that in turtle blood some of the hydrolytic activity towards acetylcholine may be due to corpuscular surface catalysis. If one is to assume enzymatic hydrolysis of acetylcholine it would appear essential to demonstrate, as a control, that it is completely inhibited by physostigmine or Prostigmin.

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Further Evidence for a Mammogenic Factor in the Rat Hypophysis.

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Gomez, Turner, and Reece[1] reported that the mammary glands of hypophysectomized male guinea pigs could be developed by the implantation of the pituitaries of estrogen-treated male rats but that no mammary growth resulted from implantation of normal male rat pituitaries. Gomez and Turner[2] reported a confirmation and extension of these results. The existence of the "mammogenic factor" described by the aforementioned authors seems to have been demonstrated in pregnant cattle pituitaries when tested on castrated rabbits[3] and rats[3] and normal mice.[4, 5] Nelson[6] reported recently

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1 Gomez, E. T., Turner, C. W., and Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 286.

2 Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 259.

3 Gomez, E. T., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 607.

4 Lewis, A. A., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 435.

5 Lewis, A. A., Turner, C. W., and Gomez, E. T., *Endocrinology*, 1939, **24**, 157.

6 Nelson, W. O., *Anat. Rec.*, 1938, **72**, 117 (Suppl.).