lower than that in the comparable offspring of the mothers immunized with the methylated bovine serum albumin aggregate ($P < .001$).

We postulate that the presence of antibody in offspring I before immunization and the enhanced ability of the postimmunization offspring to make larger amounts of antibody were due, at least in part, to stimulation of the lymphoid system of the developing fetus by antigen transmitted from the immunized mother, such that the fetal cells capable of reacting with poly-(Glu^{52}Lys^{33}Tyr^{15}) proliferated. The aggregated form of the antigen given to the mothers probably protected it from degradation so that an adequately large amount was available for stimulating the fetus over a long period of time. When methylated bovine serum albumin was used as the aggregating agent, the antibody responses of offspring IA and offspring II were less than that of offspring I (Fig. 1) ($P < .01$); thus, the passage of antigen from the immunized mother must have decreased with time. The same conclusion holds with the polylysine aggregate (Fig. 2), since the antibody response of offspring II fell to normal; however, the response of offspring IA was the same as that of offspring I.

Antibody transmitted from the mother to the fetus probably could not provide the basis of the immunological memory of the offspring. Since the half-life of rat immunoglobulin is 5 to 6 days (*10*), less than 0.05 percent of the transmitted maternal antibody would be present when the offspring were immunized. The amount that might conceivably be transmitted to the F_2 generation would, at best, be vanishingly small. It is improbable that a sufficient number of primed immunocompetent cells would cross the placental barrier from the immunized mother to colonize the F_1 offspring and, subsequently, the F_2 offspring.

Objections have been raised to the hypothesis that the persistence of antigen is responsible for the continued production of antibody and for the development of immunological memory because the antigen has generally been detected in organs other than the lymphoid system, for example, in liver, lung, and kidney. This argument is not necessarily a cogent one, however, since antigen can continually leave these organs to circulate and to stimulate the immune mechanism. In any event, the antigen in our experiments was located exclusively in immunologically competent tissues, particularly the bone marrow.

In addition to being related to the basis of immunological memory, our experiments have two other implications. First, they provide a means of differentiating between the effects of environment and heredity on the immune response. Second, our studies suggest methods of "immunological engineering" by which manipulation of the maternal environment during the development of the fetus, for example, with a long-acting vaccine, could afford the offspring an enhanced immune response against infectious diseases in the postnatal period. This technique would be applicable to certain individuals, such as those with various developmental abnormalities, who have increased susceptibility to infection. The same techniques might also be used to counter the teratogenic effects of viruses and thus prevent the development of induced congenital anomalies.

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Cooperative Control of Potassium Accumulation by Ouabain in Vascular Smooth Muscle

Abstract. *The effects of ouabain on potassium accumulation were studied in the dog carotid artery. It was confirmed that vascular smooth muscle lost potassium in the presence of ouabain greater than 10^{-9} molar. This effect could be reversed by systematically increasing potassium in the external medium. The action of ouabain on ion accumulation was represented quantitatively with the application of a recent biophysical approach.*

The inhibitory effects of cardiac glycosides on ionic fluxes are well established. Although it is generally assumed that related shifts would occur in cellular potassium contents, few systematic observations of these modifications have been reported. Moreover, the extensive investigations of transport processes have not clearly resolved mechanisms underlying the inotropic activity of these agents (*1*).

Investigations were therefore carried out on the influence of ouabain on potassium accumulative processes. In addition to the classic approaches to membrane transport, consideration was also given to a biophysical model which has recently been developed. This approach assigns a fundamental role to the cytoplasmic proteins comparable at least to that of the membrane proteins, in the control of cellular functions (*2*). A number of qualitative as well as quantitative predictions of this model have been confirmed recently on skeletal muscle (*3*) and arterial smooth muscle (*4*). The present work represents an extension of this approach to the general problem of biological control of ion accumulation via pharmacological agents.

Table 1. Experimental values used for parameters in the cooperative absorption isotherm.

