CHANGES IN MEMBRANE CHARACTERISTICS OF HEART MUSCLE DURING INHIBITION*

By W. TRAUTWEIN,‡ S. W. KUFFLER, AND C. EDWARDS

(From The Wilmer Institute, The Johns Hopkins Hospital and University, Baltimore)

(Received for publication, February 27, 1956)

INTRODUCTION

A change in the membrane characteristics of heart muscle during vagal inhibition has been suggested by numerous experiments. In 1887 Gaskell (8) had already reported an increase in demarcation potential of the turtle auricle on stimulation of the vagus. A second phenomenon found by Samojloff (20) and frequently confirmed by other authors was a shortening of the auricular action potential during inhibition (*cf.* references 2, 19, 22, and 25, for extensive references). More recently, using intracellular recording methods Burgen and Terroux (3) and Hoffman and Suckling (10) confirmed that the recovery phase of the heart muscle action potential was greatly accelerated by vagal stimulation or by external application of acetylcholine. Since then inhibitory processes during vagal activity have been studied in detail in simultaneous investigations by del Castillo and Katz (4) and by Hutter and Trautwein (14). Their results, as well as those of Burgen and Terroux (3) on the effect of acetylcholine on the resting potential, indicate that the inhibitory transmitter, *i.e.* acetylcholine, changes the permeability to specific ions, thereby leading to the observed membrane changes. In these studies no definite conclusions regarding conductance or resistance changes could be reached.

The first more direct evidence for a decrease of the membrane resistance during inhibition was shown in the crustacean neuromuscular junction. Fatt and Katz (7) found that the restitution phase of the excitatory end plate potential was accelerated by inhibitory impulses, indicating a shortened time constant of the membrane. In studies on spinal motoneurons of the cat, Coombs, Eccles, and Fatt (5) came to the conclusion that permeability changes to K^+ and Cl^- ions may be responsible for the membrane phenomena of inhibition. In the isolated nerve cells of crayfish and lobster stretch receptors, additional evidence was obtained that neural inhibition increases the conductance of the cells (17); here also Cl^- and K^+ have now been shown to participate in the inhibitory potential (Hagiwara and Edwards, unpublished observations).

* Supported by a grant from the United States Public Health Service.

‡ Fellow of the National Academy of Sciences, from the Physiologisches Institut der Universität, Heidelberg.

In view of the available information from various cells in different species, pointing to a common synaptic mechanism, it seemed desirable to attempt a more detailed approach in measuring membrane characteristics during inhibition. Therefore changes in the length constant and time constant of fiber bundles in the frog's auricle were measured during the application of acetylcholine. Absolute values of membrane resistances were difficult to obtain since the heart muscle fibers form a syncytium and therefore cannot be treated as uniform cylindrical structures, as are nerve axons or skeletal muscle fibers to which the present methods are directly applicable (9, 16). The results give, however, direct evidence that the membrane resistance during inhibitory activity is greatly reduced.

Method

Frog auricles were opened and fine cylindrical preformed strips were carefully excised under a dissecting microscope. The strips were about 5 mm. long and 0.1 mm. in diameter and had to be "clean," to avoid adhering of fluid droplets at least in the region in which recording was done. Special care was taken to avoid injury to the thin muscle bundles which were held in fine forceps at each end. The small bundles of auricle muscle did not usually beat spontaneously. The state of the preparation was judged by the size of action potentials and the vigorous accompanying contractions in response to stimulation. The amplitude of contractions usually did not decline for 5 to 10 hours. For recording the strips were raised into paraffin oil above the Ringer bath. Separate containers holding various test solutions were placed below the paraffin oil layer and the preparations were lowered into them for various periods, with the electrodes remaining in place. On occasions a drop of acetylcholine in Ringer was applied directly to a portion of the strip by a pipette, and after 20 to 50 seconds the drop was removed, the preparation remaining in paraffin oil. This method of application caused the least disturbance in recording conditions, and minimized variations in the quantity of fluid adhering to the strip due to solution changes. All measurements were made in non-beating preparations.

Four silver-silver chloride electrodes were connected to the preparation through agar-Ringer and through bristles from a "natural" bristle tooth-brush which were first boiled and then cut down to a fine tip which made contact with the muscle bundle over an area about 0.1 mm. wide.

