EXPERIMENTS ON THE ROLE OF POTASSIUM IN THE BLOCKING OF NEUROMUSCULAR TRANSMISSION BY CURARE AND OTHER DRUGS

By WALLACE O. FENN, REBECA GERSCHMAN, GEORGE FISCHER, JANE LACY, MARGARET D. BAILLY, and JOAN L. WRIGHT

(*From the Department of Physiology and Vital Economics, The University of Rochester, School of Medicine and Dentistry, Rochester, New York*)

(Received for publication, November 8, 1950)

There is a considerable amount of evidence that potassium is intimately concerned in the transmission of the nerve impulse from nerve to muscle. Thus Reginster (1938) postulates that acetylcholine, formed by the nerve impulse, liberates K from an organic compound RK and that it is the K thus liberated which stimulates the muscle. Coppé (1943) has further postulated that curare acts by previously liberating the K from RK and then forming a compound of curare with R. In the absence of RK there is then no source of K available to the acetylcholine for the stimulation of the muscle.

A theory of this sort is an attractive one for explaining the well known antagonism between K and curare (Mautner and Luisada 1941; Salama 1949; Quillam and Taylor 1947; Wilson and Wright 1936). Thus if the addition of curare served to release some K from a muscle compound RK and so caused paralysis, the excitability could be restored if the released K could be replaced by the addition of more K. In conformity with this hypothesis it has been reported that the injection of curare into guinea pigs decreases the K content of striated muscle and smooth muscle (Leulier and Vanhems, 1935). Likewise McIntyre and King (1944) have claimed that the addition of curare to Ringer's solution in which frog muscles are immersed results in greater liberation of K into the solution and a greater loss of K on stimulation. Ammonium has been found to act in the same way (Fenn *et al.*, 1944). It is known that curare does not prevent the liberation of acetylcholine at the ganglion or nerve muscle junction when the nerve is stimulated (Brinkman and Ruiter, 1925; Brown and Feldberg, 1936). It does, however, inhibit choline esterase (Harris and Harris, 1941; Fegler and Kowarzyk, 1938), and it does inhibit the response of the muscle to acetylcholine (McIntyre, King, and Dunn, 1945).

With these general theories of neuromuscular transmission in mind it seemed desirable to obtain some more direct evidence by determining whether it is in fact true that curare (or other related quarternary ammonium drugs) causes a liberation of K from muscle. For this purpose two types of experiments were tried, one with perfused and one with excised muscles.

1. Experiments with Perfused Muscles

In the experiments of series (*a*) and series (*b*) a cannula was inserted in the abdominal aorta of a frog and another in the abdominal vein. The renal portal vein was tied off so that all the venous return from the legs passed out by way of the cannula and was collected at intervals for analysis. The technique was in general similar to that described by Fenn, Koenemann, and Sheridan (1940). The perfusing solution consisted of 3 per cent acacia (dialyzed) in Ringer's solution containing 10 per cent of washed beef red blood cells. The red cells are supposed to avoid edema by supplying plenty of oxygen but they may also have blocked some of the capillaries as indicated by the considerable diminution of flow which occurred in the early stages of the perfusion. The perfusate was collected in small samples of about 2 to 3 ml., which were centrifuged and analyzed for potassium on the flame photometer. Similar analyses were made on the arterial inflow. When switching from the control to the drug solution, the control solution contained in the tubing above the cannula was withdrawn in a syringe so that the new solution entered immediately into the cannula. Care was taken also to obtain a pulsating pressure by automatically interrupting the flow at approximately 1 second intervals. This also is believed to be useful in minimizing edema. In general each experiment consisted of three 1 hour periods, before, during, and after the addition of the drug.

