J. Physiol. (1940) 98, 314–335 612.463:612.462.1

THE EFFECT OF RENIN ON URINE FORMATION[1]

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(*Received* 4 *March* 1940)

In the course of some experiments [Pickering & Prinzmetal, 1938] on renin, the protein-like pressor substance first extracted from the kidney by Tigerstedt & Bergman [1898], it was noticed that the injection of strongly pressor renal extracts often caused unanaesthetized rabbits to void abundant pale urine. In view of the implications on the function of renin which a renal action might have, it seemed worth while to investigate this point in greater detail, and this paper records the results obtained with renal extracts containing renin in preliminary experiments on anaesthetized rabbits and an unanaesthetized dog, and in more detailed observations on unanaesthetized rabbits.

The conclusion which we shall reach is that there is present in the kidney a substance which, injected into the circulation, profoundly modifies renal function. Our present evidence is that this substance is the pressor substance, renin, and we shall so term it in this paper, but it is to be mentioned that a pure preparation of this substance has not yet been obtained, and the possibility cannot be finally excluded that the renal effects are due to another constituent of kidney closely resembling renin in some physical and chemical properties.

Preliminary experiments

In six rabbits lightly anaesthetized with 0·15 g. sodium luminal per kg., in which a bladder cannula had been inserted an hour or more previously under ether anaesthesia, saline extracts of alcohol dried rabbit's kidney [Pickering & Prinzmetal, 1938] in amounts corresponding to 0·5–1 g. kidney were consistently found to raise arterial pressure and accelerate the flow of urine (Fig. 1). The same extracts boiled for 5 min.

[1] Work done on behalf of the Medical Research Council.

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had no effect on urine flow or arterial pressure. 4 mg. tyramine gave similar but more transient rise of blood pressure and smaller increases in urine flow.

Very similar experiments were described by Bingel & Claus [1910], who observed that in the rabbit, lightly anaesthetized with urethane, renal extracts raised arterial pressure and increased urine flow, the kidney volume rising simultaneously. The behaviour of the kidney is, however, greatly influenced by anaesthetics; we therefore proceeded to ascertain the effects of renin on an unanaesthetized dog, and in these observations we were fortunate to have the collaboration of Dr G. W. Theobald.

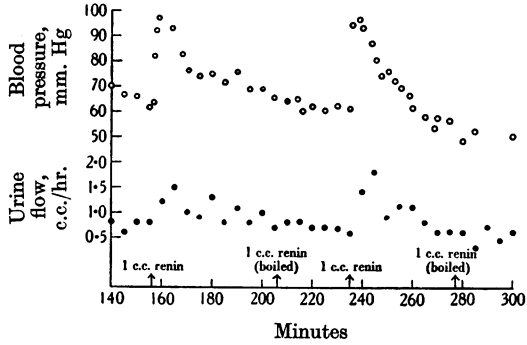

Fig. 1. Rabbit 2·0 kg. 10 Mar. 1937. 3 c.c. 10% sodium luminal injected subcutaneously and 60 c.c. water given by stomach tube at 10 a.m. Bladder cannula inserted under light ether anaesthesia at 11.30 a.m. Experiment begun at 1 p.m. Renin solution prepared by alcohol method, 1 c.c. being equivalent to 0·5 g. rabbit kidney.

The animal used was a bitch in which the urethra had been exposed by a previous perineal operation and in which urine flow was recorded from a catheter passed into and maintained in the bladder. She had been repeatedly used by Dr Theobald for the assay of posterior pituitary principle and was accustomed to the experimental procedure adopted. Adequate tissue hydration was secured by giving 250 c.c. water by stomach tube on the preceding evening and again on the morning of the experiment, before the response to renin was tested. Saline extracts of alcohol-dried rabbit's kidney, to some extent purified by half-saturation with ammonium sulphate and subsequent dialysis first against water and then against 0·9% sodium chloride, produced no increase in urine flow when injected intravenously in doses of 1–5 c.c. after the subsidence of water diuresis. Injected on the ascending limb of water diuresis 5 c.c. of this extract reduced urine flow from 250 to 15 c.c./hr., the effect lasting about 60 min. This antidiuretic action was abolished by heating the

extract for 2 hr. to 60° C., a procedure which has been previously found to inactivate renin [Pickering & Prinzmetal, 1938].

The preparation of rabbit's renin used in these experiments undoubtedly contained other protein and, to exclude the antidiuretic effect being a non-specific response to a foreign protein, extracts were made from dog's renal cortex and medulla, the former containing renin, the

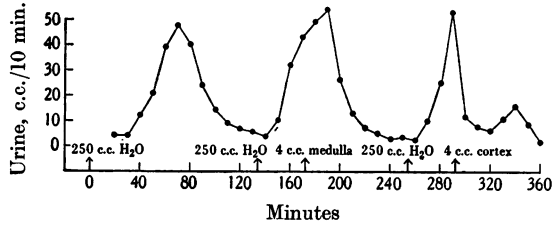

Fig. 2. Unanaesthetized bitch, 5 Jan. 1938. Urine from catheter retained in bladder. Chart showing diuresis following administration of 250 c.c. water by stomach tube, and the effects of extracts of medulla and cortex of dog kidney.

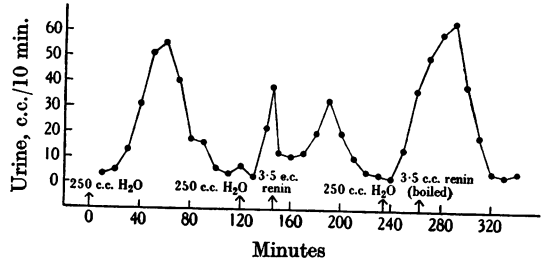

Fig. 3. Same animal as in Fig. 2. 7 Jan. 1938. Showing abolition of antidiuretic effect of cortical extract (renin) by boiling.

latter none. The fresh kidneys of a dog killed by bleeding were separated by dissection into cortex and medulla, the knife passing to the medullary side of the line of demarcation. The cortex was pulped in a mortar, treated with 2 c.c. absolute alcohol per g. for 5 hr. in the refrigerator, and filtered. The residue was dried at room temperature and the dry powder extracted with 5 c.c. 0·9% saline per g. The medulla was similarly treated. Tested on the blood pressure of an unanaesthetized rabbit, 1 c.c. of the extract of medulla was inactive, while 1 c.c. of the cortical extract produced a rise of 50 mm. Hg. The effects of these two preparations on the urine formation of the dog are shown in Figs. 2 and 3. It will be seen that 3·5 and 4 c.c. of extract made from cortex inhibited water diuresis for about 45 and 50 min. respectively, and that the effect was

abolished by boiling the solution for 5 min. No antidiuretic effect was observed from 4 c.c. extract of medulla.