Ouabain (M)	$K_{K/Na}$	$n = e^{-\gamma/2RT^*}$
Control	90	2.7
5×10^{-8}	19	2.9
10^{-7}	11	2.4
10^{-6}	3	1.0

* R, 1.986 cal mole^{-1} deg^{-1}; T, 310°K; $-\gamma/2$ (cal mole^{-1}).

Carotid arteries from dogs were dissected and cut into small strips (20 to 25 mg). These were stored overnight at 2° to 4°C in a potassium-free and glucose-free medium. When the tissues were transferred to normal Krebs solution at 37°C for recovery, the accumulation of potassium was found to be completed within 6 hours (Fig. 1). The accumulation was inhibited by the presence of ouabain in a dose-dependent manner as shown in Fig. 2. Little effect was noted at glycoside concentration of $10^{-9}M$ and the maximum effect occurred at about $10^{-6}M$. These results are similar to the dose-dependent inhibition of potassium influx produced by glycosides in other tissues (*5*, *6*).

The inotropic actions of ouabain as well as its effects on potassium influx can be antagonized by potassium in the extracellular medium (*7*). The activity of the membrane Na–K–adenosine triphosphatase system is also known to be affected similarly by ouabain. The enzyme is believed to have allosteric properties, and its interactions with ions and cardiac glycosides may involve subtle conformational changes (*8*). Experiments were designed to see whether invoking a similar concept of conformational alteration in proteins might also explain the effects of ouabain on the accumulation of potassium. This question was approached by extending the observations of ouabain effects over a range of external potassium concentrations, $[K]_{ex}$. Systematic shifts in the fundamental relation between $[K]_{ex}$ and cell potassium accumulation, K_{ad}, would be indicative of biologic resetting.

First, the accumulation of potassium was measured by varying $[K]_{ex}$ in the absence of ouabain (Fig. 3). The results confirm earlier observations in which a specific biophysical model was applied to this relation (*4*). The equilibrium levels of potassium reached by the cells in the presence of sodium followed a cooperative adsorption isotherm as shown in Fig. 4 (*4*, *9*). The isotherm was derived by assuming that ions are accumulated by a fixed number ($F_T = 120$ μmole per gram of dry solid) of sites distributed throughout the cell. The sigmoid shape of the solid curve is a result of the interaction energy, designated as $-\gamma/2$, between potassium and sodium ions on nearest-neighboring sites (*10*). The parameter, $K_{K/Na}$, represents the intrinsic equilibrium constant (selectivity ratio) for potassium accumulation in the presence of sodium. In the absence of nearest-neighboring interac-

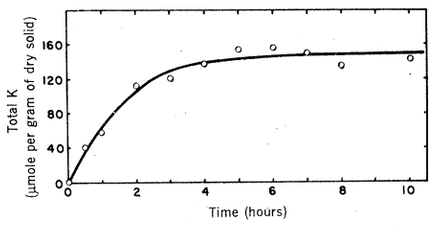

Fig. 1. Recovery of potassium at 37°C in the carotid artery after overnight cold storage in potassium-free and glucose-free Krebs solution. The solutions were bubbled with 5 percent CO_2 and 95 percent O_2. Each point is the mean of two to three determinations.

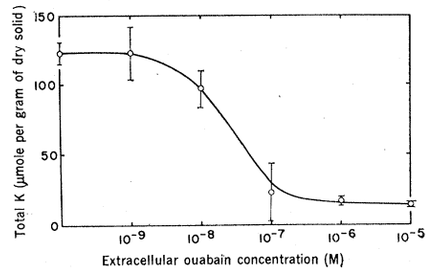

Fig. 2. The equilibrium uptake of potassium in the presence of different ouabain concentrations. The potassium-depleted tissues were recovered for 6 to 8 hours in normal Krebs solution containing (mM): Na, 145; K, 5; Mg, 1.2; Ca, 2.5; Cl, 134; HCO_3, 22.5; H_2PO_4, 1.2; dextrose, 5.6; and ouabain. Each point represents the mean of about eight determinations; the vertical bars show ± standard error of the mean.