In the experiments of series (*c*) a slightly modified technique was used. The perfusing solution contained no acacia or red cells and was perfused at constant rather than intermittent pressure. The object was not only to simplify the procedure but to avoid possible clogging of the vessels with red cells. As a result, the edema was considerably greater in series (*c*) than in series (*a*) or (*b*), the increase in weight being often over 50 per cent. The perfusing solution contained (millimols per liter) NaCl, 111; $CaCl_2$, 1.1; KCl, 4 to 5; sodium phosphate buffers, 7 (as to P, pH 7.2). Flow rate was kept as constant as possible at about 0.2 ml. per minute by adjusting the perfusing pressure. Periods before, during, and after the drug usually lasted about 2 hours each. In the tables the experiment numbers are followed by letters *a*, *b*, or *c* according to the series to which they belong.

RESULTS

1. Experiments with Perfused Muscles

(*a*) *Curare.*—The experiments with *d*-tubocurarine are summarized in Table I. The slight increase in acidity caused by this drug was carefully neutralized to the pH of the control, at least in series (*c*). The figures given represent the A-V potassium differences (inflow minus outflow concentration). In series (*c*) the potassium diffused out of the muscles into the perfusate while in series (*a*), due to the higher concentration of K in the perfusate, the reverse was true. The last column shows the average liberation of K, this being the difference between the figures for the two controls (averaged) and that for the drug period. A minus sign in the last column indicates a relative absorption of K due to the drug and *vice versa.* The average liberation of K is only 0.04 m.eq. per liter which is altogether insignificant. It can be calculated that even

the largest liberation of K which was observed represents the loss of less than 1 per cent of the potassium contained in the muscle. Therefore, the method seems to be adequately sensitive but fails to support the theory of of Coppé. If the theory is correct it must apply to minute amounts of K in highly localized spots.

TABLE I

Effect of d-Tubocurarine on Liberation of Potassium from Perfused Frog Muscles

Experiment No.	Concentration of *d*-tubocurarine	Arteriovenous K difference			Average liberation of K
		Control before	*d*-Tubocurarine	Control after	
	p.p.m.	*m.eq/liter*	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*
4(*a*)	5	0.11	0.30	0	−0.24
5(*a*)	1	0.38	−0.04	−0.63	−0.07
6(*a*)	3	0.41	0.10	0.22	+0.22
7(*a*)	3	0.38	0.19	−0.05	−0.03
15(*a*)	25	0.28	0.32	0.26	−0.05
20(*a*)	1.2	0.29	0.25	0.11	−0.05
21(*a*)	3	0.40	0.19	0.31	+0.16
22(*a*)	2.4	0.41	0.13	0.23	+0.19
13(*c*)	5	−1.05	−0.95	−0.93	−0.05
14(*c*)	5	−0.61	−0.59	−0.79	−0.11
15(*c*)	5	−0.42	−0.76	−0.99	+0.05
16(*c*)	40	−1.25	−1.19	−1.22	−0.04
17(*c*)	40	−0.80	−1.06	−1.02	+0.15
18(*c*)	40	−1.25	−1.86	−1.22	+0.63
20(*c*)	1000	−0.83	−0.83	−0.76	+0.04
21(*c*)	1000	−1.25	−1.29	−0.92	+0.20
30(*c*)	1000	−0.29	−0.41	−0.64	−0.06
31(*c*)	1000	−0.67	−0.57	−0.80	−0.16
32(*c*)	1000	−0.03	−0.19	−0.42	−0.04
Average					+0.04

(*b*) *Dihydro-β-erythroidine.*—Similar experiments with dihydro-β-erythroidine are shown in Table II. The results indicated no significant effect except in two experiments in series (*a*) in which relatively low concentrations of the drug were used. Since the much larger concentrations in series (*c*) failed to confirm this effect, this preliminary result must be regarded as possibly accidental.

(*c*) *Myanesin.*—In five experiments with this drug there was uniformly a small liberation of potassium but the magnitude was so small compared to the analytical error that the effect cannot be regarded as very important even if it is real (Table II).

