Table 1. Effect of estradiol on incorporation of glycine into endometrium in tissue culture. The number of determinations made is shown in parentheses. Each sample was counted long enough to obtain a probable error of counting of less than 1 percent.

| Growth | Cellular radioactivity per tube (count/min) | |
|---|---|---|
| | Control | Estradiol |
| 1+ | 1.20 (10) | 1.10 (4) |
| 2+ | 1.00 (5) | |
| 3+ | 1.80 (3) | 2.61 (8) |
| 4+ | 2.95 (2) | 3.86 (5) |

counted in a continuous gas flow windowless counter. The cells adhering to the tube walls were washed once with 0.9-percent sodium chloride and were then mixed with 10-percent metaphosphoric acid. The precipitate was centrifuged and transferred quantitatively, with the aid of a little water, to planchets and then dried and counted.

Attempts were made to culture nine specimens of endometrium. Four samples showed no evidence of viability after 5 days culture in the basic medium. One of these was from a postmenopausal woman, and the other three were obtained from premenopausal women shortly before their predicted menstrual periods. Of the five samples that did grow, all showed evidence of growth within 2 days. One of these was obtained 2 days after cessation of menstruation and 1 day after injection with a large dose of synthetic estrogen. Another specimen was from a patient with hyperplastic endometrium, and the other three were from women in the first 2 weeks of their ovulatory cycle. That tissue from the proliferative phase grows better than specimens from the secretory phase has been noted by other investigators (*3*).

The cultures, examined microscopically in the living state, exhibited great differences in the number of viable cells adherent to the walls of the tubes. Two basic cell types were often noted in the same tube (*3*). The greater number of cells had a long, spindle-shape, with processes which appeared to connect with other similar cells. Less often, scattered clumps of round or polygonal-shaped cells were seen.

In one incubation experiment, 43 tubes were initially planted. In addition to the basic medium, all tubes contained labeled glycine during the initial growth period; estradiol was added to 21 tubes. At the end of 3 days, there were all degrees of growth in both groups. The average amount of radioactivity per tube in the cellular material and in the medium was not significantly different in the two groups.

In another experiment three conditions were compared. The control set contained no added estradiol and the other two sets contained estradiol at concentrations of $10^{-7}$ and $10^{-6}M$. Each group of tubes was grown for 1 week in its own medium; then the old medium was replaced by fresh medium of the same type, and the tubes were left to grow for another week. At the end of this time, 8 of the 13 tubes containing the higher estradiol concentration showed complete cellular necrosis. All of the other tubes contained viable cultures. This prolonged exposure to estradiol appeared to bring about an early death of the cell cultures, but did not increase incorporation or utilization of glycine from the medium.

In a third experiment 40 tubes, grown in the basic medium for 3 days until growth was established, received fresh medium containing labeled glycine. Estradiol was added to half of the tubes at random, and the cultures were incubated for four more days. The degree of growth in each tube was judged by inspection and graded 1+ to 4+ (Table 1). The amount of glycine incorporated is correlated with the estimate of cellular growth, and in the two groups with greatest growth the estradiol significantly increased the incorporation. The mean for all tubes containing estradiol was 2.5 counts per minute while that for the control was 1.4 counts per minute. This difference is small, but it is statistically significant at the 1-percent confidence level ($t$ test). The average radioactivity remaining in the medium in all of the tubes containing estradiol was 9.8 counts per minute, while the control value was 15.9 counts per minute. This difference is statistically significant at the 0.1-percent confidence level.

This experiment indicates that estradiol can increase the uptake of glycine from the medium and its incorporation into the cellular material of human endometrium in tissue culture. This effect is dependent upon the duration of contact between the tissue and medium, as shown by the second experiment. The results suggest that the method of tissue culture will be of value for future investigations into the mechanism of action of estrogens (*8*).

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## Action of Vasopressin on the Permeability of Mesentery

*Abstract.* Contemporaneous movements of $Rb^{86}$ cations and of $P^{32}$ orthophosphate across isolated rabbit mesentery display kinetic patterns that are generally associated with passive diffusion. Vasopressin at a concentration of 100 milliunit/ml produced a significant increase in the permeability constant for $P^{32}$ and at the same time a significant decrease in the permeability to $Rb^{86}$. At lower hormone concentrations (0.1 milliunit/ml) the $P^{32}$ response was less marked but still significant ($P < .01$), while the $Rb^{86}$ effect was not ($P > .05$). Hyaluronidase did not mimic these actions of vasopressin.

The scientific literature is replete with studies on the actions of vasopressin (ADH) on transport systems. Ussing and Zerahn (*1*) demonstrated its stimulatory effect on the active sodium transport in frog skin and presented evidence for a similar action on the passive sodium flux. With the isolated urinary bladder of the toad (*Bufo marinus*) Leaf and Dempsey (*2*) showed that vasopressin increased active sodium transport, but they were unable to establish an effect on the passive flux. Sawyer (*3*) and others have explored the ability of this hormone to promote

Table 1. Effect of vasopressin (ADH) on the permeability of mesentery to $Rb^{86}$ and $P^{32}$ at 38°C.*

| ADH (milliunit/ml) | Change in $K$ (percent of control) | |
|---|---|---|
| | $P^{32}$† | $Rb^{86}$‡ |
| 100 | +132 | −33 |
| | + 93 | −24 |
| | + 46 | −19 |
| 10 | +113 | −13 |
| | + 52 | − 9 |
| | + 41 | − 4 |
| 1 | + 29 | − 7 |
| | + 68 | − 6 |
| | + 36 | 0 |
| 0.1 | + 32 | |
| | + 27 | |
| | + 5 | |

* Each pair of values represents the mesentery of a different rabbit. † Every value for $P^{32}$ except the last is significantly different from zero at the .01 level. ‡ The first three $Rb^{86}$ values represent changes significant at the .01 level. Other $Rb^{86}$ values are not significant ($P > .05$).

osmotic water transfer across the bullfrog bladder wall, an action that proved to be independent of the effect of vasopressin on active sodium transport. In the mammalian kidney it has been postulated that vasopressin enhances urine concentration by making the collecting ducts more permeable to water and so favoring the passive reabsorption of water into the hyperosmotic interstitial fluid (*4*).

