DRUG AND ION EFFECTS IN FROG MUSCLE*

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INTRODUCTION

In the course of studies which have been conducted on frog nerve (*e.g.* references 18 and 19, to be reported in detail) results were obtained which suggested that certain experimental agents which block conduction without causing depolarization (*i.e.*, "stabilizers," such as procaine, cocaine, yohimbine, pyribenzamine) reduce membrane permeability to potassium; conversely, other compounds which lead to augmented excitability (*i.e.*, "unstabilizers," such as calcium precipitants, veratrine) were found to produce diametrically opposite effects interpretable in terms of increased potassium permeability (*cf.* references 18–21). Earlier electrical and conductivity studies with related compounds support this view (*e.g.* references 11, 14, 23, and 24), but more direct data appear desirable.

Preliminary to undertaking the application of chemical methods, use has been made of the membrane properties of muscle, as elucidated by Meigs and Atwood (17) and by Boyle and Conway (2), to test for such permeability changes. The penetrability of small anions permits KCl to enter muscle, consequently when potassium replaces the sodium of the medium the muscles swell at a rate determined primarily by the rate of penetration of the potassium with the anion (4). Hence the rate of weight gain may serve as an index of the membrane permeability to potassium as well as to the anion, and has been so used in the following experiments.

An altered rate of KCl penetration in itself gives no indication as to whether the cation, anion, or both have had their mobilities changed. The initial rapid depolarization normally induced by this salt apparently is a diffusion potential dependent on the considerably greater mobility of the cation in the membrane (18); changes in the degree of such depolarization therefore offer a means of distinguishing the ion particularly affected. Consequently the action of the stabilizers and unstabilizers on the KCl depolarizability of the muscle has also

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been observed. In addition to this the effect of some of the agents themselves on muscle polarization has been examined.

Method

Only sartorius muscles from *Rana pipiens* were employed. These were removed in pairs; one of each pair served as a control, a procedure which gave good uniformity of results. Water uptake was measured as weight gain. Typically, 3 to 4 pairs of muscles were dissected out at one time and a silk thread tied to the tendon of each muscle for handling. They were immersed for 1 hour in oxygenated Ringer's solution or in the experimental solution. During this period or the succeeding hour a baseline was obtained by weighing the individual muscles successively and repeatedly 4 to 6 times on a 250 mg. Roller-Smith precision torsion balance. Determinations to 0.1 mg. were thus rapidly made. Each weighing was preceded by careful blotting of muscle and thread on filter paper. Following this the muscles were usually transferred to a solution which caused swelling—either hypotonic Ringer or Ringer with half the sodium replaced by potassium—and which for the experimental preparations usually also contained the "stabilizer" or "unstabilizer." The time course of the weight increase was observed in all cases, repeated readings being taken for 1 to 3 hours. The muscles were then returned to the previous solution to test reversibility. The experiment frequently terminated at this point. However, in a number of the potassium studies, this was followed by exposure to hypotonic Ringer for 0.5 to 1.0 hour—the time required to approach equilibrium—and then the original control or experimental solutions were restored; this served the twofold purpose of checking the integrity of the muscle fibers as osmotic systems as well as determining whether water permeability had been altered. Reproducibility of the weighings was good, the standard deviation of the mean being less than 1 per cent and that of individual weighings 1.5 per cent or better.

The effects of the experimental compounds on polarization and on potassium depolarizability were determined in a moist chamber by the method previously described (22). Three pairs of sartorii were mounted at one time with the pelvic ends either in a common trough containing 0.1 M potassium (as the chloride in excess of the other Ringer constituents to prevent swelling; *cf.* reference 2) or on the insulating ledge of this trough. In the latter case, which was more commonly used, the trough was filled with Ringer and filter paper strips inserted to establish contact with the individual muscles about 0.5 cm. from their ends. About 2 mm. of each tibial end was inserted in an individual U-tube filled with Ringer or the experimental solution. Effects of experimental solutions were evaluated by comparing the results with those of the control muscle from the same animal. In these electrical studies potassium was usually added as the chloride in excess of the other Ringer constituents.