(*d*) *Acetylcholine.*—With so many negative results obtained by this method,

it seemed desirable to try acetylcholine which has been reported to give positive effects. The results are shown in Table III. Eserine was always added to the perfusion fluid along with the acetylcholine, in a concentration of 20 p.p.m. Two experiments with this concentration of eserine alone showed a slight liberation of K in one (+0.12 m.eq. per liter) and a slight absorption (0.18 m.eq. per liter) in the other. It is felt, therefore, that the results with acetyl-

TABLE II

Effect of Dihydro-β-erythroidine and Myanesin on the Liberation of Potassium from Perfused Frog Muscle

Experiment No.	Concentration of drug	Arteriovenous K difference			Average liberation of K
		Control before	Drug	Control after	
	p.p.m.	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*
Dihydro-β-erythroidine					
8(*a*)	2	0.96	0.50	0.77	+0.36
10(*a*)	8	0.49	0.18	0.36	+0.25
13(*a*)	4	0.39	0.19	0.12	+0.07
22(*c*)	100	−0.69	−0.89	−1.08	0
23(*c*)	100	−0.89	−1.03	−1.16	0
24(*c*)	100	−0.86	−0.83	−0.95	−0.05
25(*c*)	100	−0.99	−1.00	−0.71	+0.15
35(*c*)	100	−0.25	−0.43	−0.67	−0.03
36(*c*)	100	−0.35	−0.42	−0.35	+0.07
Average					+0.09
Myanesin					
37(*c*)	1000	−0.35	−0.68	−0.80	+0.11
38(*c*)	1000	−0.42	−0.76	−0.90	+0.15
39(*c*)	1000	−0.48	−0.77	−0.99	+0.04
40(*c*)	1000	−0.88	−1.01	−1.02	+0.06
42(*c*)	1000	−0.61	−0.74	−0.83	+0.02
Average					+0.07

choline can be attributed to that drug rather than to the eserine which was simultaneously present.

Without curare, the acetylcholine caused a liberation of K amounting to 0.45 m.eq. per liter. When curare was simultaneously present in sufficient concentration to cause paralysis, the liberation of K was only 0.05 m.eq. per liter, which is scarcely enough to be significant. Without the curare, the muscles showed perceptible twitching when the acetylcholine passed through the vessels, suggesting that the liberation of K is concerned with the concentration

rather than with the process of excitation. In other experiments Fenn and Gerschman (1950) have found, however, a liberation of K from nerve treated with acetylcholine; likewise this may have been concerned with some characteristic of the response of the nerve rather than with its excitation. The loss of K from perfused frog muscles under the action of acetylcholine was demonstrated also by Kometiani *et al.* (1945) and by Cicardo and Moglia (1940). The latter authors also report that the loss of K is intensified by previous denervation of the muscles and that it is eliminated by previous treatment with curare.

TABLE III

Effect of Acetylcholine Plus Eserine (20 P.P.M.) on Liberation of Potassium from Perfused Frog Muscle

Experiment No.	Concentration of drug	Arteriovenous K difference			Average liberation of K
		Control before	Drug	Control after	
	p.p.m.	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*
Without curare					
19(*a*)	125	0.03	−0.78	−0.47	+0.56
23(*a*)	50	0.41	−0.79	−0.73	+0.63
24(*a*)	50	0.11	−0.43	−0.47	+0.13
2(*b*)	50	0.08	−0.05	0.08	+0.13
5(*b*)	50	0.71	0.20	0.59	+0.45
27(*c*)	50	−0.58	−1.16	−0.83	+0.45
28(*c*)	50	0.32	−1.01	−0.98	+0.68
Average					+0.45
With curare, 500 p.p.m.					
4(*b*)*	50	0.43	0.34	0.24	+0.09
6(*b*)*	50	−0.38	−0.92	−1.25	+0.54
9(*b*)*	50	0.79	0.15	0.24	+0.64
33(*c*)	50	−0.81	−1.05	−1.03	+0.13
34(*c*)	50	−0.68	−0.67	−0.65	0
41(*c*)	50	−0.30	−0.38	−0.36	+0.05
43(*c*)	50	−0.48	−0.51	−0.51	+0.02
44(*c*)	50	−0.38	−0.52	−0.57	+0.05
45(*c*)	50	−0.29	−0.45	−0.38	+0.06
Average					+0.05

* Only 60 p.p.m. curare; neuromuscular block not complete; figures not included in average.

