such strains, if isolated, may be important as immunizing agents against poliovirus in humans. There is little experimental data to help us choose between these alternatives and little will be gained by further discussion of the possibilities since proof in either case requires the isolation from animals of the specific infectious agent.

Summary. (1) The sera of cows and steers were shown to possess neutralizing substances in high titer against the viruses of poliomyelitis. These have been shown to be specific antibodies against the viruses of poliomyelitis. (2) The sera of horses, hogs and chickens also possessed modest neutralizing activity against the viruses of poliomyelitis. (3) The sera of dogs, cats, calves and lambs do not possess neutralizing activity against the viruses of poliomyelitis. (4) Attempts to isolate a virus from the bovine species that might explain the origin of these antibodies were unsuccessful. The antigenic stimulus giving rise to antibodies against the viruses of poliomyelitis remains unknown.

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Myasthenia gravis. I. Importance of Potassium in Inhibitory Action of Normal and Myasthenic Thymus Extracts.* (22110)

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To investigate the concept that myasthenia gravis is due to a circulating blocking agent possibly elaborated by the thymus (McEachern)(1) several investigators(2-5) have tested thymus extracts on nerve-muscle preparations from various animals.

The present study was undertaken to repeat Dr. Wilson's experiments with the eventual objective of isolating the active blocking substance. The isolated frog sartorius nerve-muscle preparation, which had been used successfully by Wilson and Stoner(6) to detect the blocking activity of serum from myasthenic patients, was used in this investigation rather than the rat phrenic nerve-diaphragm preparation.

Methods. A. Isolated Frog Sartorius Preparation: Frogs were pithed and the sartorius muscle excised with a small block of underlying muscle to support the motor nerve. Frog Ringer, containing 6.5 g NaCl, 0.14 g KCl, 0.12 g $CaCl_2$, 0.2 g $NaHCO_3$ and 0.01 g NaH_2PO_4/l, bathed the muscle during dissection. After excision, the muscle was placed at normal resting length (5 to 10 g tension) in a plastic chamber of 2 ml capacity, provided with two sets of silver electrodes to permit independent direct and indirect stimulation of the muscle and nerve respectively. The small size of this chamber is of considerable advantage when only small volumes of test solution are available. The mechanical myogram was obtained by a transducer, consisting of an aluminum bar (6.5 x 1.2 x 0.015 cm) on either side of which was cemented a strain gage element.† The myogram was recorded with a Sanborn strain gage recorder (Model 127) fed from a Sanborn strain gage amplifier (Model 140). This arrangement permitted recording of the isometric myogram. All experiments were performed at 23 to 25°C. Thresholds for nerve and muscle excitability were recorded at beginning of each experiment. After 15 minutes equilibra-

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† Baldwin-Lima-Hamilton Corp., Type A7.

tion of the muscle in oxygenated frog Ringer, the Ringer solution was removed and the muscle stimulated via the motor nerve for 5 seconds with 0.5 to 1 volt monophasic stimuli of 0.5 millisecond duration at a frequency of 2 cps. A second and third control series of contractions were obtained at 5 minute intervals before adding test solution to the muscle chamber. At one to 3 minute intervals, the test solution was removed from the chamber and the muscle stimulated as before. When the muscle failed to respond to indirect stimulation, it was stimulated directly with 5 second trains of stimuli of increasing voltage (1,3, 5,10,14 volts). The muscle was then repeatedly washed with frog Ringer and recovery of muscle tension was followed for 60 minutes. Average amplitude of 10 consecutive tracings was computed and plotted against time. Activity of each extract was calculated on the basis of time for 50% inhibition of contraction strength per g of extracted thymus tissue.