The Ringer employed contained the usual electrolytes in the following concentrations: 107 mM NaCl, 1.7 mM KCl, 1.1 mM $CaCl_2$, and a pH 7.4, all-sodium Sørensen phosphate buffer osmotically equivalent to 1 mM NaCl. Compounds used in high concentration, such as phosphate, citrate, and oxalate, replaced an osmotically equivalent amount of NaCl. The phosphate buffer isotonic with Ringer contained 1.08 gm. Na_2HPO_4 and 0.264 gm. $NaH_2PO_4 \cdot H_2O$. Additions involving a change of only a few per cent in tonicity were made without altering any other concentrations. All solu-

tions were adjusted to a pH of 7.4. Experiments were usually carried out at room temperature (20–25°C.); in a few on muscle swelling, temperatures approaching 0°C. were obtained by surrounding the beakers containing the muscles with an ice and water mixture.

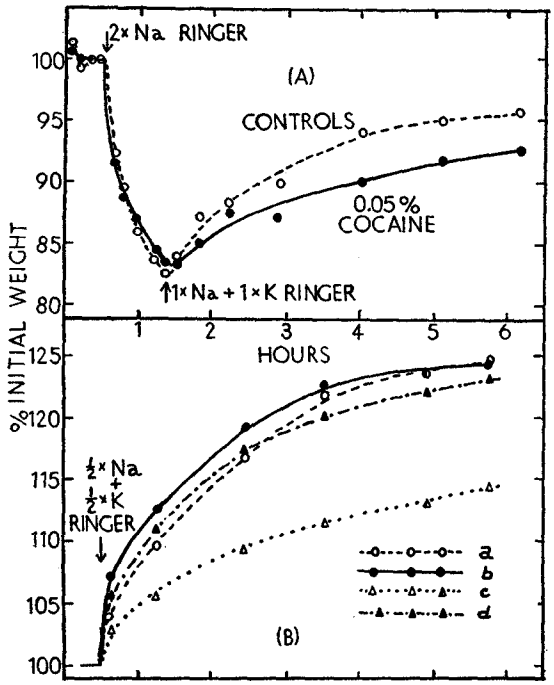

FIG. 1. (A) Weight changes of paired sartorii in Ringer, with and without cocaine present, (*a*) when the ionic strength is doubled by the addition of sodium and (*b*) when the excess sodium is subsequently replaced by potassium. Each curve is the average of 3 sartorii each of which showed the same general effects. (B) The weight increase in potassium-Ringer of (*a*) a sartorius muscle previously treated for 60 minutes with 0.0075 per cent pyribenzamine, then washed in pyribenzamine-free Ringer for 15 minutes and (*b*) its control kept in Ringer throughout. Corresponding curves are given for (*c*) a sartorius subjected to the same pyribenzamine solution continuously and (*d*) its control.

RESULTS

Stabilizers

Agents investigated were cocaine, procaine, yohimbine, and pyribenzamine.[1] Preliminary reports on the first two have appeared (18, 19). Some exploratory

[1] The hydrochlorides were very kindly provided by Dr. F. F. Yonkmann of Ciba Pharmaceutical Products, Inc.

observations have also been made with antistine,[1] which has been described as having anesthetic activity (6, 28).[2]

Swelling.—Although most experiments were performed by simple replacement of the sodium in Ringer with potassium, the procedure of first subjecting the fibers to Ringer made 2 times hypertonic with NaCl and then replacing the excess NaCl with KCl, as described by Conway and Moore (4), was also tried. A typical series is shown in Fig. 1 A. Cocaine and procaine were used in

TABLE I

The Length of Individual Sartorii after Immersion in Two Antistine Concentrations at Different Temperatures

Paired muscles are indicated by the same letters.

