atmosphere (1 g of glycol in 200,000 cc of air).

II. *Effect of Propylene Glycol in Vitro.* A. *Vegetative Forms.* Inoculation of glycol into broth cultures resulting in glycol concentrations ranging from one to 100% produced the expected effects. Growth occurred in dilutions up to 20% and killing was obtained in solutions of 80% or more.

B. *Spores.* No effect on the viability of spores resulted from the inoculation of glycol into these cultures even though spores were exposed to the glycol for as long as 72 hours.

Discussion. Although no actual proof has been demonstrated as to how the glycols exert their bactericidal action, it would appear that it is brought about by the chief physical property of glycol, namely, hygroscopicity. Due to the moisture contained in each droplet of suspended bacteria and/or the film of moisture about each bacterial cell, molecules of glycol are attracted. This develops a high glycol concentration in direct contact with the cell membrane. As shown in test-tube experiments these high concentrations produce bacterial death. The lethal effects may take place by actions which possibly include dehydration of the cell, interference with respiration, denaturation of protein, inhibition of enzyme activity, and others. Since spores are particularly resistant to drying, the possibility is suggested that removal from, or entrance into the cell body, of water, would be difficult, whereas in vegetative forms this action could occur more readily, mediated by a high glycol concentration on the cell surface.

Conclusion. Propylene glycol in its liquid or vapor state demonstrates no killing effect on spores of *B. subtilis*. This observation tends to support the hypothesis that the bactericidal action of the glycols is due to the hydroscopic properties of these substances.

14214

Effect of Hormones on Contraction of Striated Muscle and on Choline Esterase Activity.

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While sex hormones and some of the hormones secreted by the hypophysis do not contract striated muscle in low concentrations, they may modify the biochemical equilibrium of the muscle. The purpose of this study is to investigate whether or not the presence of the above substances modifies the contraction of the muscle produced by known chemical agents.

Experimental. Effect of the Hormones on the Acetylcholine Sensitivity of Striated Muscle. The *Rectus abdominis* muscle of the frog was excised and suspended in a muscle chamber containing 20 cc of Ringer's solution at pH 7 and room temperature. The muscle was immersed alternately in a 0.1 mg per 100 cc acetylcholine solution for 30 seconds and in Ringer's solution for 10 minutes until the height of contraction remained constant for 3 cycles. This contraction served as control. The height of contraction was registered with an isotonic lever on a kymograph. The muscle was then immersed in a solution or suspension of one of the hormones in Ringer's solution for 5 minutes before each immersion in acetylcholine. The hormones were used in increasing concentrations. Changes in the height of contraction of the muscle due to the presence of the hormones were measured and expressed as percent of control. Experiments were performed in each case using instead of hormones the solvents alone. The solvents alone (sesame oil, peanut oil, and water with acacia) did not modify the effect of acetylcholine on the muscle. Other solvents (alcohol, ether, acetone, bile salts) were not used

TABLE I.
Effect of Hormones on Muscle Contraction Due to Acetylcholine.

Substance	Solvent	No. of exp.	Increase of height of contraction in % of control (mean values*) Conc. in I.U. per 100 cc Traces	1	10	100
Follutein (ant. pituitary-like sex hormone, Squibb)	water	20		33	40	52
Estrone Suspension (follicular hormone, Abbott)	water and acacia	12		10	20	27
Theelin (follicular hormone, Parke-Davis)	water	10				27
Estradiol (follicular hormone, Ciba)	"	5	20			
Progestin (corpus luteum hormone, Roche-Organon)	peanut oil	15	18			
Progesterol (corpus luteum hormone, Ciba)	water	8	19			
Testosterone Propionate (Ciba)	"	12	22			
Testosterone Propionate (Schering)	sesame oil	19	20			
Pitressin (vasopressor and anti-diuretic hormone, Parke-Davis)	water	18		17	15	
Pitocin (oxytocic hormone, Parke-Davis)	"	10		—1	2	