B. *Method of extraction* was slightly modified after the method of Wilson, Obrist and Wilson(5). Thymus glands, removed at operation from patients with myasthenia gravis, were weighed and placed in cold acetone (4°C) after representative blocks had been taken for preparation of sections for microscopic examination. Each gland was chopped into small pieces and ground in a mortar with 25 ml volumes of cold acetone (4°C) until total extract volume of 1000 ml was obtained. Acetone extract (AE) was filtered and the residual insoluble material (AI) collected and dried to constant weight *in vacuo* over $CaCl_2$. The acetone fraction was evaporated to dryness *in vacuo* in a water bath at 37°C. After evaporation, the AE fraction which consisted of a yellow oil, was taken up in 5 ml of Frog Ringer. The red-brown, AI fraction was similarly taken up in 5 ml of frog Ringer. After adjusting the pH of the 2 extracts to the pH of frog Ringer, they were separately tested on the isolated frog sartorius preparation. A fresh sartorius muscle was used in each test. Sixteen thymuses from myasthenic patients, one normal child's thymus, 3 spleens and 2 samples of skeletal muscle were extracted and tested by this procedure. *C. Heat Inactivation and Dialysis Experiments*: Solutions of acetone insoluble fraction were placed in cellophane bags and dialyzed against frog Ringer. Aliquots of internal and external solutions were subsequently tested. Extracts of both acetone soluble and insoluble fractions were boiled for 2 minutes, cooled and tested on the sartorius preparation.

D. Experiments with d-Tubocurarine, $CaCl_2$ and KCl: d-Tubocurarine (0.003 to 15 γ /ml, and KCl (4 to 40 meq/L.) solutions were tested on the sartorius preparation. The effect of thymus extracts containing added $CaCl_2$ was also determined. *E.* The potassium concentration of 16 extracts was quantitatively determined by flame photometry.‡ Extracts of the acetone soluble fraction were ashed before determination of their potassium content because of fat in the solutions. *F. Thymus Pathology*: The presence of hyperplasia and germinal center formation was recorded (Castleman and Norris)(7) and the relative amounts of parenchyma, fatty and fibrous tissue was independently rated by two observers who were unaware of the activity of the extracts prepared from the glands.

Observations: *Control studies* performed

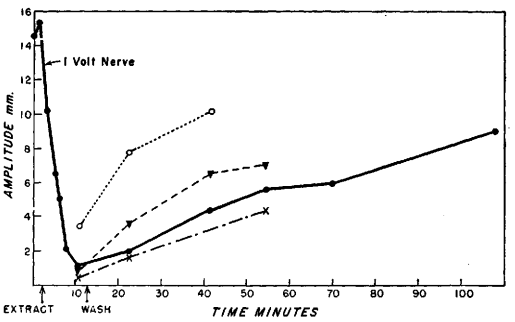

FIG. 1. Heavy circles illustrate decrease in contraction force of indirectly stimulated (1 volt) muscle resulting from immersion in thymus extract. After repeated washing, the contraction strength gradually returns. Crosses (1 volt), triangles (5 volts) and open circles (10 volts) illustrate that the muscle shows impaired response to direct stimulation. After washing, the curve of recovery of the directly stimulated muscle parallels the recovery curve of the indirectly stimulated muscle.

‡ Acknowledgment is due to Dr. Charles H. DuToit for these determinations.

TABLE I. Clinical Status of 16 Myasthenic Patients and the Activity of Extracts of Their Thymus Glands.

Age	Severity	Duration of symptoms	Activity of acetone insoluble fraction, min./g /50% inhibition
21	Mild	2.5 yr	1.33
25	"	1.5	.51
13	Moderate	3.5	.91
23	"	3.0	2.60
21	"	2.0	.20
15	"	1.5	1.33
16	"	1.0	.67
17	"	3 mo	1.14
19	"	5 yr	.73
30	Severe	7	.66
23	"	1	1.53
41	"	6	2.74 T*
13	"	2	.29
46	"	Months ?	.38 T
24	"	4.5 yr	.67
18	"	2	2.05

* T = Tumor.

with several changes of frog Ringer over periods of 60 to 70 minutes showed a maximal decrease in the tension curve of 15 to 20% of initial tension. When tissue extracts were tested, the average experiment rarely exceeded 30 minutes. Thus, decreases of the tension curve of greater magnitude are attributed to the inhibitory effects of the extracts. A dose-response curve constructed for several concentrations of d-Tubocurarine (0.003 to 15 γ/ml) showed that concentrations of 0.03 γ/ml of d-Tubocurarine could be accurately detected by this method. As expected, the muscle responded to direct stimulation; it failed to respond to indirect stimulation.