The isolated rabbit mesentery lends itself well to an investigation of passive ion fluxes (*5*). This tissue is a symmetrical membrane consisting of two indistinguishable layers of mesothelium separated by a layer of loose areolar connective tissue. Because of its symmetry in terms both of structure and embryogenesis, no transmesenteric electical potential is believed to exist under the conditions of these experiments. When the tissue is mounted in a simple diffusion cell without hydrostatic or osmotic gradients, the ions $Rb^{86}$ and $P^{32}$ orthophosphate migrate independently across the mesentery with a kinetic pattern that is generally associated with passive diffusion (*6*). The equation that describes the transfer of each isotope is

$$dQ/dt = KA(C_1 - C_2)$$

where $dQ/dt$ represents the instantaneous ion flux, $C_1 - C_2$ the difference in tracer concentration across the membrane, $A$ the area of the membrane, and $K$ its permeability coefficient. Values of the latter (*6*) are proportional to the slopes of straight lines like those in Fig. 1. One surface of the mounted mesentery was superfused with a solution containing tracer amounts of the two isotopes (source solution) and an appropriate concentration of vasopressin. The other surface was bathed with a solution of identical composition, except that initially it contained no isotope or hormone (sink solution). Since isotopes and hormone concentrations in the source solution were held constant, ion transfer was indicated only by the rising radioactivity of the sink solution. Relevant technical details, including procedures for determining the activities of the individual isotopes in the mixture, have been described elsewhere (*6*). In all experiments Krebs-Ringer bicarbonate ($p$H = 7.4) was used as the base solution to which were added radioisotopes and, when appropriate, vasopressin (*7*).

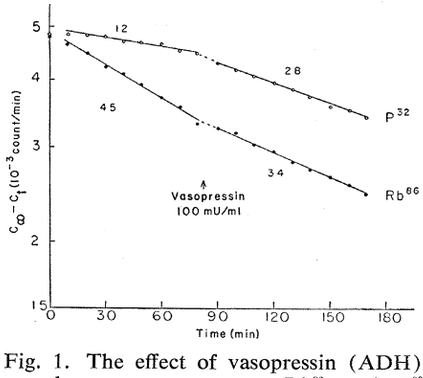


Fig. 1. The effect of vasopressin (ADH) on the contemporaneous $Rb^{86}$ and $P^{32}$ fluxes at 38°C. The concentration difference across the membrane ($C_\infty - C_t$) is plotted on a logarithmic scale. At the time indicated by the arrow, ADH (100 milliunit/ml) was added to the source solution and maintained thereafter. The numbers above the $P^{32}$ lines and below the $Rb^{86}$ lines are the calculated permeability coefficients $K$ (cm $min^{-1} \times 10^3$).

As can be seen in Fig. 1, the addition of vasopressin (100 milliunit/ml) after a suitable control period caused a prompt and sustained effect. Specifically, the anion flux increased while the cation flux decreased. In Table 1 several such experiments are summarized. With lower concentrations of the hormone both the $Rb^{86}$ and $P^{32}$ responses were less marked. With one exception all of the changes in $K$ for $P^{32}$ proved to be statistically significant. The $Rb^{86}$ effect lost significance at a vasopressin concentration of 10 milliunit/ml, although the tendency for a decrease in the $K$ value was still present. These permeability responses to vasopressin constitute what appears to be a unique pattern. Of more than a dozen neurohormones, drugs, and metabolic inhibitors tested in this system (*6*), vasopressin is the only substance that has caused a reduction in the $Rb^{86}$ permeability coefficient.

Ginetzinsky (*8*) and Dicker and Eggleston (*9*) have proposed that the antidiuretic hormone acts in the kidney by releasing hyaluronidase which increases renal tubular permeability to water. When tested on rabbit mesentery in concentrations of 10 and 100 VRU/ml, hyaluronidase did not mimic vasopressin but made the membrane more permeable to both ions. It seems certain that the action of vasopressin on the permeability of isolated mesentery is not mediated through activation of endogenous hyaluronidase. Leaf (*2*), working with the toad urinary bladder, also failed to show any similarity between the actions of vasopressin and hyaluronidase.

It has been suggested that the effect of vasopressin in promoting passive water reabsorption in the mammalian kidney may be due to the opening of pores. Since the migration of $Rb^{86}$ and $P^{32}$ across rabbit mesentery appears to be a passive process, one might predict that the number or size of channels available for diffusion would be increased by vasopressin. However, its ability to discriminate between these two tracers is impossible to explain solely by an alteration of pore geometry. The action of vasopressin on the water flux across mesentery has not yet been established. Effects on water transport may or may not prove to be consistent with a pore hypothesis (*10*).


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