Merrill, Williams & Harrison [1938] have found that renal extracts, prepared from pig's kidney by a method very similar to the alcohol method used by us, usually have a diuretic action in the unanaesthetized dog. They consider that the antidiuretic effect they occasionally observed was due to some substance other than renin in their extracts, but it is not clear on what evidence this opinion is based. In our experiments, the antidiuretic substance resembled renin in its insolubility in alcohol and in half-saturated ammonium sulphate, in its inability to pass through cellophane, in its instability at 60° or above, and its presence in cortex but not in medulla of kidney.

Although the behaviour of our dog may have been exceptional, the results are reported because they were clear-cut and because they indicate that further work in this species is desirable. It proved, however, more convenient to us to make a further investigation on the unanaesthetized rabbit.

OBSERVATIONS ON THE UNANAESTHETIZED RABBIT

(By G. W. Pickering)

Methods

Urine was obtained by catheter from adult male rabbits weighing 2–3·5 kg. and fed on a mixed diet of oats, bran and green stuff. In most rabbits a rubber catheter (size 6) proved satisfactory, but in some animals this consistently entered the seminal vesicle and in these a coudé gum elastic catheter of similar size was employed. Emptying of the bladder was ensured as a routine by massage of the hypogastrium, and by washing out the bladder with air. The bladder was washed out with 5 c.c. water in those experiments where creatinine clearance estimations were made and in a few other instances. Between catheterizations the animals were placed in wire cages small enough to make it difficult for the animal to turn round, and fitted with a drain to collect any urine which might be voided spontaneously, an event that proved infrequent.

The secretion of urine in the rabbit is greatly influenced by the degree of hydration and by the state of the alimentary canal, and we found that to obtain anything approaching consistent results it was necessary to starve the animals of food during the experiment, and for 12 hr. previously, and to allow access to about 200 c.c. of water during the night preceding the experiment; most of this was usually drunk.

Renin. The renin used in these experiments was a purified preparation made from rabbit's kidney in the following way. Fresh rabbit's kidney was pulped and treated with 2 c.c. of alcohol/g. for 24 hr. in the refrigerator. The residue separated by filtration was dried at room temperature, powdered and stored at 3° C. When about 50–100 g. had accumulated the powder was extracted with 10 c.c. physiological saline/g. for 24 hr. The extract was acidified to *p*H 4–5, and the precipitate centrifuged off. The clear yellow supernatant fluid was half-saturated with ammonium sulphate, and left for 24 hr. in the

refrigerator. The precipitate was separated by filtration, dissolved in water and dialysed through cellophane for 24–48 hr. till free from sulphate. Insoluble matter was separated by the centrifuge, and the supernatant fluid evaporated to dryness in a vacuum desiccator. A yield of about 0·7 g. of a pale yellow powder was so obtained from 250 g. fresh rabbit's kidney. This powder, kept in a sealed tube in the refrigerator, maintained its activity for several months. The dose for testing was dissolved in 1–1·5 c.c. physiological saline on the day of experiment.

The renin content of each preparation was assayed by a method previously described [Pickering & Prinzmetal, 1938] and is described in this paper in terms of units. Four preparations were used, having activities of between 0·3 and 0·6 unit per mg. One unit represents the amount of renin contained in 100 mg. of our standard power.

Chemical estimations on urine and blood. Chlorides were estimated in the urine by the modified Volhard-Harvey titration [Peters & Van Slyke, 1932], and in plasma by Claudius's [1922] method on 0·1 c.c. samples. Creatinine was estimated in urine and in the Folin-Wu filtrate of plasma by the colorimetric methods of Folin [1914] and Folin & Wu [1919]. Urea was estimated in the fresh urine by the urease method [Van Slyke & Cullen, 1916] and by the hypobromite method.

Blood samples of 3–5 c.c. were taken into powdered heparin from incisions in an ear vein, the plasma being separated by the centrifuge.

Creatinine clearance. To raise the plasma creatinine level above the accepted requisite minimum of 7 mg./100 c.c., 1–1·5 g. of creatinine in 5 or 10% solution was injected intravenously at least 30 min. before the first clearance estimation was made. Blood samples were obtained either in the middle of each period of urine collection, or at the beginning, middle and end of the total experimental period, the levels of plasma creatinine corresponding to the mid-point of urine collection being obtained from the curve relating plasma creatinine concentration to time.

Results

The effect of renin when the rate of urine formation is small

In these experiments, 100 c.c. water was given by stomach tube at 10 a.m.; 4–6 hr. later, when the urine flow was falling and had reached rates between 1·0 and 15 c.c./hr., renin was injected intravenously. Urine was collected at ½ hr. intervals.

No definite effect on urine flow was observed from doses of 0·1–0·6 unit of renin. Doses of 1 unit or more were unfailingly and often conspicuously diuretic. Thus, 1 unit of renin increased urine flow in each of six experiments on three rabbits, the average increase being fourfold in the first and twofold in the second ½ hr. A total of 2·7 units (8 mg. of the preparation used), injected in two equal doses at 15 min. interval, increased urine flow to a much larger extent in each of twelve experiments on eight rabbits, the average increase being tenfold in the first, thirteenfold in the second, and twofold in the third ½ hr. periods after the first injection. The diuresis following 4 units (12 mg.), injected in three equal doses at 15 min., was very similar in degree and duration. The rates of urine flow observed after the injection of the larger doses of renin were often enormous, 60–100 c.c./hr. being common. While the degree of

diuresis is evidently dependent upon the dose of renin, the method as we have experienced it in the rabbit is quite unsuited for assay. For, in a single animal on different days, the size of the response produced by a given dose of a single preparation varies very considerably.