Experiments with Tissue Extracts: Both acetone soluble and acetone insoluble fractions of all thymus glands tested depressed the tension curve of indirectly stimulated sartorii in 2 to 20 minutes. However, when muscles failed to respond to indirect stimulation they also failed to respond to direct stimulation (Fig. 1). After 30 to 60 minutes of washing with several changes of frog Ringer, the test muscles slowly recovered. The time course of recovery for indirect and direct stimulation was similar (Fig. 1). Thus, it appeared that the extracts acted directly on the muscle, although action at the neuromuscular junction could not be excluded. Great variability of activity of extracts occurred which failed to correlate with age of patient, duration of symptoms or clinical severity of the disease. (Table I). A normal child's thymus possessed considerable inhibitory activity as did extracts of 3 spleens and 2 samples of human skeletal muscle. Extracts of these tissues rendered the test muscle inexcitable by indirect stimulation in 5 to 10 minutes. At this time, the muscles uniformly failed to respond to direct stimulation. The time course of recovery of the muscle after washing with several changes of frog Ringer was similar to that observed with thymus extracts.

Dialysis of Extracts: After dialysis of the acetone insoluble fraction, most of the inhibitory activity was present in the solution outside the cellophane membrane. This material produced a decrease of tension curves of both indirectly and directly stimulated muscles.

Heated Extracts: The acetone insoluble extract retained its inhibitory activity after boiling for 2 minutes.

Potassium concentrations of thymus extracts ranged from 2 to 33 times the concentration of potassium in frog Ringer. When the extract containing highest concentration of potassium (66 mEq/L.) was added to the test muscle, spontaneous fasciculations of the muscle occurred. These concentrations of potassium are 33 times the normal potassium content of frog Ringer. Table II shows a rough correlation between time for 50% inhibition of the muscle by the acetone-insoluble fraction and a poor correlation with the acetone soluble fraction.

Effect of Added Potassium Chloride. When

TABLE II. Potassium Content and Activity of Thymus Extracts.

Per g P_{50}	(K^+) P	TP_{50}	TA_{50}	(K^+) A
.727	2.0	8.0	—	—
.254	4.8	6.5	5.2	7.2
1.140	8.0	11.2	12.0	—
.671	11.0	8.0	3.0	7.7
.381	13.0	5.0	6.0	7.11
2.05	13.6	8.5	10.5	20.0
.287	17.0	8.8	1.9	6.1
2.740	38.5	3.7	4.7	24.3
1.330	42.0	2.9	—	—
.675	66.0	2.6	8.7	34.0

TABLE III. Potassium Content of Ten Thymuses Compared with Histological Architecture.

K content acetone soluble fraction, mEq/L	K content acetone insoluble fraction, mEq/L	Parenchyma	Fibrosis	Fat	Germinal center formation
2.0	—	1+	3+	2+	A*
4.8	—	3+	<1+	2+	P
8.0	—	3+	2+	1+	P
11.0	7.7	2+	3+	1+	P
13.0	7.7	3+	2+	2+	T
13.6	20.0	2+	2+	1+	A
17.0	6.1	4+	1+	1+	P
38.5	24.3	4+	1+	2+	T
42.0	—	4+	1+	1+	P
66.0	8.7	3+	0+	1+	A

* A = Absent; P = Present; T = Tumor.

