mental atherosclerosis by injection of anti-atheroma antibodies remains to be demonstrated.

Summary. Rabbit antibodies were produced in response to cholesterol induced chicken atheroma and chicken normal intima. Properties of these antisera were studied by complement fixation and described by isofixation curves. It was found that anti-atheroma sera reacted best with atheroma antigen and in addition reacted well with a wide variety of other chicken tissues. Anti-normal intima sera reacted well with either intima or atheroma antigen and had a limited spectrum of reactivity with other tissues. *In vivo* activity of these antisera could not be demonstrated.

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Effect of Potassium on Membrane Current of Single Ranvier Node During Excitation. (26347)

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When a frog nerve fiber is immersed in Ringer solution, containing 30-90 millimolar potassium chloride, and the resting membrane potential of the node maintained by an applied voltage, a peculiar prolonged response may be initiated in the node by superimposing a depolarizing stimulus on the applied voltage. This response consists of a spike followed by a prolonged depolarization which remains for a considerable period of time and decays slowly to the resting membrane level(1,2). Although other explanations have been put forward(1,2), the Ionic Theory of nerve activity actually predicts such a response to be elicitable under these abnormal conditions(3,4).

The Ionic Theory postulates 2 "carrier" systems: one which reacts rapidly with sodium, the other more slowly with potassium during excitation. These systems both depend for activation upon the magnitude of the electrical potential across the cell membrane. At the resting level, the systems are relatively inactive. With reduction of the membrane voltage by depolarization, carrier activity is initiated. The sodium carrier causes the membrane to become selectively permeable to sodium while the potassium carrier actively increases membrane permeability to potassium. Consequently membrane depolarization results in ionic current flow of each specie of ion along its electrochemical gradient. Initiation of sodium carrier activity produces a rapid regenerative depolarization by inward sodium current flow. The initial membrane potential level, –80 millivolts, tends to shift rapidly toward the sodium equilibrium potential, +40 millivolts. This large polarization change inactivates the sodium system, and the outward potassium current flow along its electrochemical gradient drives the membrane potential back toward the normal potassium equilibrium potential, –90 millivolts, and to the original resting level.

What happens in the abnormal situation

when a nerve fiber is immersed in solution containing excess potassium chloride and the membrane potential is held at the resting level by an applied voltage? First of all the potassium equilibrium potential is reduced. For example, the equilibrium potential for 60 millimolar excess potassium solution is almost zero. A voltage applied to maintain the membrane potential near the normal resting value, *e.g.*, –80 millivolts inside, should induce potassium ions to flow *inward* to increase the inside to outside concentration ratio. Such an inward flow would continue until the membrane potential value approximates the equilibrium potential. However, according to the theory, the carrier systems are relatively inactive as long as the membrane potential is held at or near the resting level. Therefore, in spite of the large discrepancy between membrane and equilibrium potentials, the inward potassium current would be small in the above example. A sudden depolarization by an abrupt decrease in the applied voltage should result in activation and a rapid increase in selective permeability to sodium and potassium. Both ions should flow *inward* and depolarize the membrane. The faster reacting sodium system should produce a rapid regenerative spike deflection as in the normal situation. Then the slower reacting potassium carriers should produce a regenerative, long lasting depolarization of the membrane toward the new potassium equilibrium potential level. Consequently, if the membrane voltage of a single fiber immersed in excess potassium solution were clamped at the resting level and then suddenly shifted to a depolarized value, only an inward current comprising two phases, one developed by the rapid transient sodium carrier activity and the other by the slow system associated with potassium, should be observed.

The present investigation was set up to record, if possible, such a membrane current in a single Ranvier node of a nerve immersed in a solution containing normal sodium and high potassium concentration.