0.2 per cent antistine				
2.5°C.		Time from immersion	19°C.	
A	B		A	B
mm.	*mm.*	*min.*	*mm.*	*mm.*
28	28	0	28	29
23	25	15	15	17
20	25	35	12*	13*
Contr.*	Contr.*	55	Contr.*	Contr.*
0.1 per cent antistine				
C	D		C	D
25	26	0	27	27
25	25	30	26	27
25	25	60	26	26
25	25	130	24	23
26	26	165	20*	21*
26	26	190	15*	19*

* Appearance typical of contracture.

these experiments. The shrinking in hypertonic solution and return towards the original weight upon KCl substitution are considerably slower and the latter less complete than in the semidiagrammatic figure given by Conway and Moore, even when their conditions appear to have been duplicated. The incomplete water uptake is also evident upon substitution of potassium in normal Ringer; Boyle and Conway (2) give evidence for regarding this as a consequence of a secondary chemical change brought about by potassium. Such secondary effects render final equilibrium volumes of questionable value in the interpre-

[1] Experiments personally conducted on frog sciatic nerve have failed to demonstrate a threshold increase or a spike decrease in antistine except at concentrations causing depolarization.

tation of experimental results, hence measurements have been restricted to the first few hours. Little advantage was seen in first treating the muscles with hypertonic solutions which might cause damage; moreover the stabilizer effects

TABLE II

Average Increase in Weight (Per Cent of Initial Weight) of Control Sartorii (C), and of Experimental Preparations (X) Previously Subjected for 1 or 2 Hours and Thereafter to the Indicated Drugs, upon Exposure (A) to Ringer with Half the Sodium Replaced by Potassium or (B) to Ringer with the Sodium Reduced to the Fraction Y

The times are those at which X and C were obtained as measured from the immersion in the modified Ringer. The number of individual pairs contributing to each average is given.

Agent	Concentration	A					B					
		Exposure	X	C	X/C	No.	Y	Exposure	X	C	X/C	No.
	per cent	*min.*	*per cent*	*per cent*				*min.*	*per cent*	*per cent*		
Antistine	0.01	88	6	15	0.40	3	0.5	35	26	26	1.0	3
Procaine	0.2	135	17	27	0.63	4	0.75	65	18	14	1.3	3
	0.4	140	18	24	0.75	3						
	1.0	140	10	20	0.50	4						
Cocaine	0.05	65	6.3	12	0.53	5						
	0.05	150	18	27	0.67	3						
	0.2	126	5.3	17	0.31	6	0.5	53	39	34	1.15	3
Yohimbine	0.01	90	21	21	1.0	4						
	0.05*	78	10	10	1.0	3	0.6	30	28	26	1.1	3
Pyribenzamine	0.0025	76	9.5	11.5	0.83	2						
	0.005	76	9.0	13.5	0.67	4						
	0.0075	117	9.0	17	0.53	3						
	0.01	57	2.3	11	0.21	2	0.6	32	25	16	1.6	2
	0.025	67	0.8	11	0.07	2	0.6	32	26	22	1.2	2
	0.05	77	5.5	12.5	0.44	2	0.6	36	29	20	1.5	2
	0.1	77	4.5	13	0.35	2	0.6	36	26	20	1.3	2

* Temperature *ca.* 0°C.

appeared to be less striking. Consequently use has been made chiefly of potassium substitution in ordinary Ringer.

Contracture, usually associated with weight gain, was a complication frequently encountered at higher drug concentrations. Antistine was particularly toxic; Table I illustrates the rapid contracture, measured in terms of muscle length, which develops in 0.2 per cent. After 2 hours' exposure to this concentration, response to potassium Ringer was weak or absent, and negligible weight

gain occurred in 0.6 times Ringer; during this period the fibers lost weight continuously, although some weight gain was evident during the preliminary soaking period. Apparently the membranes were seriously disrupted by the antihistaminic.[3] It may be noted, too, that contracture development is appreciably delayed by lowering the temperature or the concentration. At 0.1 per cent antistine begins to exert its effect in about 2 hours at room temperature, whereas double the concentration acts in less than 15 minutes. Three paired muscles were therefore reexamined for antistine action with a concentration of only 0.01 per cent; final average values are given in Table II. Each pair agreed in showing a striking reduction in the swelling in potassium, with no appreciable change in the response to hypotonicity. However, even at this low concentration some damage may have been present for recovery from the hypotonic solution was poor.