frog Ringer solutions containing added potassium chloride were applied to frog sartorius preparations, the effects produced were similar to those obtained with thymus, spleen and striated muscle extracts. Low concentrations of added potassium (1.28 to 12.8 mEq/L.) gradually reduced tension response of test muscle to indirect and direct stimulation. The activity of these solutions roughly paralled activity of thymus extracts containing comparable concentrations of potassium. A T_{50} of 2.9 minutes was obtained when a thymus extract containing 40 mEq/L. of potassium was tested whereas a T_{50} of 4.5 minutes was obtained when a frog Ringer solution containing 40 mEq/L of added potassium was tested. The time course of recovery of the tension curve after washing with frog Ringer was similar to that observed after tissue extracts had been tested on the sartorius preparation. Addition of $CaCl_2$ to thymus extracts (5 to 10 times normal concentration of $CaCl_2$ in frog Ringer) decreased the inhibitory effects of the extracts on the tension curve of frog sartorii. In a typical experiment, the T_{50} of the acetone insoluble fraction was 2.6 minutes whereas after addition of $CaCl_2$ (total concentration—5 x 10^{-3} M) the T_{50} increased to 8.5 minutes.

Thymus Gland Pathology. In 15 glands tested, 9 showed hyperplasia and germinal center formation, 4 failed to show these changes and 2 were thymomas. Table III shows that thymus glands with the most parenchyma possessed the greatest inhibitory activity.

Discussion. The original intention of repeating previously published experiments concerning the muscle inhibiting effects of thymus extracts (Wilson, Obrist, and Wilson) (5) as a point of departure for isolation of the active material led to investigation of potassium content of acetone-extracts of thymus tissue. The finding of a heat stable, dialysable substance in thymus extracts and extracts of other tissues which rendered the test nerve-muscle preparation inexcitable on indirect and direct stimulation suggested that potassium might be responsible for the observed inhibitory action. Presence of potassium concentrations ranging from 2 to 44 times normal potassium content of frog Ringer confirms the idea that potassium alone could be responsible for the inhibition observed in our experiments and in experiments reported by others (Wilson, Obrist and Wilson) (5). Furthermore, correlation between potassium concentration of thymus and other extracts and their ability to depress tension curve of the frog sartorius preparation strongly suggests that this is the case. Duplication of these effects by adding equivalent concentrations of KCl to frog Ringer solutions constitutes additional evidence that potassium concentrations which occurred in thymus extracts can produce rapid decrease of muscle tension. Failure of activity of acetone-soluble fraction to correlate well with potassium content is probably due to the protective effect of lipids in the test solution.

The deleterious effect of increased potassium concentrations on muscle excitability has long been recognized (8-10). According to Gellhorn's data (10) at the sodium concen-

tration used in our experiments (0.134 M), concentrations of potassium chloride in excess of 96 x 10^{-4} M produce rapid loss of excitability. Table II shows that the thymus extracts tested contained 48 to 660 x 10^{-4} M of potassium. Gellhorn(10) showed that calcium ions also antagonized the inhibitory effect of increased potassium concentrations. Approximately equal amounts of $CaCl_2$ above the concentration (8.1 x 10^{-4} M) of $CaCl_2$ in frog Ringer antagonized approximately equal amounts of KCl added to normal frog Ringer solution. Thus, in experiments where $CaCl_2$ was added to thymus extracts, the decrease in inhibitory activity of the extract can be attributed to antagonism between high concentrations of potassium in the extracts and the added calcium ions.

It should be emphasized that the concentrated tissue extracts used contain other ions (Na^+, Ca^{++}, Mg^{++}) in undetermined concentrations which, because of abnormal ion ratios, may also contribute to inhibitory action of these extracts on isolated frog nerve muscle preparation. Therefore, we cannot conclude that elevated potassium concentrations alone are responsible for the inhibition observed. Furthermore, we cannot conclude that extracts of myasthenic thymus glands prepared in this way are unique in containing considerable quantities of potassium. There is no evidence to indicate that myasthenic thymus glands contain more tissue potassium than normal thymus glands of comparable cellularity. Since the extracts tested in this study were prepared according to the methods of Wilson and associates(5), it is probable that the results reported by these investigators may be partly, if not entirely, due to high concentration of potassium in the extracts. The combined acetone soluble and acetone insoluble extracts used by Wilson and associates may have contained considerable concentrations of potassium.