The factor in these renal extracts which is diuretic in the unanaesthetized rabbit resembles the pressor substance renin in being destroyed by boiling. Thus, the same preparation as used in the experiments just described, when boiled for 5 min., failed to give any diuretic response when injected in doses corresponding to 1 unit (three experiments on three rabbits), 2·7 units (four experiments on three rabbits) and 4 units (three experiments on three rabbits) of the unboiled solution. Boiling, however, removes protein and it seemed possible that the diuretic effects observed with the unboiled preparations were due to a non-specific foreign protein. Accordingly, an extract was made from fresh rabbit's liver by a method identical with that here used for the preparation of renin. Similar or larger quantities by weight (8 and 16 mg.) of this preparation from liver dissolved in similar volumes of saline and injected intravenously produced no diuresis in five experiments on five rabbits. Finally, in collaboration with Dr G. C. Butler, we have found that the renin preparation described can be freed of 90% of its nitrogen for a given pressor activity, by adsorption on kaolin at acid, and elution at alkaline, reaction. Such a purified preparation of renin is also powerfully diuretic in the unanaesthetized rabbit (rabbit H, Table I). This is the present evidence for the identity of the pressor and diuretic substances present in rabbit's kidney.

The urine secreted during the diuresis following renin injection is pale, and contains protein (about 0·1%). Its *p*H is similar to that of the urine secreted before the injection of renin. The other constituents may now be examined in detail.

Chloride excretion. One of the most conspicuous changes in the composition of the urine secreted after renin injection is in chloride (Fig. 4). Table I shows the changes in chloride excretion in eight experiments when a total of 2–4 units of renin were injected in two equal doses at 15 min. intervals 5–6 hr. after ingestion of 100 c.c. water. Unlike the diuresis following ingestion of water, the diuresis following renin injection is associated with a rise in the percentage of chloride in the urine, which tends to reach a value of between 0·5 and 1% NaCl. These effects on chloride excretion are abolished by boiling the solution of renin for 5 min. (Fig. 5) and are not obtained with the liver extract previously described (Fig. 6). They are still obtained when renin is further purified

by kaolin adsorption (Table I). Changes in urinary chloride thus seem to represent an integral part of the kidney's response to renin in the anaesthetized rabbit.

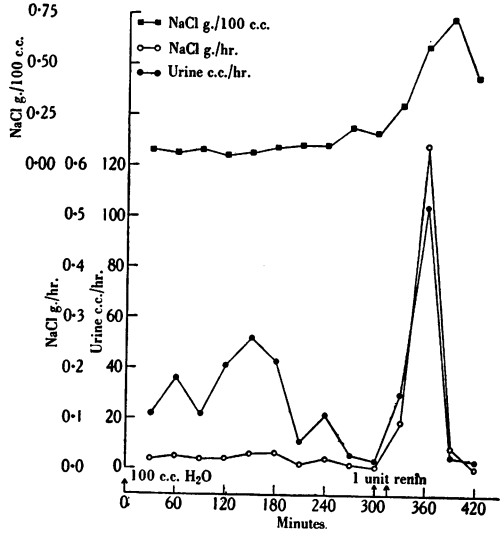

Fig. 4. Unanaesthetized rabbit H (3·2 kg.). Shows the changes in urine flow and chloride excretion induced by the ingestion of 100 c.c. tap water at 0 min. and by intravenous injection of 1 unit of renin (3 mg. of the preparation dissolved in 1 c.c. saline) at 300 and again at 315 min. In this and subsequent figures the black discs represent the rate of urine flow in c.c./hr., open circles chloride excretion in g. NaCl/hr., and black squares the urinary chloride in g. NaCl/100 c.c.

TABLE I. Showing the chloride excretion in the ½ hr. period before, and in the two ½ hr. periods after, intravenous injection of renin. 100 c.c. water was ingested 5–6 hr. previously.

Rabbit	Renin units	Urine flow, c.c./hr.		Cl. excretion, NaCl mg./hr.		Urine NaCl, g./100 c.c.	
		Before	After	Before	After	Before	After
H	2	2·6	30·4, 104	4	94, 640	0·17	0·31, 0·6
H	*	9·6	55·6, 104	4	306, 580	0·04	0·67, 0·56
193	2	15·2	20, 41·4	4	94, 210	0·03	0·47, 0·5
194	2	2·4	24, 46	8	176, 264	0·33	0·73, 0·57
191	2·7	1·4	3·8, 9·0	2	8, 72	0·14	0·24, 0·8
210	2·7	3·4	71, 78	16	414, 470	0·47	0·59, 0·6
210	4·0	3·0	29·0, 59·4	6	192, 360	0·2	0·67, 0·6
133	2·7	3·4	55, 19	24	460, 184	0·71	0·83, 0·97
133	4·0	1·8	36, 28	10	312, 260	0·55	0·87, 0·93

* This preparation had been further purified by adsorption on kaolin, and its renin content was not precisely assayed.

The known influence of the plasma chloride level on the urinary excretion of chloride (Ambard & Weill, 1912; Aitken, 1929) suggested the

possibility that renin might act by raising the concentration of chloride in the plasma. The plasma chlorides were therefore estimated before renin and at the height of the succeeding diuresis in six other experiments made primarily to investigate creatinine clearance. The results are shown in

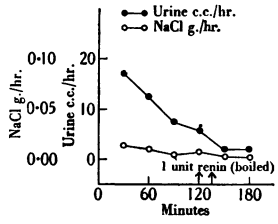

Fig. 5.

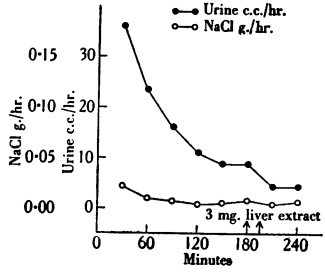

Fig. 6.

Fig. 5. The same rabbit as in Fig. 4. 100 c.c. tap water given by stomach tube 3 hr. previously. At 120 and again at 135 min. intravenous injection of 1 c.c. of a solution of renin, boiled for 5 min., the solution before boiling containing the same dose of the same preparation used in the experiment illustrated by Fig. 4.

Fig. 6. Rabbit as in Figs. 4 and 5. 100 c.c. tap water by stomach tube 3 hr. previously. Intravenous injection of 3 mg. liver extract in 1 c.c. saline at 180 and again at 195 min.

