to the University of California from the National Institute of Neurological Diseases and Blindness, National Institutes of Health (No. B-608), and from the American Cancer Society. A detailed presentation of this work is in preparation.

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Removal of Phosphorus from Hydrogen Peroxide by Kaolinite

Hydrogen peroxide has been used as an oxidizing agent in the determination of organic phosphorus in soils and also in the determination of phosphorus in a solution that contains interfering organic matter, such as the dithionite-citrate extraction of iron phosphate by Chang and Jackson (*1*). Hydrogen peroxide is often highly contaminated with phosphorus, for phosphoric acid is usually added as stabilizing agent. Merck chemically pure 30-percent Superoxol has a maximum PO_4 content of 0.005 percent, which is equivalent to about 16.3 ppm of phosphorus. Therefore it is necessary to remove the phosphorus from the hydrogen peroxide before it is used for the aforementioned determinations. It has been found that the phosphorus could be brought to a satisfactorily low concentration by adsorption on kaolinite (Tables 1, 2, and 3).

Dickman and DeTurk (*2*) used distillation to free hydrogen peroxide from phosphorus. This method requires a comparatively complicated set-up, constant care, and a longer period of time than the new method. Furthermore, the product obtained is only half of the original amount, and the concentration of the hydrogen peroxide is also considerably decreased.

Dickman and Bray (*3*) recommended treatment with $FeCl_3$ and $CaCO_3$ for removal of phosphorus from hydrogen peroxide. They claimed that colorimetric analysis of 5 ml of hydrogen peroxide showed 0.2 ppm of phosphorus. Since $FeCl_3$ causes vigorous decomposition of the peroxide, this procedure is not easily carried out successfully.

In the proposed procedure, 10 g of Merck's "colloid kaolin" (mainly kaolinite) is shaken by hand in 100 ml of hydrogen peroxide for 5 minutes. The suspension is allowed to stand for 1 minute and then is decanted through a Büchner funnel under gentle suction. The filtrate is again treated with kaolinite as before. The number of treatments depends on the original phosphorus content of the peroxide. Three or four (rarely five) treatments are sufficient to reduce the phorphorus content to about 0.1 to 0.2 ppm. It is not necessary to make the first and second filtrates clear. The final filtrate is made clear by centrifugation. Then 0.5 ml of concentrated HCl is added to every 100 ml of the clear hydrogen peroxide for stabilization.

The proposed procedure has the advantages of (i) being simple, (ii) being time saving, (iii) being able to reduce phosphorus to about 0.1 to 0.2 ppm, (iv) giving a high percentage of volume recovery, and (v) giving little loss of H_2O_2 concentration.

Table 1. Removal of phosphorus from hydrogen peroxide by different amounts of kaolinite.

Kaolin (g/10 ml of H_2O_2)	Resulting concn. of P (ppm)	
	Replication 1	Replication 2
0	6.5	6.5
0.5	3.35	3.4
1.0	1.9	1.9
2.0	1.65	

Table 2. Removal of phosphorus from hydrogen peroxide by successive treatments with kaolinite.

Treatment No.	Resulting concn. of P (ppm)			
	1 g kaolinite per 10 ml H_2O_2		0.5 g kaolinite per 10 ml H_2O_2	
	Replication 1	Replication 2	Replication 1	Replication 2
0	6.5	6.5	6.5	6.5
1	1.9	1.9	3.35	3.4
2	0.2	0.2	1.58	
3	0.12	0.12	0.50	0.5
4			0.12	0.12

Table 3. Volume recovery of hydrogen peroxide, phosphorus concentration, and hydrogen peroxide concentration after three treatments with kaolinite at a rate of 10 g of kaolinite per 100 ml of hydrogen peroxide. The percentage of hydrogen peroxide was determined by titration with standard 0.1*N* $KMnO_4$ according to Treadwell and Hall (*4*).

Time	Total volume (ml)	P content (ppm)	H_2O_2 (%)
Before treatment	500	6.5	28.5
After treatment	430	0.11	26.1

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Relationship between Membrane Potentials and Repolarization in the Rat Atrium

The variation in membrane potentials observed between cell penetrations in a single rat atrium might be interpreted as arising experimentally; on the other hand, such variation may indicate true differences between atrial cells. An inverse relationship between the magnitude and duration of the action potential in the normal atrium was observed.