Methods. Nerve fibers of frog, *R. Pipiens* and toad, *Bufo Marinus,* were dissected and

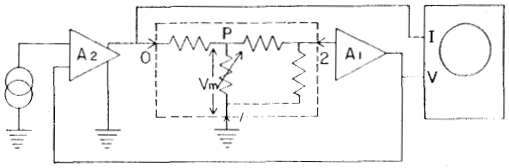

FIG. 1. An electrical schematic representation of the frog single nerve fiber set up for voltage clamp recordings. See text.

mounted in a chamber as described previously(5). Membrane current recordings were obtained by use of a voltage clamp system similar to others(6,7). Figure 1 shows an electrical schematic representation of the single nerve fiber set up for voltage clamping. The numbers 0, 1 and 2 indicate the three electrodes connected with the nodes N_0, N_1 and N_2 respectively. (See Figure 1(5)). The membrane potential of the test node, N_1, is represented by the voltage, V_m, across the variable resistor as shown. The other resistances include the internodal longitudinal resistance and a leakage resistance between electrode 2 and ground. The recording electrode 2, for membrane potential measurements is connected with the input of amplifier, A_1. The output of this amplifier connects directly with the input of the vertical amplifier for one sweep of the recording oscilloscope and with one side of the input of differential amplifier A_2, the clamping amplifier. The other input of amplifier A_2 connects with the stimulator, the output with node N_0 and the vertical amplifier of the other sweep of the recording oscilloscope. Any variation in the voltage V_m, is immediately recorded through the longitudinal fiber resistance by amplifier A_1, the resulting output signal from this amplifier fed back to amplifier A_2 and subsequently into the fiber as a compensating current which re-establishes the original voltage V_m immediately. The compensating current is actually recorded as a voltage developed between electrodes 0 and 1 or, in other words, the voltage output of amplifier A_2 measured between output and ground.

Hypertonic Ringer solutions containing 30-90 millimolar excess potassium chloride were used for most of the experiments. It was found first that solutions containing 2 times

normal concentrations of sodium chloride caused no deleterious effect on electrical activity. The effect of hypertonicity of the excess potassium chloride solutions was, therefore, not considered significant.

The potassium excess solutions were made up by adding potassium chloride to normal Ringer to bring the total amount in solution usually up to 60 millimolar. Since one millimole potassium chloride is equal to 77 mg and normal Ringer contains 2 millimoles potassium chloride, 58 × 77 mg solid potassium were added to one liter Ringer solution.

In some experiments potassium chloride was substituted for sodium chloride in Ringer solution (high potassium isotonic Ringer). To make up these solutions the ingredients sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, and potassium bicarbonate were made up in separate 117 millimolar isotonic solutions. Then test solutions were made up as follows: normal Ringer: 2 cc KCl, 1 cc $CaCl_2$, 114 cc NaCl, total 117 cc Ringer, buffer to 7.4 with $NaHCO_3$. 60 mM high potassium isotonic Ringer: 60 cc KCl, 1 cc $CaCl_2$, 56 cc NaCl, total 117 cc, buffer with $NaHCO_3$. For 100% isotonic Ringer, sodium chloride completely substituted by potassium chloride, potassium bicarbonate was used for buffering.

The temperature of the preparation mounted in the chamber was maintained at 15-18°C in which range the nerve fiber remained in good condition for many hours.

