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HUMAN URINARY KININ EXCRETION

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It has been shown that the isolated rat duodenum relaxes in the presence of low concentrations of plasma and urinary kinin. The tissue is at least as sensitive as the rat uterus. Vasopressin and oxytocin, in large doses, also caused relaxation of the duodenum whereas acetylcholine, substance P and 5-hydroxytryptamine caused contraction. It was concluded that if an extract is assayed on the rat uterus and the rat duodenum in parallel using plasma kinin as a standard, and the results agree, this is good evidence that the active principle being estimated is a kinin. This method is therefore both sensitive and specific for kinin estimations, but it will not distinguish between kinins of different origin. The urinary excretion of kinin in 14 healthy adults was found to be fairly constant. The minute output was unaffected by the rate of urine formation, urinary *p*H, or time of day. There was no increase during sweating or salivation.

The plasma kinins are polypeptides derived from plasma which stimulate certain types of plain muscle. They include the substances kallidin (Werle, 1937; Werle and Berek, 1950), bradykinin (Rocha e Silva, Beraldo and Rosenfeld, 1949) and the pain-producing substance (Armstrong, Jepson, Keele, and Stewart, 1954, 1957). There is some evidence that the same active principle may be responsible for the activity of all these substances (Werle, 1953; Jepson, Armstrong, Keele, and Stewart, 1956; Holdstock, Mathias and Schachter, 1957), and possibly also for the activity of various other bradykinin-like substances which have been reported (Hilton and Lewis, 1955, 1956; Schachter, 1956; Adam, Hardwick, and Spencer, 1957; Lewis and Work, 1957; Lewis, 1958). Human urine contains a similar kinin which could not be distinguished from the plasma kinins qualitatively or by parallel assays (Gomes, 1955; Gaddum and Horton, 1959) though there is some chemical evidence that the active principles are different (Gomes, 1957).

There is evidence for the formation of a plasma kinin during functional vasodilatation in the salivary and sweat glands (Hilton and Lewis, 1955, 1956; Fox and Hilton, 1958), and it is possible that kinin formation may occur in other parts of the body. The excretion of a kinin in normal human urine suggested a continual production, but it was not known to what extent the excretion rate varied under different physiological conditions and in different people. This has been investigated using a new method for kinin estimations which is both sensitive and specific. The amount of kinin excreted/unit time was fairly constant in both male and female urine.

METHODS

Rat Uterus.—Virgin rats weighing 120 to 200 g. were injected with stilboestrol (10 μg./100 g.) 16 to 18 hr. before use. Uteri were suspended in a 2 ml. bath at 30 to 31° and bathed in de Jalon solution containing atropine sulphate (10^{-6}).

Rat Duodenum.—The proximal 3 cm. of duodenum from rats weighing 150 to 250 g. were suspended in a 10 or 15 ml. bath containing atropinized (10^{-6}) de Jalon solution. It was observed that tissues kept at 4 to 6° for 2 to 3 hr. before use behaved particularly well, so this procedure has been used routinely. Since relaxation of the muscle was small, a high lever magnification (1 in 15) was required. A tension of approximately 0.5 g. was suitable for most tissues. A bath temperature of 30 to 31° was used to minimize spontaneous activity, but temperatures up to 37° were also satisfactory. De Jalon solution was the most satisfactory; increasing the calcium concentration did not improve performance nor did the use of Tyrode solution. A dose cycle of 3 or 4 min. was used with 30 sec. contact time. The bath was washed out by upward displacement and overflow.

Simultaneous Parallel Assays.—The rat uterus was suspended in a 2 ml. bath and the rat duodenum in a 10 or 15 ml. bath. The two tissues were used

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simultaneously for the assay of urine samples. In each case a dose cycle of 4 min. was used, doses being administered to each tissue alternately every 2 min.