Procaine, cocaine, and pyribenzamine, at the concentrations shown in Table II, resemble antistine in depressing the water uptake in potassium-Ringer, but, if anything, improve the swelling in hypotonic Ringer. Occasional preparations were observed to be unaffected by the anesthetics, particularly if obtained from animals stored for any length of time. With pyribenzamine 100 per cent consistent results were obtained.

In general these weight changes are reversible, particularly at low drug concentrations. But even in controls recovery from potassium solutions occasionally may be slow or incomplete. Some features of recovery as well as peculiarities observed in the initial swelling process were obtained in detail with pyribenzamine and are summarized in Figs. 1 B and 2. At high concentrations (0.05 and 0.10 per cent), although no weight gain is evident during exposure to the antihistaminic alone, a rapid increase ensues upon immersion in potassium-Ringer. This either fails to reverse or reverses but slightly upon return to normal potassium levels; despite the continued gradual weight increase these preparations are capable of a good osmotic response (Table II). At 0.025 per cent the weight gain in potassium is delayed, but once initiated fails to cease immediately upon return to Ringer. From this concentration down, pyribenzamine exerts a graded effect on the rate of swelling in potassium substitution solutions. At concentrations as low as 0.0025 per cent recovery from these solutions is slow; this is not attributable to the irreversible effects observed at higher concentrations, for removal of the pyribenzamine from the potassium-Ringer about 1.5 hours before return to Ringer improves recovery (Fig. 2, curve S). Reversibility from pyribenzamine effects has also been observed with respect to the swelling phase in potassium. Thus, after a 1 hour exposure to 0.0075 per cent pyribenzamine, 3 muscles were washed for 15 minutes in antihistaminic-free Ringer, and then subjected to potassium-Ringer. As may be seen in Fig.

[3] No mechanism is implied by this conventional term, which is employed solely for convenience.

1 B, the swelling at first was several per cent less than that of the controls, but by 300 minutes had overtaken the latter. A 1, 3, or 5 hour previous exposure to this antihistaminic concentration causes essentially the same depression of the swelling in potassium solutions as long as the pyribenzamine is present;

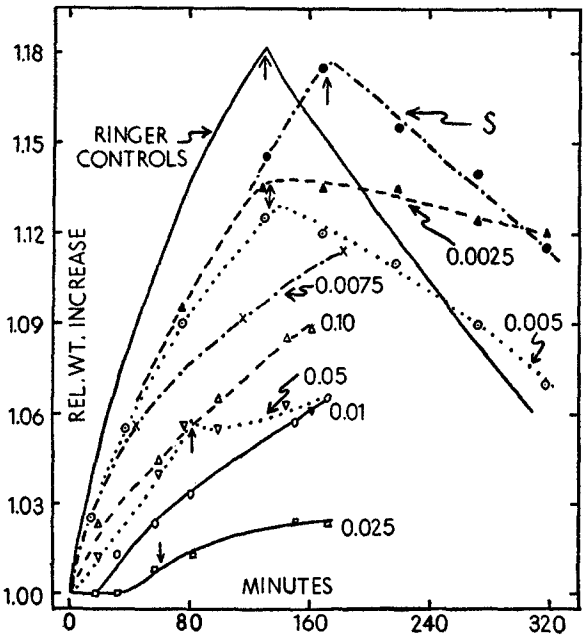

FIG. 2. The weight, relative to the initial weight, of sartorii upon exposure to Ringer with half the sodium replaced by potassium and containing the indicated percentage concentration of pyribenzamine. The antihistaminic was applied 1 hour before these runs. The control curve is the average of all the mates to the experimental preparations; the number of sartorii contributing to each experimental curve is given for individual pyribenzamine concentrations in Table II. Curve S is the average of 2 muscles for which the pyribenzamine was removed from the potassium-Ringer at time 76 minutes. Vertical arrows indicate return to the initial, normal potassium-Ringer.

from this it may be concluded that the return to normal swelling after the 1 hour soaking in pyribenzamine represents a true reversal of pyribenzamine action rather than a weaker effect resulting from the shorter period of treatment.

At comparatively high concentrations in nerve yohimbine also acts as a stabilizer. In muscle such concentrations were found to approach the level which causes contracture. Consequently one series at higher concentration was run at about 0°C. However, at neither 0.01 nor 0.05 per cent was any significant interference with water uptake observed (Table II). Nevertheless the response

to electrical stimuli (condenser discharges) was weaker even in the lower concentration of alkaloid.