Hodgkin and Rushton's (9) technique of measuring time constants by applied square pulses was used with the circuit modification described by Katz (16). Square pulses of current were passed through two electrodes, one near the end and one near the middle of the muscle bundle. The amount of current passed through the preparation was monitored on one beam of the oscilloscope. One of the recording leads was fixed near the end of the strip while the other was movable. The pulses were recorded with a conventional D.C. amplifier. The distance between the movable recording electrode and the nearby stimulating electrode was measured with a calibrated microscope eyepiece.

Validity of Assumptions.—The application of the cable equation to a strip of heart muscle must be made with caution. The analysis of Hodgkin and Rushton (9) for single fibers was used by Katz (16) for the study of aggregates of muscle fibers.

He confirmed his results on individual muscle as well as on nerve cells. The measurements reported here give an average value for the membrane constants of heart tissue and variations in the values of these constants caused by acetylcholine give a good indication of the direction of the changes. However, conversion of the results into quantitative values of the resistance changes of the muscle membrane rests on numerous assumptions.

In the ideal case, as treated by Hodgkin and Rushton, it is assumed that the current flow is parallel in the internal and external conductors which obey Ohm's law. Further, the electrode width should be negligible and the interelectrode distance infinite. In our experiments the characteristic length in Ringer was 3 to 5 times the diameter of the bundle, so that the current flow must have been nearly parallel (16). In the presence of acetylcholine the space constant was as little as twice the diameter and here the analysis becomes more questionable. The assumption of ohmic resistances was confirmed by measurements of the interelectrode potential gradient; the current was kept below the half threshold value to minimize any rectification that may have occurred due to "local" responses. The electrode width was one-fifth or less of the space constant in Ringer but only about one-third in acetylcholine; this must have introduced a slight "smearing" of the potentials. The distance between the two stimulating electrodes and the distance between the two recording electrodes were 10 or more characteristic lengths, so that interference between them could not have been important.

RESULTS

Measurement of the Characteristic Length of the Membrane (λ).—The maximal heights of the electrotonic potentials which were set up by the subthreshold square pulses were measured at different distances between the stimulating cathode and the roving recording lead. The steady state height of the electrotonic pulse (anodal as well as cathodal) is an exponential function of the interelectrode distance; thus a plot of the pulse height as a function of distance on a semilogarithmic scale gives the characteristic length directly. Fig. 1 shows some of the electrotonic potentials at different distances from the stimulating cathode. Between *A* and *B* a drop of acetylcholine (5.5 μM/liter) together with prostigmin (30 μM/liter) was applied for 30 seconds and then removed. The potentials in *B*, taken within 5 to 6 minutes of the drug application, clearly became smaller and at a distance of 0.6 mm. they could not be measured even with an increased amplification (note calibration differences). After washing in ordinary Ringer solution for about 5 minutes the original potential size was restored (Fig. 1 *C*). The complete results of the experiment of Fig. 1 are plotted in Fig. 2. The reversible decrease in characteristic length was found in all applications (Table I). In one case (strip two, Table I), for unknown reasons, the change was small, but a second application produced the usual effect. The change in λ was confirmed in numerous repeated acetylcholine applications without prostigmin; however, the changes were short lasting (see below). In the present experiments, the threshold concentration

