the RNA-containing Rous sarcoma virus in 48-hour-old primary cultures of chick fibroblast cells. The present data with IUdR indicate, as did Temin's findings with Actinomycin D, that replication of RSV is somehow associated with DNA-synthesis. The mode of IUdR action in the described test system is not yet known.

Interpretation of data is complicated by the fact that standard stock preparations of RSV, and presumably ours, contain Rous associated virus (RAV) which is serologically identical with RSV(11,12). Although DNA was shown to increase in RAV-infected chick fibroblast cells, there is no definite information on whether this virus is a DNA virus (13). RAV is known to assist in the multiplication of RSV in transformed cells, while RSV itself causes malignant transformation, but no production of complete virus(14), so that RSV may be considered an incomplete or defective virus particle.

The data indicate that IUdR does inhibit RSV; for example, no tumors formed after inoculation of the extracellular or intracellular fluids from IUdR-treated (256 μg/ml) cells. It can be assumed that IUdR-treatment of infected cells prevented the production of tumor-inducing RSV particles. Because of the inter-relationship, RSV multiplication could presumably be affected by drug action on RAV. No attempt was made, however, to assay the stock virus or cell cultures specifically for incomplete and complete RSV or RAV. Consequently, it cannot be determined whether RAV, if present, was affected by IUdR.

The ability of IUdR to suppress the malignant transformation of chick fibroblast cells infected with RSV is assumed to reflect its activity as an inhibitor of the replication of RSV (tumor-inducing) particles.

Summary. IUdR, added to primary chick fibroblast cultures at the time of RSV infection, not only inhibits virus multiplication but also appears to suppress the malignant transformation of infected cells.

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Effects of Angiotensin on the Toad Skin. (29379)

BENJAMIN H. BARBOUR,* JOHN R. GILL, JR. AND FREDERIC C. BARTTER
Clinical Endocrinology Branch, National Heart Institute, National Institutes of Health, Bethesda, Md.

Whereas angiotensin generally produces a decrease in urine volume and in sodium excretion, it occasionally produces an increase in both. The mechanism responsible for these changes may entail a direct action of angiotensin on the renal vasculature(1) or on the tubule to inhibit the diffusion of water and/or the transport of sodium(2,3). Layssac *et al*(4) found that angiotensin produced an inhibition of sodium transport in isolated kidney slices, and proposed that angiotensin is concerned with a renal auto-regulatory

* Present address: Univ. of Southern California, School of Med., Los Angeles.

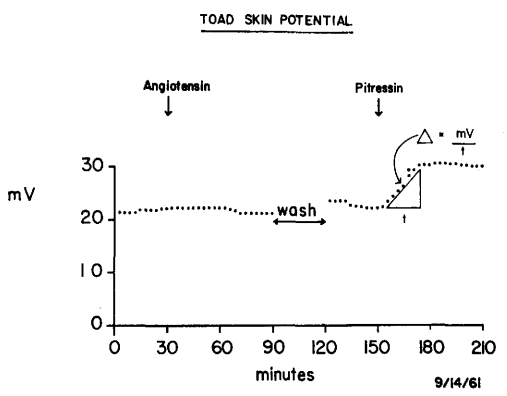

FIG. 1. Effect of angiotensin and of pitressin on potential of toad skin.

mechanism, balancing glomerular and tubular function through its combined effect on arteriolar constriction and on tubular sodium transport. Peters(5) has shown that in the rat angiotensin has a strong natriuretic effect. In the present study, the toad skin was selected to investigate the effects of angiotensin on the movement of sodium across a biological membrane. By analogy, the results may help to explain the effect of angiotensin upon renal tubules. It was found that angiotensin has no influence on short circuit current or potential, indicating that angiotensin has no influence on the transport of sodium across the toad skin. Furthermore, this lack of effect was not a result of rapid destruction of angiotensin by the toad skin.

Methods. The abdominal skin from a pithed toad, *Bufo marinus*, was dissected and washed in Ringer's solution (solution ionic content, KCl 2 mM; NaCl, 111 mM; $NaHCO_3$, 2.4 mM). The clean skin was mounted between 2 lucite chambers; potential difference and short circuit current across the skin were measured according to the techniques of Ussing and Zerahn(6). Each toad had been maintained in a moist medium for at least 10 days prior to killing. After either resting potential or current had become stable, pitressin (20-200 mU/ml) was added to each side of the bath and the changes in potential or current was recorded for 30 minutes. Measurements after pitressin were obtained by dividing current or voltage change by time required to establish "plateau" value (Fig. 1). The bath was then washed out with Ringer's solution and after current or potential had become stable, valine 5, asparagine-beta amide-angiotensin II, Ciba (40-800 mμg/ml) was added to each side of the bath. Potential and/or current was observed for at least one hour following addition of angiotensin. In some cases angiotensin was added first (Fig. 1).