			Conc. in mg per 100 cc 0.0001	0.001	0.01	0.05	0.1	0.5	1
Physostigmine	water	10	0	13	15	19	37	79	175

* The S.E. of the mean for each value was less than ±10%.

because they strongly potentiate the contraction producing effect of acetylcholine.[1-7]

The height of contraction of muscle due to acetylcholine was slightly potentiated by most of the hormones used (Table I). The potency of these hormones may be evaluated by comparison with physostigmine. Low concentrations are as potent as low concentrations of physostigmine. However, an accurate quantitative comparison to physostigmine is impossible because the slight water solubility of some of the hormones prevents the evaluation of the amount dissolved and the use of more concentrated solutions.

Follicular hormone, Progesterone, Follutein, Testosterone, and Pitressin potentiated the acetylcholine contraction of the *Rectus abdominis* muscle from 15 to 40% more if mixed with brain extract or urine than did brain extract or urine alone. Pitocin did not modify the contraction producing power of brain extract or urine. Experiments using the same hormones in the same solvents, but inactivated by heat or oxydation, were also performed and the results demonstrated that the effect of the hormones on the muscle contraction induced by acteylcholine was due to the hormones themselves.*

The potentiation of the effect of acetylcholine may be due to at least two processes: (1) increased sensitivity of the effector cells to stimuli, and (2) decreased inactivation of acetylcholine by tissue choline esterase. The first possibility was investigated by studying the effect of the above hormones on the response of the muscle to potassium, the second with choline esterase experiments.

Effect of the Hormones on the Potassium Sensitivity of Striated Muscle. The *Rectus abdominis* muscle was treated as described

1 Meng, *Chin. J. Physiol.*, 1940, **15**, 143.

2 Bernheim and Bernheim, *J. Pharm. Exp. Therap.*, 1936, **57**, 427.

3 Ettinger, Brown and Megill, *Ibid.*, 1941, **73**, 119.

4 Torda, *Ibid.*, 1943, **77**, 50.

5 Torda, *Ibid.*, 1943, **77**, 350.

6 Torda, *Ibid.*, in press.

7 Sobotka and Antropol, *Enzymologia*, 1937, **4**, 189.

* The results are not included because various aspects of the process of inactivation require further investigation and are not relevant to this study.

TABLE II.
Effect of Hormones on Muscle Contraction Due to Potassium.

Substance	Solvent	No. of exp.	Increase of height of contraction in % of control (mean values*) Conc. of substances in 100 cc: Traces	0.01 mg	0.05 mg	1 I.U.	10 I.U.	100 I.U.
Follutein	water	20				29	45	99
Estrone Suspension	water and acacia	12				24	29	43
Theelin	water	10						40
Estradiol	"	5	38					
Progestin (Roche-Organon)	peanut oil	12	26					
Progesterol (Ciba)	water	8	52					
Testosterone Propionate (Ciba)	"	12	40					
Testosterone Propionate (Schering)	sesame oil	20	36					
Doca (desoxycorticosterone, Roche-Organon)	" "	10	46					
Cholesterol	water	20		47	60			
Pitressin	"	18				17	20	
Pitocin	"	10				2	1	

* The S.E. of the mean for each value was less than ±10%.

above but was contracted by a 20 mM potassium solution instead of acetylcholine. Most of the hormones used increased the potassium sensitivity of the *Rectus abdominis* muscle (Table II). Results paralleling the above were obtained using desoxycorticosterone or cholesterol, indicating that the potentiation of the potassium sensitivity may be a general property of the sterol compounds.