This correlation was studied quantitatively in 25 atria, from each of which 70 to 80 readings were obtained, by tabulating durations and overshoots of the action potential for 5-mv ranges in the action-potential magnitude (*1*). Membrane potentials were determined with microelectrodes on atria that were stimulated electrically at a rate of 200 per minute as previously described (*2*). The results for 1866 atrial cell penetrations are shown in Table 1. The greater the magnitude of the action potential, the shorter is the duration, and this inverse relationship is linear over the entire range studied. The overshoot varied relatively little and was constant above an action potential of 75 mv.

It might be expected that the lower the action potential, the shorter would be the duration, assuming a constant rate of repolarization; however, just the opposite was observed. The repolarization rate was approximately five-fold greater for the highest action potentials than for the lowest. Since the overshoot was relatively constant over the range, contrary to that of Purkinje fibers (*3*), and since the entire atrial preparation was depolarized during each impulse, it would appear that the repolarization rate is independent of the degree of change from

Table 1. Variations in the membrane characteristics of atrial cells. The repolarization rate was calculated on the basis of a linear repolarization with values obtained from a plot of the data in the table.

Average action potential (mv)	Overshoot (mv)	Average resting potential (mv)	Duration of action potential (msec)	Repolarization rate (v/sec)
53	8.0	45.0	67.5	0.49
58	8.9	49.1	60.1	0.61
63	9.8	53.2	54.0	0.73
68	10.3	57.7	52.6	0.88
73	12.3	60.7	49.5	1.05
78	13.6	64.4	47.3	1.25
83	13.6	69.4	40.4	1.49
88	13.9	74.1	39.1	1.77
93	13.6	79.4	36.2	2.13
98	13.5	84.5	29.3	2.58

the resting potential or of the electric fields arising from adjacent polarized cells. Relative degrees of damage to cells is an unlikely explanation of the variations because of the typical bell-shaped distribution of the values and the constancy of these characteristics over several hours.

The possibility remains that the correlation observed is an expression of some basic difference between the cells. If the rate of repolarization is dependent on K^+ outflow following depolarization, and if this outflow rate is determined by the concentration gradient of K^+ inside and outside the cells, it might be assumed that a rapid repolarization is indicative of a high gradient—that is, a high internal concentration of K^+. If this is correct, cells with the highest repolarization rate should possess the highest resting potentials and, since the overshoot is relatively constant, the highest action potentials, and this is what was observed. It is possible to test this hypothesis using the simplest assumptions. If the rate of K^+ outflow is directly proportional to the concentration gradient, and if the major portion of the repolarization is linear, the repolarization rates for two groups of cells with different potentials would be proportional to the internal K^+ concentrations:

$$\left(\frac{AP-20}{APD}\right)_1 \Big/ \left(\frac{AP-20}{APD}\right)_2 = \frac{(K_i^+)_1}{(K_i^+)_2}$$

where AP is the magnitude of the action potential, $(AP-20)$ is the potential change during repolarization to the level at which the duration was measured, and APD is the duration of the action potential. Using values from a plot of the data in Table 1, and taking the lowest and highest average action potentials in the range studied, the following result is obtained:

$$RP_1 - RP_2 = 59.9 \log \frac{(K_i^+)_1}{(K_i^+)_2} =$$
$$59.9 \log 5.24 = 43.1 \text{ mv}$$

where RP is the resting potential. The actual difference in resting potential (Table 1) is 39.5 mv. Considering that the repolarization is not strictly linear but frequently deviates toward a logarithmic path (*2*) the agreement between the measured and calculated resting potential differences is satisfactory. This agreement does not, of course, prove the hypothesis, but no other reasonable explanation for the data is evident.

Certain implications of importance arise if this hypothesis is correct. Atrial cells would then not constitute a uniform population but a collection of cells with different membrane properties; whether this might reflect functional specialization is a matter of speculation, but the bearing on cardiac arrhythmias, ectopic centers, and abnormal conduction pathways is evident. Such differences would also imply quantitative differences in ion-transport activity or in other factors controlling the accumulation within cells of K^+. Finally, it would support the concept that repolarization is a passive phenomenon in terms of ionic flow, a simple diffusion dependent on concentration gradients and on the permeability properties of the membrane.

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References and Notes

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