Table II. It is clear from this table that renin, in the doses here employed, produces no significant change in the plasma chloride concentration, and we may infer that the change in urinary chloride content is not a consequence of change in plasma chloride. It is also apparent that the

TABLE II. Plasma and urine chloride before and after injection of 3 units (8 mg.) renin

	Plasma, NaCl g./100 c.c.		Urine, NaCl g./100 c.c.	
Rabbit	Before	After	Before	After
R	0·63	0·68	0·11	0·68
H	0·67	0·66	0·3	0·72
133	0·67	0·65	0·48	0·68
H	0·63	0·62	0·16	0·68
211	0·58	0·58	0·09	0·62
193	0·58	0·58	0·07	0·58

chloride content of the urine after renin injection approximates to, but tends to exceed slightly, the plasma chloride. The significance of this will be discussed later.

Excretion of sodium and other inorganic radicles. In two experiments Dr M. Maizels and Dr A. Farmer kindly estimated the urinary content of sodium, by the method described by Salit [1932], total base, by Stadie and Ross's method [Peters & Van Slyke, 1932], and chloride. The results,

TABLE III. The content of basic and acid radicles (in milliequivalents) of urine secreted in one ½ hr. period before and two ½ hr. periods after the injection of 2·7 units renin

Rabbit	Urine specimen	Volume c.c.	Sodium m.-equiv./l.	Sodium m.-equiv.	Other basic radicles m.-equiv./l.	Other basic radicles m.-equiv.	Chloride m.-equiv./l.	Chloride m.-equiv.	Other acid radicles m.-equiv./l.	Other acid radicles m.-equiv.
133	Before renin	4·7	63	0·295	115	0·54	87	0·41	91	0·43
	After renin (1)	18	154	2·76	49	0·88	149	2·68	54	0·97
	After renin (2)	14	170	2·37	58	0·82	159	2·23	69	0·97
210	Before renin	8·3	56	0·46	9·1	0·07	23·1	0·19	42	0·35
	After renin (1)	14·5	128	1·86	13·3	0·19	114	1·65	27·3	0·40
	After renin (2)	29·7	122	3·62	5·2	0·15	103	3·06	24·2	0·72

expressed in milliequivalents (m.-equiv.), are shown in Table III, where the column "Other basic radicles" represents Total base − Na, and the column "Other acid radicles" represents Total base − Cl. From this table it will be seen that an increase in percentile and absolute excretion of Na parallels, and in both experiments slightly exceeds, the increased percentile and absolute excretion of Cl. By comparison, the changes in the excretion of other basic and acid radicles are small, and are chiefly in the direction of a fall in percentile and rise in absolute excretion, changes which may possibly be a reflexion of the increased volume of urine secreted in response to renin.

The plasma sodium was also estimated in these two experiments, and was respectively 155 and 154 m.-equiv./l. in rabbit 133, and 156 and 155 m.-equiv./l. in rabbit 210 before, and at the height of the diuresis after, renin injection. It seems clear from the experiment on rabbit 133 that the sodium content of the urine secreted in response to renin may slightly exceed that of the plasma, the behaviour of sodium, in this respect also, resembling that of chloride.

Urea excretion. The urea excretion in the urine was followed in seven experiments through the diuresis produced by ingesting 100 c.c. water and, after this had subsided, through the diuresis resulting from the injection of 2·5–3 units renin. The results are summarized in Table IV. It

TABLE IV. The effect of 2·5–3 units renin on urea excretion

Rabbit	Urine flow, c.c./hr. Peak water diuresis	Urine flow, c.c./hr. Before renin	Urine flow, c.c./hr. After renin	Urea excretion, g./hr. Peak water diuresis	Urea excretion, g./hr. Before renin	Urea excretion, g./hr. After renin
133	80	2·8	29	0·12	0·08	0·16
133	51·6	4·0	41	0·14	0·08	0·18
194	48	3·0	27·6	0·08	0·08	0·09
210	46	13·0	45·6	0·1	0·08	0·09
211	50	8·6	18·6	0·16	0·12	0·09
H	42·4	4·2	25·4	0·13	0·04	0·09
L	70	3·2	98	0·14	0·12	0·18

will be seen that the urea excretion was greater during the water diuresis than after its subsidence, and again increased during the diuresis following renin injection. There seems to be no significant difference between the increase in urea excretion produced by water and by renin, sometimes the one, sometimes the other being the greater. The conclusion drawn from these experiments is that the changes in urea excretion produced by renin are of an order to be expected from the increased flow of urine.

Creatinine excretion. The excretion of endogenous creatinine was followed during the waxing and waning of water diuresis and during the subsequent diuresis produced by the injection of 1–4 units of renin in nine experiments on four rabbits. Water diuresis was not accompanied by any constant or significant change in creatinine excretion. Renin diuresis, which was usually more intense than water diuresis, was accompanied by a slight but definite increase in creatinine excretion in four experiments, by no significant change in four experiments, and by a slight but definite fall in one experiment. It is evident, therefore, that the changes in creatinine excretion during renin diuresis are inconstant and often insignificant.

Creatinine clearance. The most obvious mechanism by which renin could act as a diuretic would be through the transmission of the rise in arterial pressure to the glomeruli, with a consequent acceleration in the rate of glomerular filtration. It is generally accepted that in most animals the glomerular filtration rate may be measured by the rate at which inulin is removed from plasma to urine. In the rabbit, though not in man, creatinine also seems to be excreted in the urine entirely through the agency of glomerular filtration, and in this animal Kaplan & Smith [1935] have shown that inulin and creatinine clearances are identical at all rates of urine flow and at all levels of plasma creatinine between 6 and 125 mg./100 c.c. We have accordingly measured the effect on creatinine clearance of renin injected during the subsidence of water diuresis.