2. *Experiments with Excised Muscles*

Because of some feeling of dissatisfaction with the perfusion technique, it seemed worthwhile to attempt to confirm the liberation of K by curare reported by McIntyre and King (1944) by a technique more similar to the one which they used. Accordingly, some experiments were tried in which a number of small frog muscles (sartorius, semitendinosus, etc.) were immersed for varying periods of time in 200 ml. of Ringer's solution with and without the

TABLE IV

Effect of d-Tubocurarine on the Loss of Potassium from Muscle

Concentration of curare	Time of immersion	Initial weight of control muscles	K content of muscles after immersion		ΔK
			Control	With *d*-tubocurarine	
p.p.m.	*hrs.*	*mg.*	*m.eq./kg.*	*m.eq./kg.*	*m.eq./kg.*
4	3	602 (4)	72.4	64.0	−8.4
10	6	324 (3)	75.0	76.7	+1.7
10	6	748 (1)	84.3	78.5	−5.8
10	6	252 (3)	75.8	72.5	−3.3
10	6	750 (1)	82.7	88.3	+5.6
100	5.5	434 (3)	70.0	67.5	−2.5
100	5	360 (4)	72.5	72.5	+6.0
1000	8	379 (3)	71.5	74.5	+3.0
1000	8	652 (1)	71.8	75.5	+3.7

Paired muscles were soaked for varying periods in Ringer's solution with and without the addition of *d*-tubocurarine of varying concentrations as indicated (in parts per million). After 3 to 8 hours the muscles were removed, blotted gently, weighed, ashed, and analyzed for potassium on the flame photometer. The results were calculated in milliequivalents of K per kg. of initial weight of the muscle. Figures in parentheses indicate the number of pairs of muscles used in each experiment. The gastrocnemius was used for a single large muscle and for the small muscles the sartorius, semitendinosus, ileofibularis, peroneus, and the tibialis anticus were used. Changes in the weight during immersion varied from −6 to +8 per cent. The drug had no obvious effect on the weight changes. The difference in weight between paired sets of muscles averaged 2.3 per cent and did not exceed 5.4 per cent.

addition of curare or myanesin or acetylcholine. After varying periods of time the muscles were removed from the solution and analyzed for potassium. This method has the advantage over the perfusion technique that the loss of potassium is cumulative rather than being distributed over many samples of perfusate. Alternatively the muscles were immersed in small volumes of solution and after varying periods of time the solutions were analyzed for potassium. The results of these experiments are reported in Tables IV, V, and VI.

In Table IV are shown the results of nine experiments with *d*-tubocurarine. Details of the experiments are given in the legend. The last column shows the difference in the potassium content of the control and the experimental mus-

TABLE V

Effect of d-Tubocurarine on the Loss of Potassium from Muscles Immersed in Ringer's Solution

Initial weight of control muscles	Volume of solution	Loss of K per gm. initial weight of muscle		ΔK
		Control	With *d*-tubocurarine	
mg.	*ml.*	*m.eq./kg.*	*m.eq./kg.*	*m.eq./kg.*
379 (3)	2	2.1	4.5	+2.4
652 (1)	2	3.62	4.26	+0.64
246 (2)	3	10.15	8.4	−1.75
175 (3)	3	16.62	17.21	+0.59
315 (4)	3	8.99	8.85	−0.14
946 (4)	3	4.55	3.23	−1.32