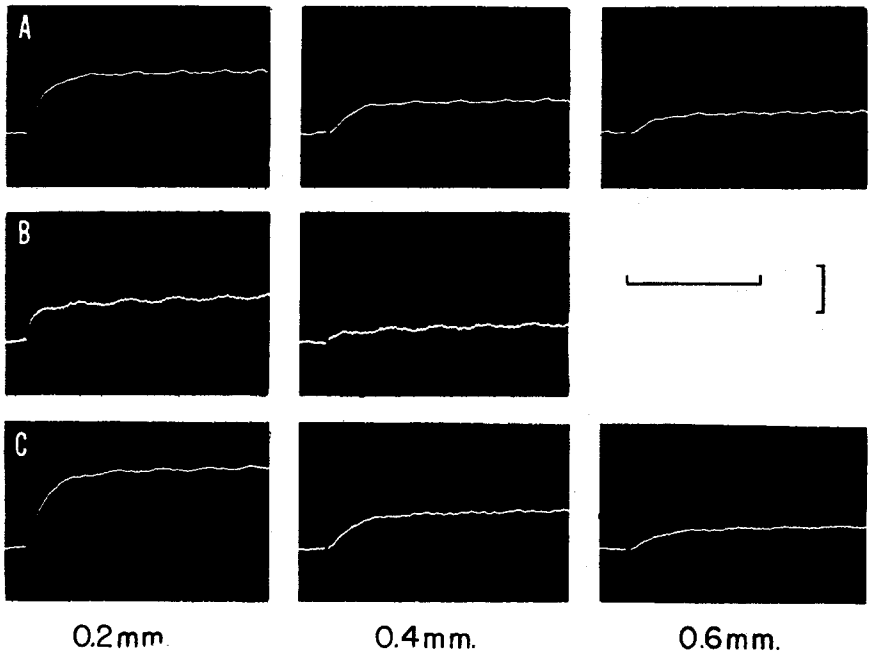

FIG. 1. Electrotonic pulses at different interelectrode distances. *A*, normal records. *B*, during action of 5.5 μM/liter acetylcholine and 30 μM/liter prostigmin. *C*, after washing the preparation in Ringer for 5 minutes. Time calibration 50 msec. Amplification, 1 mv. for lines *A* and *C*, ½ mv. for *B*.

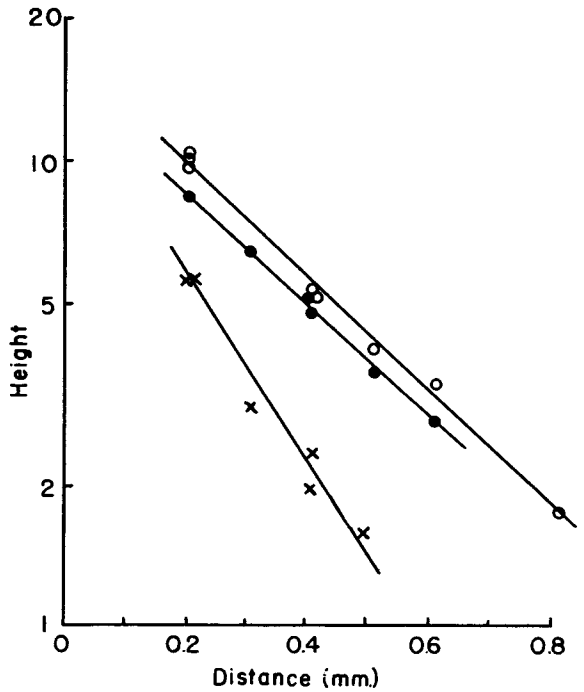

FIG. 2. Semilogarithmic plot of pulse height as a function of interelectrode distance. Closed circles, preparation normal. Crosses, during action of 5.5 μM/liter acetylcholine and 30 μM/liter prostigmin. Open circles, after washing in normal Ringer. Same experiment as Fig. 1.

of acetylcholine (tested by time and length constant changes and by shortening of conducted muscle potentials) was about 0.55 μM/liter (1 part in 10^7) without prostigmin. Almost all tests were done with concentrations of 5.5 μM/liter acetylcholine (1 part in 10^6) and 30 μM/liter prostigmin.

TABLE I

Effect of Acetylcholine on Membrane Constants

	λ	τ_{m1}	τ_{m2}
	mm.	*msec.*	*msec.*
Strip one			
Normal	0.41	6.0	6.2
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.32	4.6	3.4
7 min. in Ringer	0.40	6.5	6.0
Strip two			
Normal	0.23	3.6	3.7
Normal, 40 min. later	0.25	3.4	4.5
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.25	1.8	2.4
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.19	2.7	1.7
4 min. in Ringer	0.35	4.5	5.2
Strip three			
Normal	0.36	9.8	7.8
Normal, 1 hr. later	0.34	9.2	9.1
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.18	4.4	2.8
12 min. in Ringer	0.33	8.2	8.7
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.25	4.6	3.1
25 min. in Ringer	0.36	6.9	10.0
ACh(5.5 μM/liter) + prostigmin (30 μM/liter)	0.29	2.6	3.9
Average reduction, *per cent*.....	29	44	59

To evaluate the changes in λ it should be noted that

λ is equal to $\sqrt{\frac{r_m}{r_i + r_e}}$ in which

r_m = resistance × unit length of the surface membrane in ohm cm.

r_i = internal resistance per unit length of the fibers in ohm cm.$^{-1}$

r_e = resistance per unit length of the external fluid in ohm cm.$^{-1}$

Note also the basic constants:

$R_i = \pi a^2 r_i$ = specific resistivity of the myoplasm in ohm cm. (a = radius of fibers in centimeters).