Rydin & Verney [1938] have clearly shown the disturbing influence of strong sensory stimuli on the secretion of urine, and we experienced at first some difficulty in obtaining a regular base line of urine flow and satisfactory responses to renin in these experiments, in which the taking of blood samples introduced a considerable degree of interference. We eventually found it essential to work with animals in which the main sensory nerves to one ear had been cut, the anaesthetic ear being used for all intravenous injections and blood sampling. Urine samples were obtained by catheter at $\frac{1}{2}$ hr. intervals, the bladder being washed out with

TABLE V. Showing the creatinine clearance in the ½ hr. periods before and after injecting 3 units (8 mg.) of renin, 2–6 hr. after the ingestion of 100 c.c. water

Rabbit	Urine flow, c.c./hr.		Creatinine clearance, c.c. plasma/min.		Urinary chloride, g. NaCl/100 c.c.	
	Before	After	Before	After	Before	After
H	7·4	28·0	25·3	25·7	0·16	0·68
193	20·0, 19·0	24·0	10·7, 11·3	11·3	0·09, 0·7	0·58
194	18	21·6, 18, 4·4	9·4	9·4, 8·1, 10·7	0·02	0·28, 0·11, 0·23
R	14·0	70·0	10·3	10·1	0·16	0·48
R	8·2	60·0	22·0	19·0	0·11	0·68
133	15·0, 6·0	45·8	17·2, 17·2	15·0	0·4, 0·53	0·83
210	4·4, 3·6	10·0	19·0, 16·2	18·3	0·19, 0·11	0·96

5 c.c. water. Table V summarizes the results obtained in experiments where the plasma creatinine remained above 7 mg. % during the entire experimental period; other experiments in which the creatinine had fallen below this figure gave essentially similar results but have been excluded because clearances at this plasma level would not be generally accepted as indicative of glomerular filtration rates. Kaplan & Smith [1935] have pointed out that in the rabbit the glomerular filtration rate, as measured by the inulin and creatinine clearances, varies with the rate of urine flow. It seemed, therefore, desirable to have fairly large as well as small rates of urine flow before the injection of renin, and the experiments in Table V include observations in which renin was injected at varying points on the descending limb of water diuresis from 2 to 6 hr. after the ingestion of 100 c.c. water. It will be seen that the characteristic changes in urine flow and chloride concentration were obtained in all experiments, though both changes in rabbit 194 were small, as were the changes in urine flow in rabbits 193 and 210. Nevertheless, the results in all rabbits agree in showing that when renin is injected during the subsidence of water diuresis, the increase in urine flow and urinary chloride in the succeeding ½ hr. is unaccompanied by any significant change in creatinine clearance.

Relationship of diuretic response to blood-pressure change

For technical reasons no determinations of blood pressure were made during those experiments in which urine was collected for measurement. As we have previously pointed out [Pickering & Prinzmetal, 1938] and since repeatedly confirmed, the response of a single unanaesthetized rabbit to a given dose of renin usually remains fairly constant from day to day, and in several animals we have therefore recorded the blood-pressure changes to the dose of renin used in the experiments on urine flow. It is to be remembered that the maximum rise in arterial pressure after

intravenous renin is reached in about 2 min., the rise then subsiding, at first rather rapidly, later more gradually, the total duration being of the order of ½–1 hr., depending on the dose injected. Examples will be found in Figs. 8 and 10. 0·3 unit renin gave rises of arterial pressure of 8, 9, 11 and 18 mm. Hg in four rabbits in which this dose produced no definite diuretic response. 1 unit renin gave rises of 30, 33, 39 and 44 mm. Hg in four animals in which the same dose produced a conspicuous diuresis lasting over ½ and less than 1 hr., the rises of blood pressure at the end of ½ hr. being 7, 12, 16 and 16 mm. Hg. The conclusions we would draw from these and similar observations are that the dose of renin necessary to produce a diuretic effect is greater than that producing a detectable change in arterial pressure; and that the duration of the diuretic effect and that of the pressor effect are, within the limits of our measurement, similar. The intensity of the diuretic action does not, however, run parallel to that of the pressor effect. Thus, when 2·7 units of renin are given in two equal doses at 15 min. interval, the maximum diuretic effect is observed in the second ½ hr. after the first injection, whereas of course the maximum pressor effect is in the first ½ hr. This relationship is pursued further in the next section.

Tyramin acid phosphate injected in three equal doses, each of 2, 4 and 6 mg. at 10 min. intervals, was found to produce no consistent increase in urine flow or chloride excretion in the unanaesthetized rabbit (six experiments). These doses gave rises of blood pressure which were similar in degree to those given by renin in diuretic doses, but the rise of pressure with tyramine is of very much shorter duration (about 5 min.).

The effect of renin when urine formation is high

The results obtained in the unanaesthetized dog suggested the possibility that renin might be antidiuretic under some circumstances, and its action in the unanaesthetized rabbit was therefore investigated when given between the 45th and 60th min. after water ingestion, that is, just before the anticipated peak of water diuresis. Here again was introduced the complicating factor of disturbances in urine flow due to sensory stimuli, to obviate which we finally made our intravenous injections into an ear, the sensory nerves of which had been cut days or weeks previously. Four such rabbits gave consistent control curves of water diuresis when urine was obtained at 10–15 min. intervals, the curves being apparently uninfluenced by intravenous injections of saline, boiled renin or the liver extract described previously (Fig. 7). In all these rabbits renin produced an antidiuretic followed by a diuretic response, the antidiuretic action

being the more conspicuous with small, and the diuretic with large, doses (Figs. 8–10). Thus, 0·3 unit produced an antidiuretic effect lasting 20 min. which was followed in one rabbit only by a slight diuretic effect.

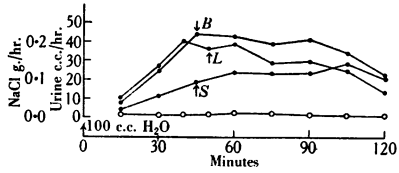

Fig. 7.

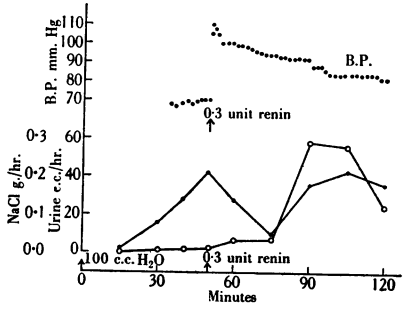

Fig. 8.

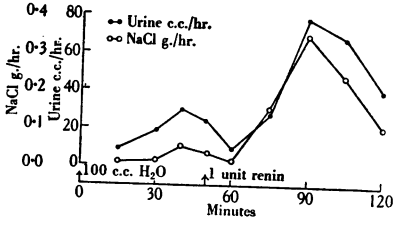

Fig. 9.