Polarization.—It has already been pointed out that cocaine can appreciably reduce the depolarizing action of potassium (18). The data which have been obtained for procaine and pyribenzamine are in general agreement with those

TABLE III

(A) The Maximal Decline of Potential of Individual Sartorii, in an Excess of Potassium 20 Per Cent of Isotonic Strength, in the Presence (X) or Absence (C) of Cocaine and Procaine. (B) Corresponding Recovery upon Return to Normal Potassium-Ringer

Agent	Concentration	Exposure	A		B	
			X	C	X	C
	per cent	*hrs.*	*mv.*	*mv.*	*mv.*	*mv.*
Cocaine	0.05	5*	4.7	23.2	3.7	17.5
			1.4	32	—	—
			4.3	13.8	2.6	14.9
	0.05	3‡	6.6	17.4	6.1	18.0
			8.0	20.3	8.0	19.9
	0.01	3‡	16.1	22.3	—	—
Procaine	0.1	1§	15.9	16.2	16.9	17
			11.2	13.1	11.2	13.8
			9.9	14.2	7.6	15.1
	0.2	¾‖	11.5	20.6	10.4	22.7
		1	11.9	18.1	13.8	20.6

* Potassium excess one-third of isotonic strength.

‡ Potassium *substituting* for 20 per cent of the sodium.

§ Procaine locally applied; caused depolarization of 1.75, 0.6, 2.1 mv. respectively (reading downward).

‖ Procaine locally applied; caused a depolarization of 2.8 mv.

for cocaine (Tables III and IV). The decrease in depolarization in procaine was occasionally so small that it may have been attributable to the depolarization by procaine itself. Pyribenzamine, which required considerably lower concentrations to depress potassium depolarization, was studied in greatest detail. Most experiments were performed with potassium present in addition to the other Ringer constituents. In several experiments in which potassium replaced 20 per cent of the sodium the depressed depolarization in the antihistaminic as well as the depolarization of the control were of the same order as when potassium was in excess. In Table IV are shown the KCl depolarizations of in-

dividual sartorii, previously soaked for 2 to 3 hours in various pyribenzamine concentrations, compared with the average depolarization of the corresponding controls. The variability of the controls is expressed as the standard deviation of the individual samples. A consistent, significant depression of KCl depolarization is evident over a wide range of K concentration and at as low a pyribenzamine concentration as 0.0025 per cent. At still higher antihistaminic concentrations depolarization in KCl becomes negligible.

TABLE IV

Millivolts Maximal Decline of Potential of Individual Sartorii upon Exposure to Potassium-Ringer after 2 to 3 Hours' Soaking in Pyribenzamine

KCl concentration (per cent of isotonic strength)	Pyribenzamine concentration, *per cent*					
	0.05	0.015	0.005	0.0025	0.001	0.0
	mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
15		0	0.9	10.9		11.8 ± 4.0
		0	1.5			
20	0*	0*	1.5*	11.3	15.7	19.9 ± 5.4
	0	0	6.6			
			3.8			
40		1.3	6.4	19.7		29.2 ± 4.9
		0.7	5.5			
75	0	2.4	3.7	25.0	32.4	34.4 ± 7.8
	1.7	4.1	15.8			
			13.0			
93		3.9	17.4	27.1		35.0 ± 5.4
		4.4	13.0			

* K replacing Na.

Cocaine, procaine, and pyribenzamine, in contrast to the hyperpolarization induced in frog nerve (unpublished), produce a depolarization in muscle. Thus, in 5 of 6 sartorii treated with 0.1 and 0.2 per cent procaine, the resting potential declined an average of 1.8 mv. in 0.5 to 1 hour whereas the controls treated with fresh Ringer showed a slight rise in potential in as many instances. Lower concentrations had no significant effect. Pyribenzamine, at a concentration of 0.1 to 0.03 per cent, depolarized sartorii at a rate of 3 to 5 mv. per hour, and this depolarization continued at least an hour or two following return to Ringer. It is noteworthy that muscles treated simultaneously with 0.025 to 0.1 per cent pyribenzamine and potassium-substituted Ringer continued to gain weight after return to Ringer. Apparently these higher drug concentrations,

particularly under the influence of an elevated temperature and potassium level, set off a process which is not readily stopped or reversed.