Matched muscles were immersed in small volumes of solution with and without the addition of 0.1 per cent *d*-tubocurarine. After varying periods of time the muscles were removed, blotted gently, and weighed, and aliquots of the solutions were analyzed for potassium. The increased amounts of potassium found in the solutions (after allowing for the changes in the volumes of the solutions due to swelling of the muscles) were calculated as a loss of potassium in milliequivalents per kg. of initial weight of muscle. All the muscles gained slightly in weight during immersion. The gain varied from 0.8 to 6.3 per cent of the muscle weight in different muscles (average 4 per cent). The average difference between two paired muscles was 1.8 per cent and never exceeded 3.5 per cent. Figures in parentheses indicate numbers of muscles used in each solution. The gastrocnemius was used for a single large muscle and the small muscles were sartorius, ileofibularis, tibialis anticus, peroneus, or semitendinosus.

TABLE VI

Effect of Myanesin (0.1 Per Cent) on the Loss of Potassium from Muscle

Initial weight of control muscles	Volume of solution	Loss of K from muscle		ΔK
		Control	Myanesin	
mg.	*ml.*	*m.eq./kg.*	*m.eq./kg.*	*m.eq./kg.*
724 (1)	2	3.52	4.91	+1.39
715 (4)	3	3.50	3.38	−0.12
552 (4)	3	5.71	6.76	+1.05
973 (1)	3	2.14	2.19	+0.05

Matched pairs were immersed in small volumes of Ringer's solution with and without the addition of 0.1 per cent myanesin. After varying periods of time the muscles were removed, blotted gently, and weighed, and aliquots of the solution were analyzed for potassium. The observed increase in the amount of K found in the solution (after allowing for any changes in volume due to movement of water into or out of the muscle) was calculated as a loss of K per kg. of initial weight of the muscle. Changes in the weight of the muscles during immersion varied between −1 and +3 per cent and the differences between paired muscles were always less than 1 per cent of the muscle weight. Figures in parentheses indicate the number of pairs of muscles used in each experiment.

cles after the period of immersion. About half of the experiments showed no absorption and half showed a liberation of K due to the drug, the maximum

differences being in the order of 10 per cent. It is difficult to know why the differences are so large, but in any event there is no consistent effect due to the drug.

A similar conclusion results from the six experiments of Table V in which analyses were made in the solutions rather than in the muscles. Details are again provided in the legend under the table. The differences in the K contents of the two solutions were calculated in milliequivalents per kilo of muscle and are shown in the last column. In this case a plus sign indicates a liberation of K into the solution by the drug. Again, half the experiments showed a gain and half a loss of K by the muscles. The maximum loss of K amounted to only 2.14 m.eq. per kg. of muscle, which is about 3 per cent of the muscle content. The differences must be regarded, therefore, as merely random errors. It should be mentioned that in all these experiments except the last four in Table V, the *d*-tubocurarine was not neutralized and was found later to be rather acid. An increase in acidity is known to cause a liberation of K. These experiments failed to show any extra liberation of K in spite of the slightly increased acidity due to the drug.

In Table VI are shown the results of some similar experiments in which muscles were soaked in Ringer's solution with and without the addition of myanesin. Here again the results were negative, there being no consistent changes in the potassium which could be correlated with the drug. Two other series of similar experiments were tried. In the first there were 7 pairs of muscles immersed, each muscle in 5 ml. of solution; and in the second series 6 pairs, each muscle in 100 ml. of solution. In both cases one muscle of each pair had 0.1 per cent myanesin added to the Ringer's and the immersion periods were 2.7 hours at 23°C. Each muscle was analyzed separately. The average percentage differences (and standard errors) in the two series were respectively 4.5 ± 3.1 and 2.9 ± 2.8. Here again, therefore, no significant effect of this drug could be observed. This is not surprising since myanesin is supposed to exert its effect centrally rather than on the myoneural junction.