It is of interest that in our study a rough correlation was found between cellularity of thymus glands tested, their potassium content and the activity of the extracts. Extracts prepared from glands which contained little fatty and fibrous tissue contained more extractable potassium and possessed greater activity. Thus, it might be expected that a myasthenic or a normal child's thymus might contain more inhibitory activity than the fatty, fibrous glands of adults as reported by Wilson and associates(5). However, it is not possible to explain on this basis the positive correlation between extract activity and the post-operative course of the patients reported by these investigators. The contractures observed by Wilson and associates(5) when high concentrations of thymus extracts were added to frog rectus abdominis muscle preparations may have been due to potassium in the extracts(11-13). The potentiation of acetylcholine action on the frog rectus preparation by thymus extracts reported by Wilson and associates(5) may also be due to potassium ions in the extracts.

Since simple acetone and saline extraction of tissue breis results in crude mixtures of many components including small ions in varying proportions, extreme caution must be exercised in interpreting the effects produced by these mixtures on nerve-muscle preparations. Of particular importance is potassium which in low concentrations blocks the neuromuscular junction (Overton)(14) and in high concentrations abolishes the excitability of the muscle.

It is possible that the inhibitory activity of myasthenic thymus extracts may not entirely be due to potassium in the extracts, but inhibitory activity not due to potassium cannot be determined in the presence of excessive potassium concentrations or abnormal concentrations of other ions in the test solutions. Although desalting procedures might eliminate this source of error, it is probable that other extraction methods should be used in searching for inhibitory activity in thymus glands from patients with myasthenia gravis.

Summary. 1. A method is described which permits recording of the isolated myogram of indirectly or directly stimulated isolated frog sartorius muscle. 2. Acetone extracts prepared from thymus glands obtained from 16 myasthenic patients, one normal child's thymus, 3 spleens and 2 samples of striated muscle were tested on the isolated frog sartorius

preparation. 3. Both acetone soluble and acetone insoluble fractions markedly decrease the muscle tension curve in 2 to 20 minutes. When the muscle becomes inexcitable on indirect stimulation, it is also inexcitable on direct stimulation. 4. The active material is heat stable, dialysable and is antagonized by $CaCl_2$ added to the extracts. 5. Concentrations of potassium 2 to 44 times the potassium concentration of frog Ringer were found in the extracts. These concentrations of potassium are sufficient to produce the muscle inhibition observed. 6. The significance of these results relevant to the search for a blocking agent in the thymus glands of myasthenic patients is discussed.

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Serum Complement in Hepatobiliary Diseases.* (22111)

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There is considerable suggestive evidence in the literature that the liver may be involved in the formation of serum complement and that the latter is diminished in liver disease and in experimental hepatic injury. Reports prior to 1929 were reviewed by Goldner(1). They indicate diminution of serum complement (C′) in experimental phosphorus or alcohol poisoning and its disappearance following hepatectomy in rabbits. In perfusion experiments with heat-inactivated serum, guinea pig liver contributed complement to perfusing serum, enabling the latter to hemolyze sheep red cells. On the other hand, complement remained normal in abdominally eviscerated dogs which had retained their livers. Goldner, using a rabbit hemolysin-sheep cell system in which *complete* hemolysis was used for the C′ titer, found that C′ was subnormal in 8 of 20 cases of "icterus catarrhalis" and in 10 of 11 cases of (cholangitic and portal) cirrhosis; furthermore, 3 of 7 cases of metastatic tumor of the liver showed diminution or absence of complement, one case of acute yellow atrophy complete disappearance of C′, while 6 cases of cholecystitis revealed no change from normal. Recently, Jordan(2), using his hematocrit method, reported marked diminution of C′ in all of 18 cases of cirrhosis studied; 22 cases of hepatitis showed some drop in C′ near the peak of the disease, while 12 patients with obstructive jaundice yielded high values. In contrast to these findings, Dulaney(3), using a 50%-hemolysis endpoint as determined pho-

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