Reversibility of the pyribenzamine effect on potassium depolarizability was seen in experiments performed with 0.015 and 0.005 per cent pyribenzamine. After 45 minutes' exposure to the drug the muscles were returned to Ringer while the mates were kept in the antihistaminic. As may be seen in Table V, the former respond to potassium almost normally whereas the latter show the typical depressed response.

TABLE V

Potential Decrease in Potassium-Ringer (A) of Individual Sartorii after 3¾ Hours' Exposure to 0.015 and 0.005 Per Cent Pyribenzamine and (B) of Their Mates after Three-Quarter Hour Exposure Followed by 3 Hours' Washing in Ringer

KCl (per cent of isotonic strength)	A		B	
	0.015	0.005	0.015	0.005
	mv.	*mv.*	*mv.*	*mv.*
15	0	1.5	6.9	10.5
	0		10.8	
40	0.7	5.5	13.3	20.6
	1.4		22.3	
93	4.0	13.0	20.6	32.2
	4.5		32.8	

Unstabilizers

Under this category will be described observations made with calcium precipitants—chiefly the Sørensen all-sodium phosphate buffer—and veratrine.

Swelling.—Table VI summarizes the results which have been obtained. An interesting effect was the practically complete obliteration of the weight gain in potassium substitution solutions in the presence of phosphate; on the other hand, a brief priming in the calcium precipitant, followed by calcium-free Ringer prior to the potassium solution, left the swelling rate of winter preparations (November to February) unaltered but augmented that of August muscles. The difference in response is apparently related to a seasonal difference in the controls. An appreciable delay in water uptake normally occurs with the summer material, but treatment with the precipitant restores the immediate gain in weight which ensues with winter muscles in potassium-Ringer. Water uptake in hypotonic solution also was reduced in the presence of phosphate, and returned to normal levels when the precipitant was washed out with Ringer.

Oxalate likewise augmented the rate of swelling of August tissue in potassium-Ringer; this was observed even with oxalate present. A tendency to develop

contracture was noted in potassium solutions containing oxalate or, to a lesser degree, phosphate.

TABLE VI

Average Increase in Weight (Per Cent of Initial Weight) of Control Sartorii (C), and of Experimental Preparations (X) Previously Subjected for ½ or 1 Hour and Thereafter to the Indicated Agents, upon Exposure (A) to Ringer with Half the Sodium Replaced by Potassium or (B) to Ringer with Sodium Reduced to the Fraction Y

The times are those at which X and C were obtained as measured from the immersion in the modified Ringer. The number of individual pairs contributing to each average is given.

Agent	Concentration	A				B				
		Exposure	X	C	No.	Y	Exposure	X	C	No.
	per cent	*min.*	*per cent*	*per cent*			*min.*	*per cent*	*per cent*	
Sørensen buffer*	50	75	3	20‡	3	0.5	60	21	32‡	3
	50					0.4	55	15	21‡	4
	0§					0.4	65	21‡	16‡	4
	50‖	85	3	12‡	4	0.5	60	19	40	4
	50‖					0.5	70	18	40	4
	0§, ‖					0.5	60	34	29	4
	20¶	90	15**	8	3					
Oxalate*	20¶	77	14	9	3					
	20¶	70	16**	8	3					
Citrate* (=X) *vs.* Buffer* (=C)	40	90	0	4	4	0.5	33	11	20	4
Veratrine	0.0005	140	18	21	2					
	0.001	140	13	20	1					
	0.001	110	13‡‡	15‡‡	3					
	0.01	130	13‡‡	15‡‡	3					

* Concentrations expressed in per cent of isotonic strength.

‡ Calcium-free Ringer.

§ Same preparations as immediately above after washing out of phosphate in X with calcium-free Ringer.

‖ Temperature *ca.* 1.5°C.

¶ Summer (August) preparations.