Since it has been shown that nerves lose potassium when immersed in solutions containing acetylcholine (Fenn and Gerschman, 1950) and since perfused muscles lose potassium when acetylcholine is added to the perfusing solution, it seemed probable that other tissues including muscle would also lose potassium more rapidly when acetylcholine is added to the solution surrounding the muscles. A number of experiments were carried out to test this possibility by immersing 2 comparable pieces of frog tissue in 50 ml. of Ringer's solution each, one with and the other without 1/20,000 acetylcholine bromide plus 1/50,000 eserine salicylate. After about 3 hours in the cold room at 4°C. the tissue was removed, weighed, and analyzed for potassium. Weight changes were small and not influenced by the drug. The percentage differences in K between the experimental and the control (100 E-C)/C) for liver were +4.2

and −7.3; for kidney −2.46 and −11.6; for skin −19.6 and 0; for muscle 0, −8.7, +3.6, and +3.9. A later series of experiments using stronger acetylcholine (0.1 per cent) gave percentage differences of +1.1, +8.7, −7.5, and +5.0. Many more experiments would obviously be needed to establish a statistically reliable conclusion for liver, kidney, and skin, although no constant difference seems indicated by these experiments. In muscle, however, it might be concluded that the loss due to immersion in the acetylcholine is on the average 0.6 ± 2 per cent (standard error) which is a totally negligible difference.

This negative result is rather surprising since the perfused muscles have regularly shown a loss of K with addition of acetylcholine. It seems probable, however, that this can be attributed to the difference in the conditions of the two types of experiments. Exposure of muscle to acetylcholine causes a contracture followed by a relaxation which is complete in 6 to 8 minutes. Since curare prevents the liberation of K it may be supposed that the period of liberation of K lasts little longer than the actual contracture, after which the K may be reabsorbed in recovery. The recovery uptake of K may be so slow as to be imperceptible in the perfusion experiments but the cumulative effect in prolonged immersion experiments might be sufficient to restore the muscle K to normal. This suggests that a very brief immersion period might reveal a loss of K which was not evident in longer experiments. To test this, four experiments were tried in which the muscles were removed from the solution and analyzed when the contracture is at its peak, 3 minutes after exposure to acetylcholine 0.1 per cent (without eserine) at 23°C. These experiments also showed no significant differences (−8.7, −7.4, +0.7, and +2.2 per cent). In so short a time as 3 minutes, of course, no loss of K could be expected to occur except from the most superficial fibers.

In these last experiments it will be noted that eserine was omitted so that any acetylcholine which penetrated the muscle might have broken down into choline and acetic acid. The resulting increase in acidity inside the muscle might tend to prevent the loss of K (*cf.* Fenn and Cobb, 1934). It seemed possible also that eserine might have a metabolic effect, since Greig and Holland (1949) have shown that breakdown of acetylcholine provides energy for the transport of K into the interior of red cells. To test the possible effect of eserine in muscle, a series of fourteen experiments was tried in which matched muscles were immersed in Ringer's plus acetylcholine, one muscle having eserine (0.02 per cent) added and the other not. No significant differences were found in the K contents of the two muscles after immersion for 5 hours at 5°C. The acetylcholine concentrations used were 5, 50, and 1000 parts per million, the number of experiments with each being three, nine, and two respectively. Half of the experiments showed a greater loss with eserine and the other half a gain so that on the average there was no effect.

It seems evident that acetylcholine does not control the K intake in muscle as it does in red cells. From these experiments it must be concluded that the K liberated by acetylcholine in perfused muscles is a rather transitory affair and may come from a very small fraction of the muscle, perhaps the myoneural junctions or the nerves contained in the muscle. The immersion technique is evidently not appropriate for the demonstration of this phenomenon. It might be mentioned also that the perfusion technique is much more sensitive for this type of exchange because a very small percentage of the muscle K can cause a relatively large percentage change in the concentration of K in the perfusate. Assuming a flow of 0.2 ml. per minute for 20 minutes and a change in K concentration due to acetylcholine of 0.45 m.eq. per liter (as in Table III), it can be calculated that the total K removed was 0.0108 m.eq. Assuming that there are 10 gm. of muscle in the hind legs of a frog, this represents only 1.3 per cent of the K contained in the perfused muscles. Therefore, unless acetylcholine causes a continuing loss of K throughout the period of immersion, the loss expected from the perfusion data would be much too small to measure by analysis of the muscle itself. Acetylcholine 0.1 per cent represents 6 m.eq. per liter which by cation exchange should have liberated eventually nearly 10 per cent of the K in the muscle. Evidently, therefore, this mechanism does not operate and the loss of K observed in perfused muscles depends upon the physiological response of the muscle and is too small and/or too transitory to be observed in immersed muscles.