$R_m = 2\pi a r_m$ = transverse resistance of the surface membrane in ohm cm.^{2} (9).

C_m = capacity per unit area of the surface membrane in fd./cm.^{2}.

On the average, the characteristic length with 5.5 μM/liter acetylcholine and 30 μM/liter prostigmin application was reduced about 30 per cent. If we as-

sume that the internal and external resistances are unchanged, this indicates that the membrane resistance r_m must have been reduced even more, because λ is proportional to the square root of r_m.

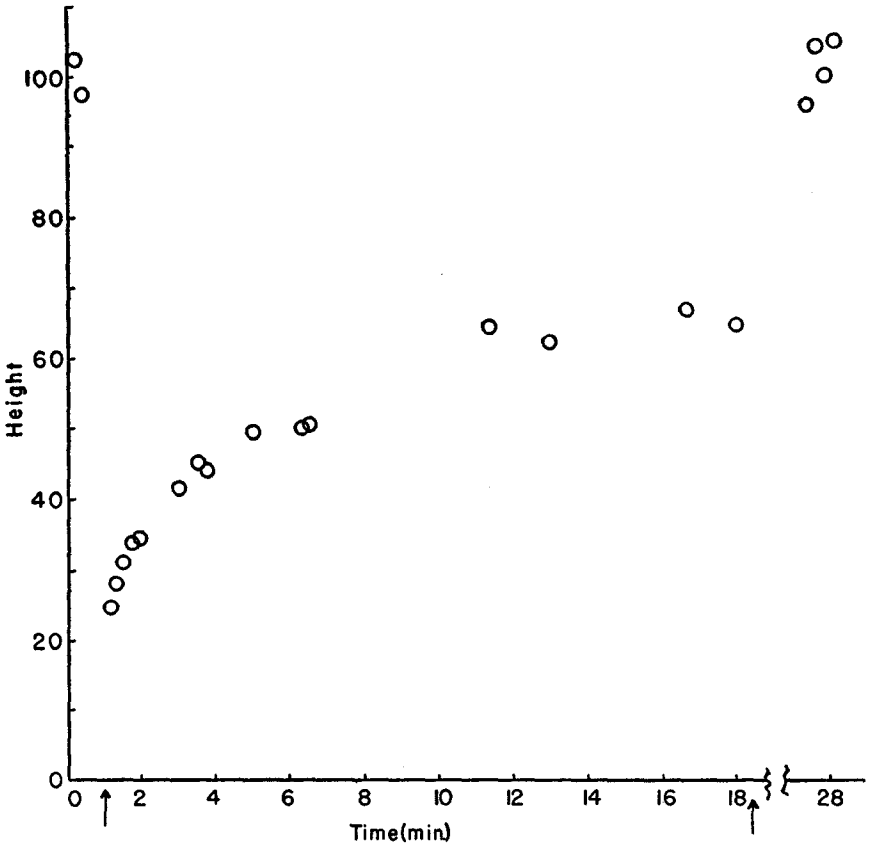

FIG. 3. Height of electrotonic pulse as a function of time. Position of recording electrodes fixed. At the first arrow a drop of 55 μM/liter acetylcholine without prostigmin was applied to the preparation for 20 seconds. The preparation was bathed in Ringer for about 10 minutes starting at the second arrow and afterwards measurements were repeated.

Measurement of the Time Constant of the Membrane (τ_m): There are several ways to evaluate τ_m from such records as are shown in Fig. 1 (9). One method is to measure the time from the beginning of the pulse to the point of half the maximal height; the slope of the plot of this time as a function of interelectrode distance is $\tau_m/2\lambda$ (τ_{m1} in Table I). An alternative method is to fit

one or more points on the experimental curves to the theoretical formula (9) (τ_{m2} in Table I).