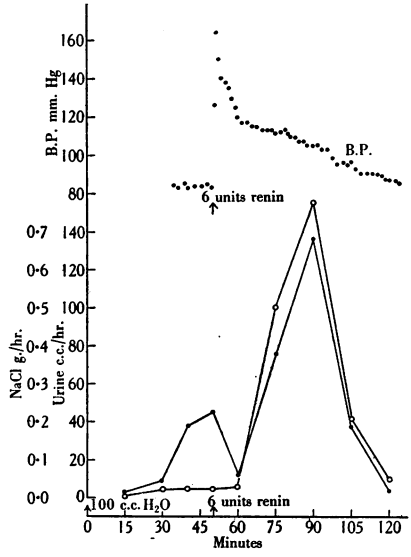

Fig. 10.

Figs. 7–10. Rabbit B (2·5 kg.). 100 c.c. of water given by stomach tube at 0 min.

Fig. 7. Three control curves obtained on separate days. Intravenous injection at *B* of 15 mg. renin in 1 c.c. saline, the solution having been boiled for 5 min.; at *L* of 15 mg. liver extract in 1 c.c. saline, and at *S* 1 c.c. saline. Chloride excretion corresponding to Curve *L*.

Figs. 8–10. Changes in urine flow and chloride excretion produced by 0·3, 1 and 6 units renin dissolved in 1 c.c. saline and injected at the 50th min. after water ingestion. In Figs. 8 and 10 are shown the changes in arterial pressure produced on a separate occasion by 0·3 and 6 units renin.

One unit produced a fall in urine flow lasting 10–20 min. followed in three rabbits by a rise in urine flow above the value expected from control curves. The antidiuretic effect following 6 units was shorter, being detectable in one rabbit only when urine collections were made for 15 min.

periods, but pronounced in all when collecting periods were reduced to 10 min.; this antidiuretic effect was followed in all rabbits by a very conspicuous diuresis, urine flow frequently reaching the enormous rates of 150–180 c.c./hr.

It is evident that the threshold dose for the antidiuretic effect of renin is lower than that for the diuretic effect. Thus 0·3 unit has a definite inhibiting effect on water diuresis, but has no detectable diuretic action, be the initial rate of urine formation high or low. The diuretic action does not become conspicuous until the dose of renin is raised to 1 unit.

In every case the injection of 0·3–6·0 unit renin was followed by a rise of the chloride percentage in the urine, particularly in the specimens collected between 15 and 45 min. after the injection, the rise being observed even in those experiments in which the dose of renin was small, and the rate of urine flow never rose above the level expected from control curves. In the second 15 min. period after renin injection the plasma chlorides in three experiments were 0·69, 0·63 and 0·61 mg. NaCl/100 c.c., while the corresponding urines contained 0·64, 0·61 and 0·58 mg. NaCl/100 c.c. Again we find, therefore, that the chloride content of the urine secreted in response to renin tends to approximate to that of the plasma, but in this instance, with a large water load, urine chloride is slightly lower than plasma chloride.

In Figs. 8 and 10 are shown the changes in arterial pressure produced, in separate experiments on the same animal, by the same dose of renin used to produce the changes in urine flow. The rise of arterial pressure is greatest during the phase of reduced urine flow and continues, but is of less degree, during the succeeding phase of diuresis, or of raised urinary chloride.

It is evident from these experiments that the unanaesthetized rabbit may with suitable dosage of renin give a response similar to that which we observed in the unanaesthetized dog.

Creatinine clearance. Four additional experiments were made in three animals to determine the effect of large doses of renin (6 units) on the creatinine clearance, the plasma creatinine being raised to between 8 and 35 mg./100 c.c. by previous injection of creatinine. Urine collections were made during two 10 min. periods before the injection of renin at the 50th min., and for one period of 10 and one of 15 min. afterwards, blood being taken at the beginning, middle and end of the experimental period. From Table VI, which summarizes the results, it may be seen that in each experiment a profound fall in the creatinine clearance rate occurred during the phase of reduced urine flow immediately following renin injection.

TABLE VI. Urine flow and creatinine clearance, in c.c./min., before and after 6 units renin intravenously at the 50th min. following water ingestion

Min. since water ingestion	Rabbit 211		Rabbit 211		Rabbit B		Rabbit H	
	Urine flow	Creatinine clearance	Urine flow	Creatinine clearance	Urine flow	Creatinine clearance	Urine flow	Creatinine clearance
30–40	0·85	15·4	1·76	22	0·30	9·2	1·10	9·3
40–50	0·42	16·2	0·60	17	0·58	11·8	0·72	10·5
50			6 units renin intravenously					
50–60	0·28	9·6	0·10	5·5	0·15	3·9	0·20	7·0
60–75	3·00	12·0	2·60	17·8	1·10	10·0	2·70	17·8

During the subsequent phase of increased urine flow, the creatinine clearance rate was smaller in one experiment, larger in one experiment and unaltered in two experiments, as compared with the values before renin injection. These changes in creatinine clearance, corresponding to the phases of decreased and increased urine formation after renin, raise the possibility that the unchanged clearances observed in the preceding section may be the summation of an initial decreased and subsequent increased rate. Thus the results obtained in the previous section do not entirely exclude the possibility that the increased urine flow, which is the predominant effect of large doses of renin, may always be associated with some increase in the rate of glomerular filtration. This possibility does, however, seem to be excluded by the results obtained in this section on rabbits 211 and B, and particularly by the first experiment on 211 in which a urine flow at the very high rate of 3 c.c./min. was associated with a creatinine clearance considerably lower than normal. We would conclude, therefore, that the antidiuretic phase of the response to large doses of renin is accompanied by a fall in the rate of glomerular filtration, but that the diuretic phase is not necessarily associated with an increase in this rate.

The effect of renin when chloride excretion is high

Experiments were made on four animals, in which 100 c.c. 1·5% sodium chloride were given by stomach tube on the evening before and again on the morning of the experiment, 3–6 units of renin being injected 4–4½ hr. later. Fig. 11 exemplifies the results obtained. In all animals, the urinary chloride rose to 2·0% or more following the ingestion of saline, but there was little diuresis except in one animal. Renin produced the usual increase in urine flow, but the percentage of chloride, instead of rising, as in the other experiments, fell, rising again as the diuretic effect of renin subsided. The lowest concentrations of chloride in the urine in these experiments were found in the largest ½ hr. urine specimens, and

were in the four animals 0·75, 0·76, 0·84 and 0·86 g. NaCl/100 c.c., values which were thus very similar to those previously found for urine secreted in response to renin by animals not dosed with salt.