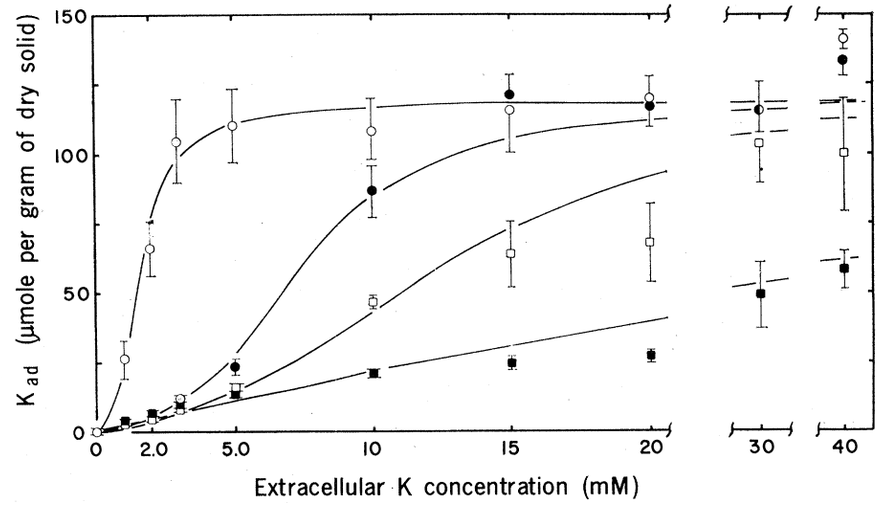

Fig. 3. The accumulation of potassium by the cells at varying $[K]_{ex}$ (and $[K]_{ex} + [Na]_{ex} = 150$ mM) and at different concentrations of ouabain. Each point is the mean of three to six determinations. The solid curves passing through the experimental points are obtained theoretically according to the equation in Fig. 4. The values of K_{ad} were found by correcting the total tissue potassium for the 4-minute fast-exchanging ^{42}K (*4*). The following doses were employed: control (○); ouabain, $5 \times 10^{-8}M$ (●); $10^{-7}M$ (□); $10^{-6}M$ (■).

$$K_{ad} = \frac{F_T}{2}\left[1 + \frac{\left(\frac{[K]_{ex}}{[Na]_{ex}} \cdot K_{K/Na} - 1\right)}{\left\{\left(\frac{[K]_{ex}}{[Na]_{ex}} \cdot K_{K/Na} - 1\right)^2 + 4\,\frac{[K]_{ex}}{[Na]_{ex}} \cdot K_{K/Na} \cdot e^{\gamma/RT}\right\}^{\frac{1}{2}}}\right]$$

Fig. 4. The cooperative adsorption isotherm for the uptake of potassium.

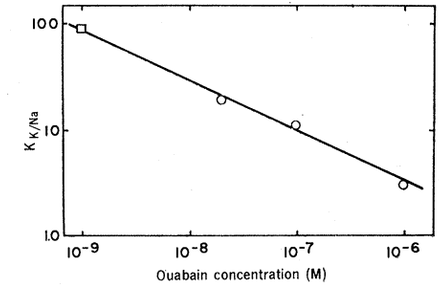

Fig. 5. Log-log plot of the intrinsic equilibrium constant compared to the concentration of ouabain. Control value indicated by open square. The empirical relation ($-\log K_{K/Na} = 0.47 \log$ ouabain concentration $+ 2.3$) was derived by regression techniques.

tion (that is, $-\gamma/2 = 0$) the equation in Fig. 4 reduces to the familiar Langmuir adsorption isotherm.