SUMMARY

1. Experiments with perfused frog muscles and with isolated frog muscles immersed in Ringer's solution have failed to show any effect of curare in liberating potassium from muscle tissue. This makes it difficult to suppose that the paralytic effect of curare can be attributed to cation exchange between curare and K whereby a labile potassium compound needed for stimulation is removed from the neuromuscular junction.

2. Similar negative results were obtained with dihydro-β-erythroidine and myanesin.

3. A small liberation of K from perfused muscle does result from treatment with acetylcholine. This is probably due to the contracture of the muscle since the effect is largely eliminated by previous treatment of the muscle with curare. The amount of potassium lost in this way from perfused muscles is too small to detect when muscles are analyzed after immersion in Ringer's solution with and without acetylcholine. It is concluded that there is no significant cation exchange between acetylcholine and K in muscle, but only a small loss of K due to the contracture produced by the acetylcholine.

The authors wish to make grateful acknowledgment to the National Foundation for Infantile Paralysis for a grant which supported a part of the expenses of this work.

REFERENCES

Brinkman, R., and Ruiter, M., *Arch. ges. Physiol.*, 1925, **208,** 58.

Brown, G. L., and Feldberg, W. J., *J. Physiol.*, 1936, **86,** 10P.

Cicardo, V. H., and Moglia, J. H., *Nature*, 1940, **145,** 551.

Coppé, G., *Arch. internat. physiol.*, 1943, **53,** 327.

Fegler, J., and Kowarzyk, M. H., *Compt. rend. Soc. biol.*, 1938, **127,** 1149.

Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, **17,** 629.

Fenn, W. O., and Gerschman, R., *J. Gen. Physiol.*, 1950, **33,** 195.

Fenn, W. O., Haege, L. F., Sheridan, E. T., and Flick, J. B., *J. Gen. Physiol.*, 1944, **28,** 53.

Fenn, W. O., Koenemann, R. H., and Sheridan, E. T., *J. Cell. and Comp. Physiol.*, 1940, **16,** 255.

Greig, M. E., and Holland, W. C., *Arch. Biochem.*, 1949, **23,** 370.

Harris, M. M., and Harris, *R. S.*, *Proc. Soc. Exp. Biol. and Med.*, 1941, **46,** 619.

Kometiani, P. A., Dolidze, S. V., and Klein, E. E., *Biol. Abstr.*, 1945, **19,** 2039 (No. 18509).

Leulier, A., and Vanhems, G., *Compt. rend. Soc. biol.*, 1935, **118,** 256.

Mautner, H., and Luisada, A., *J. Pharmacol. and Exp. Therap.*, 1941, **72,** 386.

McIntyre, A. R., and King, R. E., *Fed. Proc.*, 1944, **3,** 81.

McIntyre, A. R., King, R. E., and Dunn, A. L., *J. Neurophysiol.*, 1945, **8,** 297.

Quillam, J. P., and Taylor, D. B., *Nature*, 1947, **160,** 603.

Reginster, A., *Arch. internat. physiol.*, 1938, **47,** 71.

Salama, B., *J. Physiol.*, 1949, **108,** 50P.

Wilson, A. T., and Wright, S., *Quart. J. Exp. Physiol.*, 1936, **26,** 127.