Results. A typical experiment, results of which are shown in Fig. 1, was as follows: A single fiber was dissected and mounted in the chamber and Ringer solution allowed to flow past the node. The condition of the fiber was examined carefully by noting a normal "threshold" for stimulation and a normal action potential response. Then the feed back system for voltage clamping was switched into the circuit and, without applied voltage, the membrane potential was clamped at the resting level. That is, the clamping amplifier was balanced for zero output with the resting node connected to the input through the potential amplifier (6). The flow system was then switched from Ringer to excess potassium solution and the single node rapidly immersed in this solution. This did not affect the clamped resting membrane potential level but a small inward current was sometimes seen. The membrane potential was then shifted to a depolarized level by a pulse applied to the clamping amplifier input. The current recordings obtained at 3 different voltage clamp levels are shown in Fig. 1, A, B, C. Each picture (A, B, C) in the figure shows 2 traces. The upper trace indicates the current flow, a downward deflection denoting an inward current direction (note reference line). The lower trace indicates the clamped voltage, an upward deflection showing depolarization of the membrane. In Fig. 1, A, with a shift of the voltage clamp in the depolarization direction the current deflection first shows a small artifact and then a slowly developing prolonged inward current. Increased depolarization B, results in an early large spike-like inward current, not seen in A, followed by the slow prolonged inward current. The latency of the slow current has decreased. With still greater depolarization, C, these same 2 inward currents are discernible but merged together. The recording appears as one inward current comprising 2 phases. At this depolarization level the initial spike-like phase of the inward current oscillates. The reason for the apparent merging of 2 separate currents is a decrease in latency of the slower current with increasing depolarization. This decrease in latency is made clear by comparing the onset time of the slow current in each picture. In picture C 2 sweeps have been superimposed, one with and one without depolarization for reference. It is important to note the continuation of the slow inward current after the membrane potential level has returned to the original value.

The ionic currents producing the deflections shown can be directly identified by varying the ionic content, sodium and potassium, of the test solutions. Detailed results of a series of experiments devised for this purpose will be reported later. Briefly it

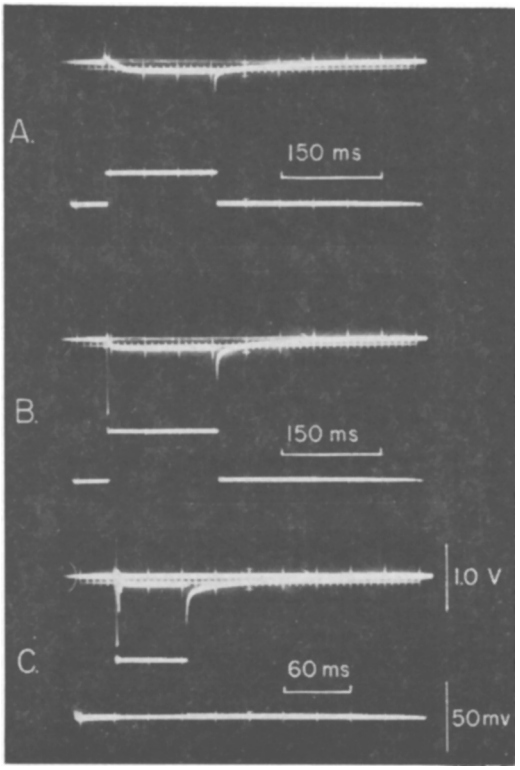

FIG. 2, A-C. Membrane currents, upper tracings, and membrane voltage, lower tracings, recorded from a single Ranvier node in excess potassium solutions under voltage clamp. A. Slowly developing inward current (downward deflection) during abrupt 25 millivolt, 150 millisecond depolarization of membrane. Note prolonged inward current after membrane potential reset to original resting level. Normal base line drawn in white ink for reference. B. Rapid short duration spike-like inward current followed by slow inward current with 40 millivolt, 150 millisecond depolarization of membrane. Normal base line drawn in white ink for reference. C. Oscillatory initial current followed by slow inward current with 50 millivolt, 60 millisecond depolarization. Two sweeps superimposed, one without depolarization for reference. The current measurement is actually voltage output of clamping amplifier and is about 0.25 volts in all the records. Assuming an overall resistance of 50 megohms, the slow currents shown in Fig. 2, A, B, C, are about 5×10^{-9} amps, the transient spike about 2×10^{-3} amps.

can be stated here that in the absence of sodium (100% potassium chloride isotonic Ringer), the early inward spike-like current deflection was never seen. Thus the early rapid transient inward current is to be associated with sodium in accord with other reports(4,6,7). This conclusion is further substantiated by potential recordings which under similar conditions showed only the slow prolonged depolarization of the membrane without the initial spike. The slow inward current could be obtained only with the fiber immersed in high potassium concentration solutions. The magnitude of the current varied directly with potassium concentration. In normal Ringer solution only a small outward current is obtained during continual depolarization(4,6,7). Thus the slow current is to be associated with an inward flow of potassium ions across the node membrane. The 2 currents, sodium and potassium, are apparently independent, though initiation and development of each is related to the membrane potential level as predicted by the theory.