The solvents alone (sesame oil, peanut oil, and water with acacia) did not modify the effect of potassium on the muscle. Other solvents (alcohol, ether, acetone, bile salts) were not used because they strongly potentiate the contraction producing power of potassium. Follicular hormone, Progesterone, Follutein, Testosterone, and Pitressin potentiated the contraction of the *Rectus abdominis* muscle from 15 to 50% more if mixed with brain extract or urine than did brain extract or urine alone. Pitocin did not modify the contraction producing power of brain extract or urine. Experiments using the same hormones in the same solvents, but inactivated by heat or oxydation, were also performed and the results demonstrated that the effect of the hormones on the muscle contraction induced by acetylcholine was due to the hormones themselves.*

Experiments with Choline Esterase. (A) *Biological Method.* The choline esterase of steer brains was concentrated by the method of Bernheim and Bernheim.[2] Acetylcholine was added to the suspension of choline esterase to form a final concentration of 0.1 mg per 100 cc. The *Rectus abdominis* muscle was immersed for 30 seconds in this mixture immediately after the addition of acetylcholine and again 10 minutes later. The suspension of choline esterase was prepared in such concentration that 50% of the acetylcholine added was destroyed during the 10 minutes of contact. The same procedure was followed with mixtures containing hormone, choline esterase and acetylcholine.

(B) *Experiments with Warburg's Method.* Brains of steer, rabbit and pig were frozen and finely ground. The tissue was pressed through muslin and suspended in bicarbonate Ringer's solution at pH 7.4. Two cc of the suspension with or without hormone were placed in the body of the vessel and 0.2 cc of a 5 mg per 100 cc acetylcholine solution was placed in the side cup. The choline esterase activity of the brain was then determined by the method of Warburg (Ammon[8]). The solvents alone (sesame oil, peanut oil, and water with acacia) did not modify the activity of choline esterase.

The results of the biological test indicate that hormones potentiating the acetylcholine contraction of the striated muscle decrease the activity of choline esterase (Table III). These

[8] Ammon, *Pflüger's Arch.*, 1933-34, **233**, 486.

TABLE III.
Effect of Hormones on the Choline Esterase Activity. (Biological Method.)

Substance	Conc. in 100 cc	Decrease of activity of choline esterase in % of control: Mean (of 5 exp.)	S.E. of mean
Follutein	100 I.U.	—26	±2.0
Estrone Suspension	500 "	—23	±2.9
Progestin	Traces	—20	±2.3
Testosterone Propionate	"	—21	±1.9
Pitressin	50 I.U.	—12	±2.2
Pitocin	50 "	0	±1.0

TABLE IV.
Effect of Hormones on the Choline Esterase Activity. (Warburg's Method)

Substance	Conc. used	No. of exp.	Decrease of activity of choline esterase in % of control: Mean	S.E. of mean
Follutein	5 I.U.	25	—24	±1.8
Estrone Suspension	100 "	10	—22	±2.1
Progestin	1 "	15	—18	±1.6
Testosterone Propionate (Schering)	25 mg	10	—19	±1.9
Pitressin	2 I.U.	10	—13	±2.2
Pitocin	5 "	10	0	±2.2

observations were confirmed by the chemical test (Table IV). An evaluation of the anti-choline esterase potency of the above substances may be attempted by comparison with the anti-choline esterase activity of physostigmine considering that $2.5/10^4$ mg% physostigmine inhibits the choline esterase activity by 50%.[8] This evaluation is of necessity an approximate one because the very slight water solubility of most of the hormones used prevents an accurate evaluation of the amount dissolved or the amount entered in contact with the enzyme.

Discussion. Some of the sex hormones and some of the hormones secreted by the hypophysis potentiate the sensitivity of the effector cells to certain chemical stimuli and decrease the choline esterase activity of the tissue. The lowest concentrations required to exert these effects are comparable with biological concentrations of the hormones. It is possible that the potentiation of the response of the *Rectus abdominis* muscle to chemical stimuli is due to impurities of the substances used or to physicochemical properties of the large molecules of the hormones distributed in the extracellular spaces, but experiments of Pompen[9] and Reynolds and Foster[10,11] show that at least the follicular hormone has a cholinergic effect *in vivo*. The above results suggest that sex hormones, hypophyseal hormones and cholesterol may play a part in the maintenance or change of excitability of effector cells.

