References and Notes

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4. Unpublished observations.
5. This has been done by Curd and Davey [*Brit. J. Pharmacol.* **5**, 25 (1950)] with Antrycide in rabbits.
6. Visking dialysis tubing, flat width 0.390 inch, average pore radius 24 Å.
7. By J. M. Vandenbelt of these laboratories.
8. O. D. Bird and A. J. Glazko, Parke, Davis & Co., Ann Arbor, Mich., unpublished data.

27 May 1963

Angiotensin Pressor Inhibition by Aldosterone in the Rabbit

Abstract. *Rabbits pretreated with aldosterone exhibited lessened response to the pressor agent angiotensin. This observation may help to explain the absence of hypertension in certain instances in which both the formation of angiotensin and the secretion of aldosterone are above normal.*

In this study we show that aldosterone dampens the pressor effect of angiotensin in the rabbit. Angiotensin, a polypeptide resulting from the interaction of kidney renin and a plasma protein, produces an increase in the secretion of aldosterone by the adrenal gland (*1*). We reported previously that aldosterone, in turn, has a blocking action on the effect of angiotensin by increasing the renin content of the rat kidney (*2*). The observation, as well as examples of the formation of increased angiotensin in the absence of hypertension in man and experimental animals, led us to the supposition that aldosterone might have inhibitory effects on angiotensin. Rabbits of a New Zealand strain were used in experiments to test this hypothesis.

On two successive days we injected adult rabbits subcutaneously with 40 μg/kg of aldosterone (Ciba *d*-aldosterone, diluted to 0.1 mg/ml in 33-percent ethyl alcohol). The control animals were given the solvent only. Several hours after the second injection, we anesthetized the animals with 20 mg/kg sodium pentobarbital (Nembutal, Abbott) and catheterized the femoral artery for recording blood pressure by the Sanborn electromanometer system. In the first group of experiments, graded doses of angiotensin II (1-L-asparaginyl-5-L-valyl angiotensin octapeptide, Ciba) were given intravenously, and the amount necessary to produce rises of 20 and 30 mm-Hg in the blood pressure was determined by titration. The average dose of angiotensin which produced these responses is given in Table I. Eight rabbits treated with aldosterone and seven control rabbits were used, but only five of the control rabbits were studied at the response level of 20 mm-Hg. The amounts of angiotensin required to produce responses of 20 and 30 mm-Hg were significantly greater in the animals pretreated with aldosterone than in the control animals.

We then used another group of five rabbits pretreated with aldosterone and five controls to determine the maximum responses in blood pressure caused by continuous infusion of angiotensin over the range of 0.2 to 1.2 μg/kg per minute. The infusion rate of a solution containing 25 μg percent of angiotensin II in 0.9 percent sodium chloride was kept constant at each dose level for a period of 5 minutes, the flow being controlled with a Sigma motor peristaltic pump. For each animal the response to the various dosages was taken as the maximum rise in blood pressure during the last minute of the infusion period. (Three control animals and two animals treated with aldosterone developed cardiac arrhythmias and respiratory distress at the 1.2 μg/kg dose rate, necessitating interruption of the infusion.) Figure 1 is constructed from the average responses in the ten animals. As can be seen, the animals treated with aldosterone were consistently less responsive to the infusion of angiotensin than the control animals. Findings similar to these have been noted in the rat and will be reported in a more extensive treatment of this subject elsewhere.

Table 1. The effect of pretreatment of rabbits with aldosterone on the response of the blood pressure to angiotensin. Logarithmic transformation of the individual doses was used to stabilize the variance. The figures in parentheses indicate the number of animals in each treatment.

Rabbits	Mean dose ($\bar{x}$) angiotensin* (μg/kg)	*t*-test	*P* (one tail)
20 mm-Hg rise in blood pressure			
Treated with aldosterone (8)	0.245	2.091	<.05
Controls (5)	0.134		
30 mm-Hg rise in blood pressure			
Treated with aldosterone (8)	0.480	3.213	<.005
Controls (7)	0.258		

* The average amount required to cause the indicated rise in blood pressure.