Discussion. Apparently it is possible to record 2 inward currents in the single nerve fiber membrane under a voltage clamp in excess potassium Ringer solution. The independence of these 2 currents or current sources is clearly shown by the fact that one can be initiated without the other. This suggests that there are 2 carrier systems which, when activated, cause the membrane to become selectively permeable to sodium and potassium ions respectively.

The sodium system reacts rapidly whereas the potassium system is relatively slow. The evidence certainly indicates that the prolonged potential response observed in a fiber in excess potassium chloride solution cannot be attributed to a broadening of the action potential *per se*. Two depolarizations, first the sodium spike and then a slow potassium depolarization potential, overlap and comprise the peculiar response observed under such abnormal conditions. This explanation conforms with the Ionic Theory of nerve activity(4).

Summary. The response of the frog single nerve fiber immersed in Ringer solution containing excess potassium has been studied under a voltage clamp. Two clearly discernible inward ionic currents have been observed. These two currents have been associated with sodium and potassium respectively. The evidence presented strongly supports the presently accepted Ionic Theory of nerve activity.

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In vitro Inhibition of Glycogenesis by D-α-Tocopherol.* (26348)

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During a recent study on the glycolytic cycle in Vit. E-deficient rabbits(1,2), it was observed that D-α-tocopherol lowered glycogen levels and lactic acid production in muscle. Zierler *et al.*(3) reported a similar finding for rat diaphragm after *in vivo* administration of α-tocopheryl phosphate. On the basis of data from several pertinent experiments, these authors concluded from indirect evidence that the phosphorylated tocopherol inhibited at the phosphoglucomutase site. It was the purpose of the present investigation to study more directly the action of free tocopherol on glycolysis.

Experimental. Colonies of white, New Zealand rabbits were reared on a Vit. E-deficient diet as previously reported(4). Half the animals (control) received oral supplements of DL-α-tocopheryl acetate and muscle strips were prepared as previously described(1). Oxygen uptake was determined for one hour in presence or absence of D-α-tocopherol with the usual Warburg technics. The tocopherol solution was prepared immediately before use by homogenizing 10 mg of the compound in 0.2 ml of propylene glycol in a 1 ml all glass homogenizer. At termination of the one hour incubation interval, suitable aliquots of tissue were used for estimation of glycogen and lactic acid(1). Isooctane extractions of the medium after incubation were shown to contain 70 to 90% of the added tocopherol as the intact chroman or para quinone by their characteristic ultraviolet absorption.

D-α-tocopherol was also tested on individual purified enzyme systems containing commercially available phosphorylase a, glucose-6-phosphate dehydrogenase and hexokinase. Phosphoglucomutase was also studied and this material was generously supplied by Dr. O. Bodansky.

Phosphorylase a activity was followed by estimating the release of inorganic phosphate while absorption at 340 mμ by reduced triphosphopyridine nucleotide was used to measure the activity of glucose-6-phosphate dehydrogenase(1). The hexokinase reaction was studied by determining the disappearance of glucose according to the method of Nelson(5) as modified by Somogyi(6). The phosphoglucomutase procedure was that of Bodansky(7) in which the disappearance of glucose-l-phosphate (or inorganic phosphate) was followed.

Results. The *in vitro* influence of tocopherol on glycogen levels and lactic acid production in rectus femoris of rabbit is shown in Table I. In the presence of exogenous glucose (0.1 ml of a 10% solution) tocopherol caused a distinct decline (19 to 43%) in glycogen levels in both control and Vit. E-deprived animals. This fall in titer of muscle glycogen was not completely alleviated by increasing the rate of glucose-6-phosphate synthesis from glucose. Addition of 1 mg hexokinase and 1 mg adenosine triphosphate

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