** Treated only ½ hour with agent, which was subsequently removed with calcium-free Ringer.

‡‡ 0.2 per cent procaine present.

Citrate appeared to be even more effective than phosphate in reducing the water uptake from potassium solutions. However, damage apparently was done by this precipitant, for the reduced response to hypotonic solution was found

to be poorly reversed, and in fact was followed by continued swelling in citrate-Ringer.

Veratrine had no striking, consistent effect on the swelling of muscles from winter frogs in potassium solution (Table VI). Water uptake may be depressed slightly, but additional data are needed to check this point.

TABLE VII

Potential Decrease of Individual Sartorii in Ringer with Excess Potassium at 20 Per Cent of Isotonic Strength (C) Compared with That of Their Mates (X) Previously Subjected for the Indicated Time, and Subsequently, to Unstabilizers

Agent	Concentration	Exposure	X	C
	per cent	*min.*	*mv.*	*mv*
Veratrine	0.0001	180	5	11.6
	0.0002		9.4	14.2
	0.0002		8.1	—
	0.0002	100	21.5	19.0
	0.0005		12.0	18.5
	0.0005		8.3	—
Sørensen buffer*	40	190	0‡	9.5‡
			2.0‡	10.4‡
	40	150	2.7§	10.0§
			6.7§	11.7§
			6.5§	13.6§
	0‖	115	12.5	13.5
			13.9	14.5
			19.5	15.6
	50	110	1.1‡	10.1‡
			2.9‡	8.3‡
			0.7‡	—

* Concentration in per cent of isotonic strength.

‡ 0.2 per cent procaine present.

§ 0.1 per cent procaine present.

‖ Same preparations as immediately above and in same order, after being washed in Ringer for the indicated time.

Polarization.—A high concentration of phosphate buffer reduces the depolarizing action of potassium (Table VII). In view of the depressed swelling in potassium, this provides further support for the dependence of the depolarizing action on the penetrability of KCl. Table VII also shows that washing out the precipitant with Ringer restores the depolarizing effect.

The buffer itself causes a negligible change in muscle polarization. In these experiments it was particularly essential to run a blank, that is, a Ringer-soaked filter paper strip in place of muscle; the phosphate buffer in 50 per cent of isotonic strength itself gives rise to a diffusion potential of about 5 mv.,

which equals the "depolarization" that seems to appear when it is applied to muscle.

The possibility that the augmented swelling in potassium after brief exposure to a calcium precipitant is associated with an increased depolarizing action was also examined. Three experiments performed in August with potassium concentrations of 5 per cent of isotonic strength uniformly demonstrated a one and a half to threefold greater effect after phosphate treatment but a negligible difference at higher concentrations. A repetition of these experiments in midSeptember failed to duplicate the earlier observations; contrary to the summer series, a good response to low potassium concentrations was frequently obtained in the controls. Within the limits of these rather restricted data, then, the electrical measurements are consistent with the seasonal differences observed with respect to weight.

Table VII demonstrates that veratrine has either no effect or depresses the depolarization in potassium. The alkaloid mixture itself is known to depolarize (concentrations of 0.0002 per cent and higher were observed to cause a depolarization), which may account for the weaker response to potassium. These experiments were performed only on muscles from winter animals.

DISCUSSION

In the light of direct analytical evidence that the swelling of muscle in potassium substitution solutions is in proportion to KCl penetration (2, 4), the weight experiments with low concentrations of the stabilizers demonstrate a reduced ionic permeability. The absence of a decrease in either the rate or extent of water uptake in hypotonic solutions under otherwise similar conditions rules out (*a*) a non-specific porosity change involving reduced permeability to water and (*b*) a decrease in the osmotic strength of the fibers. An alteration in membrane charge—such as studied by Wilbrandt (27) in artificial systems—may be involved. The reduced effectiveness of KCl as a depolarizing agent would therefore follow from a proportionately much greater decrease in potassium than chloride mobility in the membrane (*cf.* reference 18). Höber has reported a similar depressant effect by carbamate on the depolarizability of muscle by KCl as well as by other electrolytes (13).