Summary. 1. Both the acetylcholine and potassium sensitivity of striated muscle and the activity of choline esterase of brain tissue were investigated in the presence of sex hormones and some of the hormones secreted by the hypophysis. 2. The acetylcholine sensitivity of the muscle is somewhat potentiated by the anterior pituitary-like sex hormone (Follutein, Squibb), the follicular hormone (Estrone Suspension, Abbott; Estradiol, Ciba; Theelin, Parke-Davis), the corpus luteum hormone (Progestin, Roche-Organon; Progesterol, Ciba), Testosterone Propionate (Ciba, Schering), the vasopressor and anti-diuretic hormone of the posterior part of hypophysis (Pitressin, Parke-Davis), but not by the oxy-

9 Pompen, *De Invloed van Menformon op de Baarmoeder*, Theses, Amsterdam (cited by Reynolds, *Physiology of the Uterus*, Harper & Brothers, New York and London, 1939).

10 Reynolds and Foster, *J. Pharm. Exp. Therap.*, 1940, **68**, 173.

11 Reynolds and Foster, *Am. J. Physiol.*, 1939, **128**, 147.

tocic principle of the posterior part of hypophysis (Pitocin, Parke-Davis). 3. The activity of the choline esterase is somewhat decreased by Follutein, Estrone Suspension, Progestin, Testosterone Propionate, Pitressin, but not by Pitocin. 4. The potassium sensitivity of the muscle is potentiated by Follutein, Estrone Suspension, Estradiol, Theelin, Progestin, Progesterol, Testosterone Propionate, Doca (desoxycorticosterone, Roche-Organon), cholesterol, Pitressin, but not by Pitocin. 5. A correlation between threshold of excitability of the effector cells and the presence of the above substances was suggested.

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14215 P

A Simple Method for the Concentration of Blood Plasma and Serum.

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In the course of the preparation of plasma for human use it was observed that on the thawing of frozen plasma, separation into a number of layers takes place; the lowermost deep-yellow layer is rich in proteins, while the uppermost, nearly colorless layer, contains only a small amount of protein. On repeated freezing and thawing, the separation becomes more distinct. A more rapid and sharp separation of plasma into layers is obtained by centrifuging the frozen material.

It is interesting that Bujwid[1] used the freezing method for the concentration of diphtheria and tetanus anti-toxin in 1897. His results were confirmed by Ernst, Coolidge and Cook.[2] Rossi[3] used the freezing and centrifuging procedures for fractionating plasma and other colloidal solutions, and Hati[4] used this technic for the concentration of complement, opsonins and anti-sera. In view of the above observations, the question arose as to whether these procedures might be employed for the concentration of plasma without excessive loss of solids.

The following is a representative fractionation and analysis of plasma: 100 cc were placed in a separatory funnel and frozen at —10°C; on partial thawing at 4°C, a deep yellow fluid was obtained with a colorless ice block floating on the surface. The freezing and thawing process was twice repeated. During the thawing which followed the third freezing, the melting fluid was collected directly into graduated cylinders. Three fractions were obtained in this manner. The first fraction of 25 cc was deep-yellow; the second

TABLE I.

	cc plasma vol.	% total protein	% albumin	mg% non-pro-N	mg% choles-terol	mg% chlor-ides	agglu-tinins
Original fraction	100	5.3	3.5	40	125	400	1:16
No. 1	25	15.0	9.3	83	255	1107	1:64
No. 2	17	6.5	4.3	40	185	382	1:32
No. 3	58	0.7	0.5	17	28	100	1: 8

1 Bujwid, *Z. f. Bakt.*, 1897, **22**, 287.

2 Ernst, Coolidge and Cook, *J. Boston Med. Soc.*, 1898, **2**, 166.

3 Rossi, G., *Arch. di Fisiologia*, 1904, **2**, 638.

4 Hati, S., *Z. f. Bakt.*, 1909, **48**, 203.