To study the possibility of inactivation of angiotensin by the toad skin, 25 ml of Ringer's solution containing 1 μg/ml of angiotensin was incubated with the abdominal skin of a toad at 37°C for one hour. Samples of the solution were assayed in a vagotomized, pentolinium treated rat by measuring the carotid arterial blood pressure response.

Response. The results are shown in Table I. After the skins were mounted, the mean potential and current were 18 mV and 70 μamps, respectively. During the first 30 minutes after mounting the resting toad skin potential decreased rapidly at an average rate of —0.106 mV/min (N-8). However, during the second 30 minutes after mounting (control period) the skin potential was stable, changing at an average rate of only —0.023 mV/min. In each experiment pitressin was added either just before or after angiotensin, to show that each skin was re-

TABLE I. Effects of Angiotensin and of Pitressin on Potential and Short Circuit Current.

	Control	After angiotensin	After pitressin
	Potential, ΔMV/min		
Mean	−.023	−.064	+.390
Range	.00 to −.100	.000 to −.160	+.280 to +.530
n = 8		P ≥.1	P <.01
	Current, $\Delta\mu$amp/min		
Mean	−.097	−.060	+4.440
Range	−.290 to +.160	−.160 to +.170	+1.660 to +9.800
n = 6		P ≥.1	P <.01

sponsive. Addition of pitressin caused the potential to increase rapidly at a mean average rate of +0.390 mV/min. There was no change in potential for 30 minutes after addition of angiotensin (mean change, —.064 mV/min, P>.1).

During the control period the short circuit current decreased at a mean rate of —0.097 μamp/min. When the pitressin was added, the short circuit current increased at a mean rate of +4.44 μamp/min; after angiotensin was added, the mean rate of change was —0.06 μamp/min. The latter was not significantly different from the control figure (P>.1) (Table I).

Incubation of a solution of angiotensin for 30 or 60 minutes with toad skin caused no loss of potency of the angiotensin, as judged from the result of assays in a vagotomized, pentolinium treated rat. Finally, when pitressin was added to the bath containing angiotensin, an increase of potential occurred in the same manner as when pitressin was added alone.

Discussion. The difficulties encountered in defining the precise site of action of a given substance in the intact mammalian kidney have led numerous investigators to select isolated tissues for study. Angiotensin is known to affect urinary excretion of water and sodium. Although these effects may be secondary to the well-established vaso-constrictive action of angiotensin, a direct tubular effect is by no means excluded. The toad skin mimics the renal tubule in its ability to transport sodium actively and to respond to pitressin by allowing water to move more freely. It is likely that its responsiveness to a given substance will be similar to the responsiveness of the renal tubule; thus, the behavior of the toad skin may, by analogy, give insight into the behavior of the renal tubule. Changes in active transport of sodium can be calculated from measurements of the short circuit current across the membrane(6). In the studies reported here, angiotensin did not influence either current or potential. The lack of influence was seen when angiotensin was used with pitressin or alone: angiotensin did not block the action of pitressin or have an independent effect on the toad skin.

The possibility that the toad skin might destroy angiotensin, and thereby mask its effect was investigated by measuring the residual pressor activity after incubation of the toad skin with angiotensin. Loss of activity did not occur within 60 minutes. Thus, the lack of an effect on the toad skin is not a result of destruction of the peptide.

These data support the hypothesis that the characteristic antidiuresis and sodium retention that are produced by angiotensin are solely the result of effects on the renal vascular tree. The natriuresis which occurs with angiotensin under certain conditions(2,7,8) may represent an abnormal response of renal blood vessels with relatively greater vasoconstrictor action at one site within the kidney (*e.g.*, that supporting reabsorption) than that at another (*e.g.*, glomerular efferent arteriole). They do not, however, exclude the possibility of a direct effect on the mammalian renal tubule(3).

Summary. Angiotensin, added alone or in conjunction with pitressin, did not influence sodium transport by the toad skin. Incubation with toad skin did not destroy the pressor effects of angiotensin. These results support the concept that angiotensin produces changes in sodium and water excretion through its action on the renal vascular system.

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