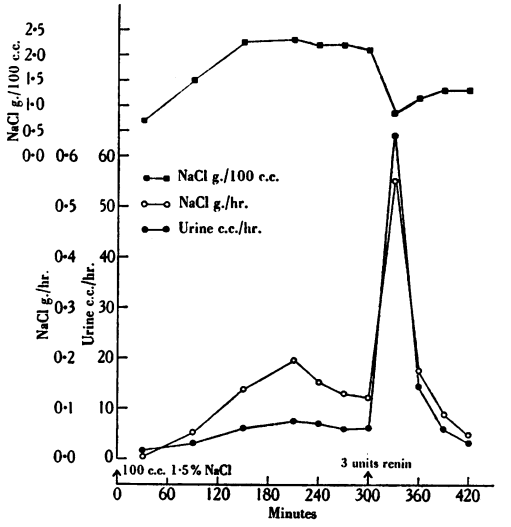

Fig. 11. Rabbit 211 (3·1 kg.) received 100 c.c. 1·5% NaCl solution on previous evening and at 0 min. 3 units renin produced increased urine flow and chloride excretion, the chloride content of the urine falling from 2·1 to 0·86% and rising again to 1·3% NaCl.

Discussion

The observations described in this paper have shown that there exists in rabbit's kidney a substance or substances which, intravenously injected into the unanaesthetized rabbit, profoundly modify the arterial pressure, the rate of formation of urine and its sodium and chloride content. Further progress in chemical separation may show that one or more of the changes in renal activity produced by our extracts are due to a substance other than that which raises arterial pressure. Particularly relevant are observations by Govaerts & Cambier [1930], who have shown that a diuresis characterized by a considerable rise in the rate of excretion of chloride is produced in the unanaesthetized dog by intravenous injection of distilled water or Witte's peptone; in both cases the animal exhibits profound somatic disturbances such as trembling, nausea, vomiting and defaecation immediately after the injection, while the diuresis does not begin until half an hour or more later. The diuresis is attributed not to any specific substance in the injected fluids but to the profound reaction in the animal which they produce. We would point

out that we have seen no signs of upset in the general well-being of our rabbits following the injection of even the larger doses of the preparations here employed; the animals sit apparently contentedly and, if permitted, move about and eat freely; they show no more than the faintest paling of the ground tone of the ears, whereas with toxic injections, intense and persistent blanching is a conspicuous feature. Nor have we been able to reproduce these changes in the volume and composition of the urine by protein containing extracts prepared similarly from rabbit's liver. If the changes in renal function which we have observed following the injection of renal extracts are not due to renin, then it seems clear that they are due to some substance which resembles it in physico-chemical properties and in its presence in kidney, and absence, or relative absence, from liver. While we must remain uncertain until chemical isolation is achieved, our present evidence favours the view that the several effects produced by our extracts on arterial pressure and renal function are due to a single substance, since they remain associated during successive stages of purification. With this qualification we may refer to these effects as those of renin.

In its powerful diuretic action, renin stands apart from other known constituents of animal tissues. Adrenaline may in small doses slightly increase the rate of urine formation in unanaesthetized rabbits [Addis, Barnett & Shevky, 1918] and in the perfused rabbit's kidney [Richards & Plant, 1922*b*], while in large doses urine flow is diminished, but there is little information concerning its effect on chloride excretion. Tyramine has been found by us to have little if any diuretic effect. Postpituitary extract resembles renin in that, in suitable doses, both may inhibit water diuresis in the unanaesthetized animal and raise the percentage of chloride in the urine [Frey & Kumpriess, 1913]. In the anaesthetized animal both may increase urine flow, and successive injections of both substances produce diminishing rises of arterial pressure. Fromherz [1923] and McFarlane [1926] claim that under certain circumstances pituitrin may increase urine flow in the unanaesthetized animal, but the effects they obtained were small, and Motzfeldt [1917] observed only an inhibition of urine flow in the unanaesthetized rabbit with intravenous injections varying between 2^{-6} and 2 c.c. of a post-pituitary extract. From their effects on urine formation, it may be concluded therefore that renin and the post-pituitary principle are not identical, a conclusion which is supported by differences in chemical properties and in their effects on the ear vessels of the unanaesthetized rabbit [Landis, Montgomery & Sparkman, 1938].

The nature of renin's action on the kidney. During the few minutes immediately following the injection of renin, a phase in which urine flow is diminished, a great rise in arterial pressure is accompanied by a fall in the rate of glomerular filtration, at least when the dose of renin is large. A fall in the rate of glomerular filtration, despite raised arterial pressure, indicates constriction of the afferent glomerular arteries or diminution of the number of open glomerular capillaries, effects which we may infer are due to the action of renin on these vessels. It is to this fall in the rate of glomerular filtration that we would attribute the diminished rate of urine formation which immediately follows the injection of renin.

The increased secretion of urine which follows the inhibitory phase and which is the predominant effect of large doses of renin in the unanaesthetized rabbit cannot, on the evidence submitted here, be attributed to increase in the amount of the glomerular filtrate, in so far as this may be inferred from the creatinine clearance rate. It must therefore be attributed to a diminished reabsorption of water from the renal tubules. This is not the only change in tubular activity, since the composition of the urine during renin diuresis contrasts sharply with that during water diuresis in its sodium and chloride contents. Evidently there is also, under ordinary circumstances, a diminished tubular reabsorption of sodium and chloride. Diminished absorption of sodium chloride in the tubules would be expected by its osmotic action to diminish water reabsorption also, and we considered at one time the possibility that the increased water excretion was simply a secondary effect of diminished reabsorption of salt. This possibility has, however, been excluded by the experiments in which 1·5% saline was ingested before the injection of renin and in which the chloride concentration of the urine fell during renin diuresis. Evidently reabsorption both of water and of sodium chloride is inhibited.

Renin in diuretic doses has a pronounced and prolonged pressor effect in the unanaesthetized rabbit, and it is interesting to note that the composition of the urine during renin diuresis has many of the properties of the urine in the pressure diuresis observed in the isolated kidney. Thus, the urine during renin diuresis tends to approximate in composition to the plasma from which it is formed, at any rate so far as chloride, urea and creatinine are concerned, and these are the only constituents fully studied. The proposition that renin diuresis represents a pressure diuresis may now be considered in greater detail.