The above experiments were repeated at three different concentrations of ouabain in the recovery medium. If the action of glycosides were mediated through the cooperative mechanism, it must be reflected by systematic shifts in $K_{K/Na}$ and $-\gamma/2$. The results have confirmed this prediction (Fig. 3). It is seen that the same cooperative adsorption isotherm can describe the equilibrium uptake of potassium at all concentrations of ouabain. The curve is, however, shifted to the right; that is, the selectivity is progressively lowered with increasing concentrations of ouabain. The values of the parameters used in these plots are summarized in Table 1. The empirical relationship between the intrinsic equlibrium constant and ouabain concentration (Fig. 5) was derived by means of log-log plot and standard regression techniques.

One explanation of the above findings is that the sites responsible for ion accumulation undergo changes of conformation in the presence of ouabain. The mechanism for inducing such changes is not known. It is attractive, however, to consider that ouabain may act on ion accumulation by binding noncompetitively to specialized sites (cardinal sites) (*2*) on cytoplasmic as well as membrane proteins. This could alter the pattern of electronic distribution (*11*) over an extended group of sites. This affects the field strength of the anionic adsorptive sites. On a theoretical basis it is known that the intrinsic equilibrium constant (the selectivity ratio for potassium over sodium) is controlled by the field strength of the anionic sites [(*2*); see also (*12*)].

The presence of more than one type of site for glycoside activity offers potential for the integration of different pharmacological effects. Indeed, the uptake of cardiac glycosides in red blood cells (*13*), unmyelinated nerve (*14*), and other mammalian cells (*6*) consists of at least two fractions: surface transport sites and nonspecific sites. Surface sites may control some aspects of cell function as exhibited by the inhibition of sodium efflux in the presence of external ouabain (*15*). A separate set of sites could explain the inotropic activity of ouabain injected into crab muscle fiber (*16*). The shifts of potassium accumulation in vascular smooth muscle also reflect that the site of cardiac glycoside action may be on cytoplasmic proteins. In conclusion, the above results indicate that the application of cooperative adsorption isotherm offers a quantitative basis for interpreting biological resetting induced by the action of drugs.

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$$\log \frac{K_{ad}}{F_T - K_{ad}} = n \log \frac{[K]_{ex}}{[Na]_{ex}} + n \log K_{K/Na}$$

This equation is the approximate form of Fig. 4 and holds when K_{ad} approaches half saturation ($F_T/2$). Therefore, once F_T is found from the experimental data, the value of n ($= e^{-\gamma/2RT}$) and $K_{K/Na}$ is fixed according to the above relationship; n is the slope of the straight line on log-log plot, and $K_{K/Na}$ is the inverse value of abscissa at ordinate value of unity.
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Specificity of Allogeneic Cell Recognition by Human Lymphocytes in vitro

Abstract. *Human lymphocytes proliferate in vitro in response to foreign histocompatibility antigens that are present on allogeneic lymphocytes. Within a population of immunocompetent lymphocytes there are specific subpopulations that respond to allogeneic cells from different individuals. A means of selectively eliminating such subpopulations is suggested.*

The major histocompatibility (H) systems in several species are complex in that each system is highly polymorphic with respect to alleles (haplotypes) and the number of different antigens associated with each system. Studying the graft-versus-host response in the chicken, in which lymphocytes respond to foreign H system antigens, Simonsen noted that up to 3 percent of lymphocytes respond to a single difference in H locus (*1*). A similarly high frequency of initially responding units in homograft reactions was subsequently found when the mixed leukocyte culture (MLC) technique (*2*) was used as a model of the recognition phase of the homograft or graft-versus-host reaction.

These two observations—the high frequency of initially responding units to single major differences in the H system where no overt sensitization had taken place, and the large number of antigens associated with each system—have led to the suggestion that the cells responding in this "primary" immune response are either extensively pluripotent or in fact totipotent. Alternatively, different cells may respond to different allogeneic antigens, and the high frequency of responding units can be explained in other ways (*1–3*). We have presented human MLC data consistent with this latter model. Stimulation in the MLC test—a measure of antigenic disparity at HL-A, which is the major H system