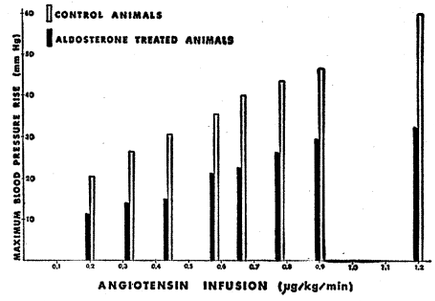

Fig. 1. Mean responses of rabbits treated with aldosterone and of control rabbits to continuous infusion with angiotensin II. The difference in the level of response of the blood pressure in the two groups is statistically significant ($P < .005$) as tested by comparison of the *y*-intercepts of the two fitted-least-squares regression lines.

In contrast to the observed effect of aldosterone on angiotensin, Raab *et al.* (*3*) noted previously that in man, the pressor effect of norepinephrine was increased after treatment with synthetic deoxycorticosterone, a salt-retaining steroid. Davis *et al.* (*4*) observed a decreased blood pressure response to angiotensin in dogs with constriction of the inferior vena cava. These animals had an increased secretion of aldosterone and presumably increased blood levels of angiotensin, yet their blood pressures were in the normal range. An increase in the activity of angiotensinase in the plasma of patients who had secondary aldosteronism was observed by Hickler *et al.* (*5*), suggesting that aldosterone may cause an increase in the activity of plasma angiotensinase and in this way may lessen the capacity of the blood pressure to respond to angiotensin.

Despite the intriguing possibility suggested by the observation of Hickler *et al.*, the actual mechanism by which aldosterone lessens the full effect of angiotensin is uncertain at present. Nevertheless, the noted antagonism of aldosterone to angiotensin provides another example of a substance acting, in turn, to inhibit or to modulate the effect of its stimulant (*6*).

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6. Supported by grants from the Orange County Heart Association, the Southern California Chapter, National Kidney Disease Foundation, and the U.S. Public Health Service, grant HE-08100-01. One of us (Y.J.K.) was an established investigator of the American Heart Association while this work was in progress. Angiotensin II (Hypertensin) was kindly supplied by the Ciba Pharmaceutical Company, Summit, N.J.

6 June 1963

Antibody Combining Site: The B Polypeptide Chain

Abstract. *The antibody molecule consists of several polypeptide chains. Peptides, which appear to have been derived from the binding region of the rabbit-antibody molecule directed against* p*-azobenzenearsonate, have been isolated. The particular polypeptide chain from which these peptides are derived has now been identified as the B chain described by Fleischman, Pain, and Porter.*

Gamma-globulin molecules of several animal species consist of several polypeptide chains linked by disulfide bonds. A question of interest has been the relation of the combining sites of the antibody molecule to these chains. Edelman and Benacerraf (*1*) separated what they called H and L chains from the antibody molecule and indicated that the L chain seems to be important in the antibody-combining site. Fleischman, Pain, and Porter (*2*), by a different method, separated the molecule into chains A and B which appear to be, respectively, the H and L chains described by Edelman and Benacerraf and they suggested that antibody activity resides in the A chains. Our findings are that a peptide apparently from the binding region of rabbit antibody against *p*-azobenzenearsonate (anti-R_p) comes from that part of the antibody molecule which falls into fraction B of Fleischman, Pain, and Porter (*2*). This was shown through use of the "paired-label" technique (*3*). In applying this technique, one portion of antibody is iodinated with I^{131}-labeled iodine in the presence of hapten and another portion is iodinated (to the same extent) with I^{125}-labeled iodine in the absence of hapten. The preparations are mixed and digested and the peptides are isolated. Any peptide for which the ratio of the two isotopes differs from that in the original mixture is derived from a portion of the molecule in which the antibody-hapten complex has affected the iodination.

A solution of 30.1 mg of specifically purified rabbit anti-R_p (*4*) antibody in 1.9 ml of borate buffer of *p*H 8 was added to 1.5 ml of 1*M* glycine buffer (*p*H 9) and iodinated at 0°C by the dropwise addition of 2.80 ml of the glycine buffer containing 6.5 μmoles of I^{125}-labeled hypoiodite (1.4×10^8 count/min). Another portion of the same antibody solution containing in addition 60 μmoles of sodium benzenearsonate was treated similarily except that the hypoiodate was labeled with I^{131} (4.9×10^8 count/min). After 2 hours at 0°C, both preparations were individually transferred to dialysis bags. Dialysis was carried out at 5°C against 2 liters of saline buffered with borate; the dialyzate was changed twice daily for 3 days. During the second dialysis, 0.25 g of potassium iodide was added to the outer solution. The I^{125}-labeled preparation contained 25.9 iodine atoms per molecule and the I^{131} preparation, 26.1 atoms per molecule.