Collection of Urine Specimens.—Urine was collected over a timed period as follows. The subject emptied his bladder and noted the time, the specimen being discarded. At the next micturition, the whole specimen was collected and the time again noted. The volume and *p*H (B.D.H. narrow range indicator paper) of the urine were measured immediately. A 10 ml. aliquot was transferred to a boiling tube and frozen by immersion in a mixture of acetone and solid carbon dioxide ("Drikold") at −70° within 30 min. of collection. It was then stored in the deep freeze until the time of the assay.

Alumina Columns. — All urine specimens were passed through an alumina column before assay. Columns 6.5 × 1.0 cm. were prepared by suspending 5 g. alumina (Savory and Moore) in water and allowing it to settle by gravity. 5 ml. urine was added and allowed to run through at 1 ml./min. When all the urine had passed into the column, distilled water was added. Washing was carried out at 1 ml./min. and 5 ml. fractions were collected; the first was discarded, while the second and third fractions which contained all the activity were pooled and used for biological assays. If the interval before the assay was to be longer than 30 min. the filtrates were frozen and stored in the deep freeze.

Standard Urinary Kinin.—This was prepared from human urine by IRC-50 adsorption and butanol extraction (Gaddum and Horton, 1959). 1 mg. of the original master standard was called 1 unit.

Standard Plasma Kinin.—This was prepared by the action of human urinary kallikrein on acid-treated human plasma, the kinin (kallidin) being extracted with alcohol (Gaddum and Horton, 1959).

Substance P.—This was prepared and kindly supplied by Dr. T. B. B. Crawford.

Wasp Kinin and Ox Bradykinin.—These materials were prepared and kindly supplied by Dr. M. Schachter.

Vasopressin, Oxytocin, and Angiotensin. — The following commercial preparations of these substances were used: Pitressin (Parke, Davis), Pitocin (Parke, Davis), and Angiotonin (Eli Lilly).

Results

The Rat Duodenum

If a tissue extract is assayed on a single preparation, for example the rat uterus, against standard plasma kinin, there can be no certainty that the active principle being estimated is really plasma kinin; some other test of identification is necessary. One of the best ways to ensure specificity is to assay an unknown in parallel on several different tissues. If similar results are obtained in this way it is reasonably certain that the active principle in the extract and the standard preparation are identical (Chang and Gaddum, 1933).

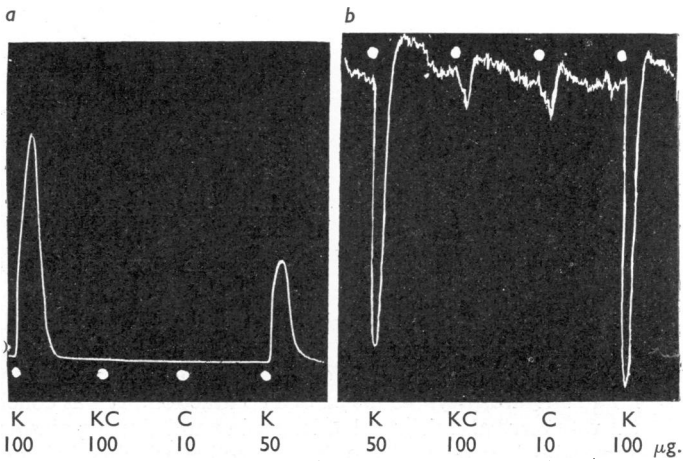

FIG. 1.—(*a*) Isolated rat uterus; (*b*) isolated rat duodenum. Each tissue was suspended in a 10 ml. bath containing atropinized (10^{-6}) de Jalon solution at 30°. ***K***= standard plasma kinin extract. ***KC***=mixture of plasma kinin (100 μg.) and chymotrypsin (10 μg.) incubated for 1 hr. at 37° and then heated in a boiling water bath for 10 min. ***C***=control containing no plasma kinin treated as ***KC***. All doses in μg.

It has been found that the rat duodenum responds to small doses of plasma kinin, being at least as sensitive as the rat uterus, and usually more sensitive. