observations on the effect of vitamin A on cell membranes(14) it is interesting to speculate that this effect of vitamin A on increased glucose utilization may be the result of increased permeability of cell membranes to glucose.

Summary. Intravenous glucose tolerance studies showed no impairment of glucose utilization in atherosclerotic dogs. It was observed, however, that administration of vitamin A to atherosclerotic dogs did produce a significant increase in glucose utilization.

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Effect of Aldosterone on Transmembrane Potentials of Frog Skeletal Muscle.* (32234)

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The importance of aldosterone in sodium and potassium regulation by the kidney of mammals is well established(1). More attention has been given to its renal than to its extra-renal activity. Its role in sodium transport is most clearly delineated by studies on amphibian skin and bladder(2,3). Since the effect of aldosterone is on sodium transport, it is not unexpected that administration of this substance to mice produced a decrease of intra-cellular sodium and an increase of intra-cellular potassium(4). Administration of the hormone to rats raised the resting membrane potentials of muscles which had been previously depressed by adrenalectomy (5). In experiments reported here aldosterone is shown to have an acute effect on resting potentials of amphibian skeletal muscle *in vitro*.

Methods. Resting potentials were examined in sartorius muscles isolated from small (2-3 inch) *Rana pipiens* which had been confined for 24 hours in cages containing a moderate amount of distilled water at 4°C. Potentials were determined at room temperature (25°C) with glass microelectrodes filled with 2.7 M KCl and connected to the input probe of a negative-capacity electrometer amplifier (Medistor Instr. Co., Model A-35) through a saturated KCl solution and calomel half-cell. The reference lead in the solution bathing the muscles was an agar-Ringer bridge connected to the amplifier through a second saturated KCl solution and calomel half-cell. Microelectrode resistances were between 8 and 15 megohms. Output of the amplifier was monitored on an oscilloscope and recorded on a servo-recorder (Heath Co., Model EUW-

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20A). Potentials from surface fibers were rejected, as were those which could not be maintained and recorded for a minimum of one minute.

In all, 3 experiments were conducted. For the first experiment, I, paired sartorii were removed and threads placed on the distal tendons and around the pelvic ends of the muscles. Each muscle was then placed into a separate lucite chamber and tied down at a length corresponding to 120% that measured *in situ*. The chambers were filled with Ringer's solution (NaCl, 110 mM/l; KCl 2.5 mM/l; $NaHCO_3$ 12.5 mM/l; $CaCl_2$ 1.25 mM/l; dextrose 5.5 mM/l) and were gassed continuously with a mixture of 95% O_2-5% CO_2. After a 30 minute period of equilibration, fresh Ringer's solution was placed in each chamber. At the end of the next hour, "0" hour in Table I, resting potentials were recorded from each muscle. Subsequently, fresh Ringer's solution was introduced into both chambers. One-tenth ml of an ethyl alcohol-aldosterone solution was then added to one chamber to yield a final concentration of 2.8×10^{-6} M aldosterone. One-tenth ml of ethyl alcohol only was added to the control muscle chamber. Five to eight resting potentials were recorded at the end of each hour thereafter, for a total period of 3 hours. Of the 6 pairs of muscles studied, aldosterone was applied to 3 left and 3 right muscles in an alternating sequence. Muscle wet and dry weights were determined.

For the second experiment, II, a double chamber was devised which permitted a pelvic block dissection to be used. The origins of both muscles at the pubic crest were intact, yet each muscle occupied a separate chamber. This technique minimized injury due to dissection and decreased the variability between muscles, as reported by Draper *et al*(6). After being stretched and allowed to equilibrate for 30 minutes, the normal Ringer's solution was flushed from the chambers and each muscle then was exposed to Ringer's solution containing 10 mM/l of KCl (substituted for an equivalent amount of NaCl) for one hour, when 10 resting potentials were determined for each muscle. Afterwards the incubation medium was exchanged for fresh, high-potassium Ringer's solution and aldosterone added as described for Experiment I. Ten resting potentials were recorded from each muscle at the end of the next 2 hourly periods. The sequence of initial recording alternated between control and treated muscles in the successive muscle pairs of the 10 animals studied.

The protocol for Experiment III followed that of Experiment II except that ouabain was present in the 10 mM/l potassium-Ringer's solutions to a final concentration of 10^{-4} M. Ten potential records were obtained each hour for 3 hours after adding aldosterone; 5 pairs of muscles were examined. Data were analyzed by use of Student's t test; a probability of $\leq .02$ was accepted as statistically significant.

Results. Table I summarizes the results of all 3 experiments. Resting potentials recorded at the end of the hour preceding the addition of aldosterone are tabulated under the "0" hour heading. At this time, the observed differences in potentials between Experiments I and II correspond to the predictable changes due to the high-potassium Ringer's solution. Although the differences in potentials between Experiments II and III at this time are not large, they are significant ($p \leqq .02$).

In Experiment I, the addition of aldosterone maintained the resting potentials at or near the original ("0" hour) value throughout the 3-hour observation period. The control muscles potentials were significantly lower ($p < .002$) than their original value during hours 1, 2 and 3 and significantly lower ($p < .001$) than the aldosterone-treated muscles potentials during hours 1 and 2.

Control muscle potentials in Experiment II were reasonably consistent throughout the 2-hour observation period. The addition of aldosterone produced a slight, but significant, ($p < .02$) increase in resting potential during hour 2.

The presence of ouabain in the high-potassium Ringer's solution, Experiment III, produced a progressive decline in resting potentials of both control and aldosterone-treated muscles throughout the observation period.

Discussion. The hyperpolarization exhibited by the ouabain-treated muscle fibers in ex-

TABLE I. Transmembrane Potentials of Frog Skeletal Muscle Fibers Exposed to Aldosterone.

		Hour			
		0	1	2	3
Exp I					
(6) Ringer (2.5 mM K)	(a)	90.8 ± .3 (58)	85.8 ± .4 (34)	87.0 ± .3 (38)	88.4 ± .7 (30)
(6) Ringer + aldosterone	(b)	91.1 ± .3 (79)	91.0 ± .5 (47)	91.0 ± .2 (40)	90.0 ± .6 (37)
Exp II					
(10) Ringer (10 mM K)	(c)	58.2 ± .8 (100)	58.0 ± .8 (100)	57.7 ± .7 (100)	
(10) Ringer + aldosterone	(d)	58.2 ± .8 (100)	58.1 ± .8 (100)	60.0 ± .7 (100)	
Exp III					
(5) Ringer (10 mM K) + ouabain	(e)	61.1 ± .9 (50)	60.4 ± .9 (50)	57.7 ±1.0 (50)	55.1 ± .9 (50)
(5) Ringer + ouabain + aldosterone	(f)	61.3 ± .9 (50)	60.6 ± .8 (50)	58.0 ± .8 (50)	55.4 ± .9 (50)
Probability Aldosterone *vs* no aldosterone	(a *vs* b)	>.40	<<.001	<<.001	>.07
	(c *vs* d)	>.90	>.90	<.02	
	(e *vs* f)	>.90	>.90	>.80	>.80
Probability Aldosterone *vs* time	(b)		>.80	∞	>.13
	(d)		>.60	>.07	
	(f)		>.50	<.05	<.05

periment III at the "0" hour, when compared to the potential values of untreated fibers in Experiment II, can be attributed to an increase in K-ion permeability. If it is assumed that after 1 hour of equilibration $[K]i/[K]o = [Cl]o/[Cl]i$(7) and that ouabain in addition to inhibiting active Na-K transport also increases the K-ion permeability, application of the constant field equation for membrane potential(8,9) yields calculated resting potentials for Experiment III which depending upon the absolute concentrations stipulated are 1, or more, millivolts larger than for Experiment II. More direct evidence for such a proposed increase in K-ion permeability due to ouabain has been reported by Gourley(10). In frog nerve, ouabain appears to nearly double the K-ion permeability.† The progressive decline in the resting potentials of ouabain-exposed muscle fibers during hours 1, 2 and 3 is a consequence of slow, but steady, Na-ion influx.