The absence of any increase in the rate of glomerular filtration, despite the presence of raised arterial pressure during the diuretic phase of renin's

action, indicates that the renal vessels are not unaffected by renin at this stage, but undergo constriction. And it may be suggested that during this phase there is a total closure of some of the originally open glomerular tufts; while in those remaining open intra-glomerular arterial pressure is greatly increased, partly by increased systemic pressure and partly by constriction of the efferent glomerular arterioles. This conception receives support from recent experiments on the unanaesthetized dog by Corcoran & Page [1939], who conclude that renin decreases renal blood flow and increases intra-glomerular arterial pressure either by increasing general arterial pressure or by efferent arteriolar constriction. Evidence for efferent arteriolar constriction has previously been obtained in dogs anaesthetized with nembutal by Merrill *et al.* [1938], who observed decreased renal blood flow and swelling of the kidney in response to renin. Friedman, Abramson & Marx [1938] in similar preparations observed a preliminary fall followed by a rise in kidney volume following renin injection.

While it might be possible on this basis to consider renin diuresis as a form of pressure diuresis in which relatively few nephrons participate, a real difficulty is encountered when we consider more closely the chloride content of the urine. Thus in the isolated kidney, when the concentration of chloride in the urine is initially low, a rise of arterial pressure is accompanied by a rise in the concentration of chloride in the urine towards, but not up to, its concentration in the blood [Richards & Plant, 1922*a*; Starling & Verney, 1925]. In renin diuresis there is a similar rise in chloride concentration, but this reaches and may surpass the plasma chloride level. It is difficult to know how much importance to attach to this difference, since the comparison is between the behaviour of the intact animal and that of the isolated kidney, and for this reason no final decision may be taken. Nevertheless, the disparity between the behaviour of urinary chloride in the renin diuresis on the one hand, and in the pressure diuresis in the isolated kidney on the other, suggests that renin may not act purely on the renal vessels, but may directly influence the renal tubule cells, inhibiting the reabsorption of water, of sodium and of chloride.

Starling & Verney found identical chloride contents in plasma and the urine secreted by an isolated dog's kidney after tubular action had been abolished by cyanide. Westfall, Findley & Richards [1934] have found identical chloride contents in plasma and glomerular urine of the frog and necturus when backflow from the tubules was prevented, though in earlier experiments when this precaution was not taken, a tendency

was observed for glomerular urine to have a higher chloride content than plasma. More recently Walker, Hudson, Findley & Richards [1937] have found, both in the frog and necturus, that the chloride content of the urine in the proximal convoluted tubule is about 10% higher than in the plasma, while that in the distal tubule becomes progressively lower. In view of the technical difficulties of the experiment these workers did not feel justified in trying to explain this difference between plasma and proximal tubule urine, but it would seem possible from their findings that the urine secreted after renin represents, as far as its chloride content is concerned, an approximation to that of the proximal convoluted tubule rather than to that of the glomerular capsule.

General. The existence of a substance apparently present in normal renal cortex and capable of profoundly influencing renal function may help to explain some otherwise obscure chapters in the physiology of the kidney. Recent work has shown that, in some respects at least, the behaviour of the kidney is independent of its nervous connexions and is determined by the concentration in the plasma of substances which it excretes, and by chemical substances elaborated elsewhere in the body, for example in the posterior lobe of the pituitary and in the suprarenal cortex. Exactly how these various influences are integrated, and what part, if any, renin plays in the final product are questions as yet unanswered. In this connexion it is relevant to consider dosage. It has been found in other experiments in progress in this laboratory that the renin content of rabbit's kidney varies between about 0·4 and 5·0 unit/g., a very common value being 1 unit/g. If renin exerts its diuretic action solely by its effects on the general arterial pressure and glomerular vessels, then it would presumably act by being released into the general circulation, and the experiments here described indicate that the amount released would have to be of the order of 1 unit. An average pair of rabbit's kidneys weighing 15 g. would thus contain only about 15 times the diuretic dose. If, however, renin acts directly on the tubule cells, then its presence in normal renal cortex might enable it to act on the kidney without entering the general circulation, and the amount requisite to change renal activity would be small; in this case also it might be possible for renin to affect the activity of the kidney without influencing the arterial pressure.

Summary

1. In the anaesthetized rabbit, saline extracts of alcohol dried rabbit's kidney raise blood pressure and acclerate urine flow. Both effects are abolished by boiling the extracts.

2. In the hydrated unanaesthetized rabbit with a small initial rate of urine flow, renal extracts containing renin, in doses of 1 unit or more, produce a conspicuous diuresis lasting about an hour. The diuresis is not accompanied by any significant change in the excretion of endogenous creatinine or in the rate at which injected creatinine is cleared from the plasma; changes in urea excretion are similar to those occurring in water diuresis. There is, however, a very large increase in the excretion of sodium and chloride, the percentage of chloride in the urine tending to rise to, and slightly to exceed, that of the plasma.

3. In the unanaesthetized rabbit, renin injected on the ascending limb of the curve of water diuresis produced a transient inhibition followed by an increase in the flow of urine. With doses of less than 1 unit, the antidiuretic effect alone may be observed, but with larger doses the diuretic effect is the more conspicuous. The rate at which injected creatinine is cleared from the plasma is decreased during the phase of diminished urine flow, and is not constantly or significantly altered during the phase of increased urine flow. The chloride content of the urine during the diuretic phase rises towards that of the plasma.

4. When in the unanaesthetized rabbit the chloride content of the urine is artificially raised by 1·5% saline ingestion, renin produces an increased urine flow accompanied by a fall in the percentage of chloride in the urine towards that of the plasma.

5. Injected intravenously, the dose of renin necessary to produce diuresis is greater than that producing a rise of arterial pressure.

6. The antidiuretic action of renin in the unanaesthetized rabbit is attributed to its action on the glomerular vessels reducing the rate of glomerular filtration.

7. The diuretic action of renin in the unanaesthetized rabbit is due to an inhibition of tubular reabsorption of water, sodium and chloride. This may represent the effects of a pressure diuresis in which relatively few nephrons are involved, but it is more probably due in part to a direct action of renin on the activity of the renal tubule cells.

Our thanks are offered to Prof. J. H. Gaddum for the hospitality of his department during the observations made on the dog, and to Dr Hans Heller for some helpful suggestions.

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