The failure to obtain an increased polarization of muscle with graded concentrations of pyribenzamine, procaine, and cocaine merits emphasis. The same technique with frog nerve is consistent in demonstrating a hyperpolarization, which in the case of procaine correlates closely with threshold increase (unpublished). Crescitelli (5) has suggested that the augmented polarization found in nerve may be characteristic of stabilizer action in other tissues; he notes, however, that Höber *et al.* (15) and Guttman (12) failed to obtain such an effect in other excitable systems, as confirmed recently for crab nerve (21) and in the present study. Hyperpolarization therefore cannot be considered an

essential element in stabilization, although under the special circumstances operative in vertebrate nerve the two usually occur together (*cf.* discussion in reference 21).

An important question arising from the depolarization observed with the stabilizers is whether this may be responsible for the reduced electrical activity of potassium. It appears disposed of particularly for pyribenzamine by the very low concentrations which proved effective. It would be desirable to check Höber's early study of the depressant action of carbamate on polarization changes (13) from this standpoint.

Fleckenstein and Hardt (9), by a different approach, present evidence for a reduction in the potassium permeability of muscle in local anesthetics, antihistaminics, and calcium. Direct evidence has now been obtained in frog nerve that cocaine reduces the anoxic release of potassium.[4] It seems quite possible, therefore, that all these agents, insofar as they exhibit the characteristics of stabilizers, act by way of the same membrane alteration rather than by virtue of an antagonism to any specific compound or enzyme. This is further indicated by the fact that antihistaminics are anesthetics (6, 28) and, conversely, that narcotics may act as antihistaminics (7). Similarly, Verzár's observation (25) that iodoacetate delays the swelling of muscle in isotonic KCl may represent a physical effect quite independent of metabolic inhibition.

Under normal conditions the mobility of the anions is the limiting factor in KCl penetration of muscle (2, 4), hence an increase in cation permeability such as might occur in unstabilizers may leave both the rate of diffusion of KCl and its ability to depolarize unaltered. Such appears to have been the case with veratrine and also the case following short exposures to phosphate buffer. The delayed KCl penetration in summer nerves, associated with reduced depolarizability, and the return to the winter condition by brief treatment with a calcium precipitant, suggest that calcium itself can act to varying degrees as a stabilizer in reducing potassium penetration and, further, that potassium may counteract calcium stabilization. Höber's discussion of such ionic interaction (14) is quite applicable. The disagreement between Fenn and Cobb (8) and Conway (3) as to whether potassium at low concentrations causes muscle to swell may be due to preparation differences such as have been described. Fleckenstein and Hertel (10) have noted a similar delaying action by calcium on potassium contracture. In view of the seasonal effects which appear to have been present, it would be desirable to reexamine the action of veratrine on summer muscles, for evidence is available that this alkaloid mixture displaces membrane calcium (20, 21, 26); such displacement would account for the greater sensitivity of muscle to potassium (16) and for the increased potassium loss it causes in resting muscle (1) and nerve (unpublished).

[4] Described at the 34th annual meeting (April 18, 1950) of the Federation of American Societies for Experimental Biology (*Fed. Proc.*, 1950, **9**, 116).

The action of strong phosphate buffer, although resembling that of the stabilizers in reducing water uptake in potassium substitution solutions and in depressing the depolarization by KCl, differs in reducing water uptake from hypotonic solution. Here, then, a non-specific permeability change appears to be involved; the possibility of a "clogging" of membrane pores (*e.g.*, by precipitated calcium phosphate?) or of a surrounding sheath such as the sarcolemma may merit study.

In conclusion, the experiments described focus attention on physical chemical effects produced by stabilizers as one possible basis for their action. The permeability to the potassium ion has been singled out in this study because of its probable involvement in impulse transmission; permeability with respect to other substances may require consideration in other phenomena.

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SUMMARY

Procaine, cocaine, pyribenzamine, antistine, and phosphate decrease the uptake of water by frog sartorii in a Ringer solution in which potassium has been substituted for sodium; all but the last two leave the swelling in hypotonic Ringer practically unaltered. They also decrease the depolarizing action of potassium. These effects are considered indicative of reduced membrane permeability to potassium.

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