In Experiment I control muscle fiber potentials decline in accord with Boyle and Conway's(7) observation that isolated frog muscle in normal Ringer's solution steadily gains sodium and loses potassium. It is also probably due, in part, to dissection technique and temperature(6). Despite this, it emphasizes the effect of aldosterone on the contralateral muscles. Addition of aldosterone to the Ringer's solution prevents the decline in potential with time presumably by stimulation of the active Na-K transport mechanism. Maintenance of resting potential(5) and even hyperpolarization(11) in rat skeletal muscle fibers treated with aldosterone has been reported.

When muscle fibers are depolarized by an increase in external potassium, as in Experiment II, the consequent stimulation of active Na-K transport(12) prevents or diminishes the deterioration of resting potential with time which occurs in normal Ringer's solution. Application of aldosterone, in fact, appears to exert a significant additive effect within 2 hours. Antagonism of ouabain by aldosterone(13) was not apparent in Experiment III.

Summary. Resting potentials of frog muscle fibers incubated in normal or high-potassium Ringer's solutions are maintained for longer periods of time by the addition of aldosterone. This response is absent in ouabain-Ringer's solution. Although based upon separate groups of muscles, the results indicate that muscle fibers in high-potassium Ringer's solution are slightly hyperpolarized when ouabain also is present.

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† F. P. J. Diecke, personal communication.

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Stimulation of Interferon Production in Human Lymphocytes by Mitogens. (32235)

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Recent studies have shown that a number of non-viral substances elicit the *in vitro* production of antiviral proteins with many of the properties of interferon (reviewed in ref. 1). One of the most interesting of these non-viral inducers of interferon (or interferon-like substances) is the phytohemagglutinin (PHA) obtained from the kidney bean, *Phaseolus vulgaris*. In human lymphocyte cultures PHA stimulates cellular enlargement and mitotic activity(2) and in addition initiates the production of an interferon-like protein(3). A variety of other substances are also capable of inducing lymphocyte growth *in vitro*. Some of these, like PHA, are non-specific, in that they are effective with cells from all subjects including cells obtained from umbilical cord blood(4). Other materials have been termed specific, since they are only active when the lymphocytes tested are obtained from a subject who has been immunologically sensitized to the substance in question(5,6).

It was of interest to determine whether the production of interferon by lymphocytes was a generalized concomitant of lymphocyte growth or whether the phenomenon was peculiar to stimulation by PHA. In this study the interferon-inducing effect of an extract of the poke weed (*Phytolacca americana*) which behaves as a non-specific mitogen(7) and that of streptolysin-O, which behaves as a specific mitogen(4), were investigated and compared with the effect of PHA. In addition, the kinetics of PHA-stimulated interferon production were studied in some detail.

Materials and methods. The preparation of purified human lymphocyte cultures has been previously described(8). Cells were cultured at a density of 2×10^6/ml in Eagle's MEM (spinner modification) with 10% autologous plasma.

Mitogens employed were PHA, poke weed mitogen (PWM), or streptolysin-O (SLO). PHA was obtained from Burroughs Wellcome, Inc. (PHA, batch x5) and was used at 10 μg/10^6 cells; SLO, from Difco Laboratories (0.4 ml standard solution/10^6 cells). Purified PWM was generously provided by Drs. L. Chessin, J. Börjeson, and R. Reisfeld, NIAID, NIH, and was employed at 1.25 μg/10^6 cells. PWM and PHA stimulated cultures were incubated for 2 to 48 hours before harvesting; SLO cultures, for 4-5 days. Since little or no mitotic activity was observed before 48 hours after addition of PHA or PWM, all of the observations made on those cultures were probably referable to the originally cultured cells.

All lymphocyte culture fluids to be assayed were routinely passed through Nalgene 0.2 μ