Portions of each solution equivalent to 19 mg of protein were mixed, concentrated by pervaporation, and then dialyzed against 0.55*M* tris-HCl buffer of *p*H 8.2. The protein concentration was adjusted to 20 mg per ml. The reduction (in 0.75*M* mercaptoethanol), alkylation, and fractionation by chromatography (1*M* propionic acid and a 51 × 2.8 cm column of Sephadex G-75) were all carried out according to Fleischman *et al.* (2). The results are shown in Fig. 1 where the radioactivity of I^{131} in counts per minute per tube is shown as a function of the tube number. Seventy-four percent of the I^{131} was in tubes 21 to 28 and 26 percent in tubes 29 to 45. Tubes 22 and 23 were pooled as fraction A_1, tubes 24 to 26 as fraction A_2, and tubes 30 to 40 as fraction B. The fractions were each dialyzed against 9 volumes of distilled water. Essentially no radioactivity appeared in the outer solutions during dialysis. The solutions were lyophilized and the residues were taken up in a volume of 0.5*M* formic acid containing 0.33 mg of pepsin per milliliter, resulting in a calculated concentration of 10 mg of iodinated protein per milliliter in each case. The solutions were incubated at 37°C for 16 hours. Dialyzed and lyophilized portions of the protein before and after reduction and alkylation were also digested.

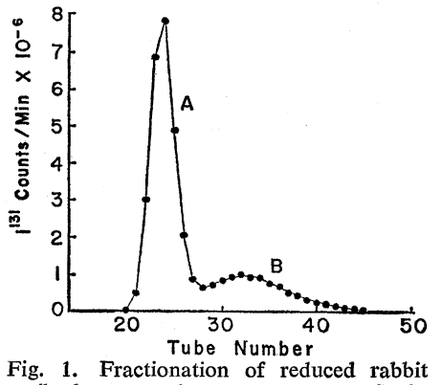

Fig. 1. Fractionation of reduced rabbit antibody to *p*-azobenzenearsonate on Sephadex G-75 in 1*M* propionic acid. Four ml fractions were collected. The antibody had been "pair-iodinated" in the presence and absence of hapten before reduction.

The digests were lyophilized and the peptides were separated by paper chromatography and high-voltage paper electrophoresis (*5*). Radioautographs were prepared and areas on the papers corresponding to spots on the radioautographs were cut out. The concentration of I^{125} and I^{131} (in counts per minute) on each piece of paper were determined and the ratio of the amount

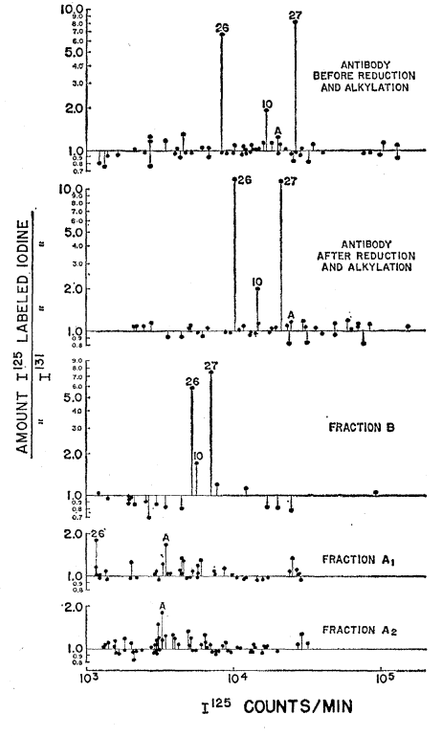

Fig. 2. Iodinated peptides derived from "pair-iodinated" rabbit antibody to *p*-azobenzenearsonate. Each dot represents a spot on the radioautograph.