The time constant was measured by both methods for the same series of experiments in which the characteristic length was measured (see records in Fig. 1). The results are given in Table I. There was a reversible decrease of the time constant due to acetylcholine application, and the average decrease was about 50 per cent.

Transient Changes after Acetylcholine Application.—In the absence of prostigmin the acetylcholine effect was transient. An example is given in Fig. 3. A drop of 55 μM/liter (1 part in 10^5) acetylcholine was applied for 20 seconds and the changes in the electrotonic potential were recorded at a fixed distance from the polarizing cathode. As a result of reduction of the length constant the electrotonic potential height was reduced by about 70 per cent, measured within 1 minute of the application. Within 14 minutes half recovery had occurred; *i.e.* the effectiveness of acetylcholine was reduced by 50 per cent. Immersion in Ringer for 10 minutes restored the original potential height. In contrast, in the presence of 5.5 μM/liter (1 part in 10^6) acetylcholine but with 30 μM/liter prostigmin added, the potential showed only a small change over the same period (Fig. 4).

The curve of Fig. 3 may well represent the time course of hydrolysis of the small quantity of acetylcholine which remained after the drop was removed. Some sort of adaptation, *i.e.* a loss of effectiveness of the drug may occur in addition, as observed by Thesleff (23) in skeletal muscle.

Values of the Membrane Constants.—The evaluation of the so called basic constants (R_i, R_m, and C_m) is quite difficult for the syncytial system of the heart. By the method of Hodgkin and Rushton (9) r_i and r_m were measured. The specific resistance of the myoplasm (R_i) was assumed to be 100 ohm cm. (value given by Weidmann (24) for kid heart Purkinje fibers). From these figures the radius of the fibers was calculated to be about 9 μ and the membrane resistance R_m to be 280 ohm cm.^{2}. R_m was reduced to 220 ohm cm.^{2} in the presence of 5.5 μM/liter acetylcholine and 30 μM/liter prostigmin. The membrane capacitance in Ringer was about 3.0 μfd./cm.^{2}.

Effect of Acetylcholine on Threshold.—When the electrotonic potentials near the cathode attained a critical height, first partially and then fully conducted impulses arose after varying latent periods. With stimuli well above threshold only fully propagated impulses were seen, the latent period decreasing with increasing stimulus strength. If a drop of acetylcholine (5.5 to 550 μM/liter) in Ringer was applied and then removed after 30 to 60 seconds, the threshold stimulus current strength for conducted impulses was always increased (*cf.* reference 3). At the same time the height of the electrotonic potential at which conduction occurred was considerably reduced. We were unable to determine, however, whether the "firing level" of the muscle fiber had ac-

tually changed. The alteration of the electrotonic potential could be due to a reduction of the length constant as the recording electrode did not lead accurately from the site of the impulse origin. Under present conditions the monophasically recorded action potential was greatly shortened after acetylcholine application. Complete block of conduction, expected according to results with vagal stimulation (14), did not occur in the present experiments

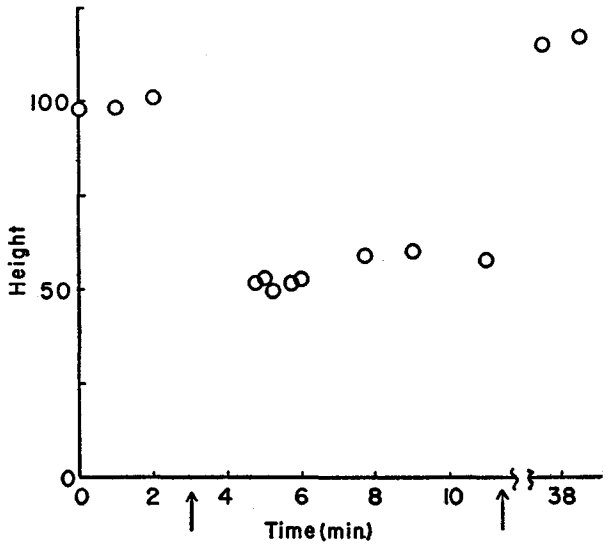

FIG. 4. Height of electrotonic pulse as a function of time. At the first arrow a drop of acetylcholine (5.5 μM/liter) and prostigmin (30 μM/liter) was applied for 30 seconds. The tissue was bathed in Ringer for about 25 minutes starting at the time indicated by the second arrow and afterwards measurements were repeated.

even with the highest concentration of acetylcholine (5.5 mM/liter or 1 in 10^3). Only a reduction of the action potential height was seen.

DISCUSSION

Resistances across membranes can be measured satisfactorily by inserting two microelectrodes into a single muscle fiber, passing current through one and determining the resulting membrane potential changes through the other (6). This method proved too difficult in the present preparation although it has been done on the relatively large Purkinje fibers (24). A simpler approach, similar to that of Araki and Otani (1), consisted of inserting only one electrode and using it simultaneously for recording and for passing current through the cell. Although direct readings of membrane resistance were thus obtained, fluctuations in the resistance of the micropipettes during insertions into the heart muscle cells and during passage of even small currents, reduced

the usefulness of this method. Kahn (15), using the method of Schaefer, Schölmerich, and Haass (21), similar to ours, measured successfully electrical properties of heart muscle strips but at that time no clear-cut conclusions could be reached about the action of acetylcholine on the length and time constants.

The present study has shown that inhibition produced by acetylcholine increases the conductance of the membrane. At the same time the membrane potential was not greatly altered with such effective concentrations as 5.5 μM/liter, because conducted action potentials were not appreciably changed in height. This is not unexpected, since the inhibitory transmitter tends to shift the membrane potential of heart muscle fibers towards the "resting" level (4, 14). Therefore it may be assumed that only certain ions are permitted to flow during inhibition. The K^+ and Cl^- ions, whose equilibrium potential is presumably near the full membrane potential level, are most likely to be involved (for recent results see 3, 4, 5, 14, 17). Earlier evidence (13, 18) has pointed to an increased K^+ liberation during vagal stimulation in mammalian and tortoise hearts and more recently Holland, Dunn, and Greig (11, 12) measured increased rates of K^+ loss in K^+-free solution. In K^+-rich solutions the uptake was reported to be increased. The importance of Cl^- in inhibitory action as suggested by Coombs *et al.* (5) in the spinal motor neuron has also been found recently in inhibitory junctions of sensory nerve cells of crayfish. Although inhibitory action persists in the absence of this ion, the inhibitory equilibrium level is changed if Cl^- is replaced by various other anions (Hagiwara and Edwards, unpublished observations).

In the present discussion a similarity of action between acetylcholine when applied externally and acetylcholine liberated by vagus stimulation has been assumed. A certain amount of caution is necessary here in view of the very high concentrations which are frequently needed to produce the present effects. Both the vagus and acetylcholine effects fluctuate appreciably in different preparations and at different seasons. This makes strict comparisons, unless made on the same preparation, difficult. In many isolated auricle strip preparations in which innervation was preserved conduction of impulses was readily blocked by vagal stimulation while 5.5 mM/liter acetylcholine did not block completely. Concentrations of acetylcholine of 5.5 μM/liter and higher reduced the long duration of the conducted action potentials, an effect which is characteristic of vagus action (4, 10, 14). It is possible that the acetylcholine concentration at the nerve-muscle junctions actually can be quite high during vagal stimulation. This aspect of acetylcholine action was not studied, although it was clear that the length constants and time constants were progressively changed by increased drug concentrations, for instance from 5.5 to 55 μM/liter. If 5.5 mM/liter was applied, it was difficult to obtain electrotonic potentials, indicating that the membrane resistance was decreased much more than in the average values presented here for lower concentrations.

SUMMARY

Membrane characteristics were studied in isolated muscle strands from auricles of frogs using the "square pulse" technique. Changes in the time course and spatial spread of subthreshold electrotonic potentials were measured. If acetylcholine is applied in concentrations which cause slowing or stoppage of the heart beat, the following changes are produced: (*a*) the length constant (λ) of the membrane is reduced, (*b*) the time constant is shortened. The effects are reversible and increase with acetylcholine concentration. The membrane changes caused by acetylcholine diminish with time.

It is concluded that during acetylcholine inhibition, as well as during vagal inhibition, the conductance of the muscle membrane is increased. Appreciable changes in the resting membrane potential need not accompany inhibition.

We wish to thank Mr. R. B. Bosler for his continued valuable technical assistance.

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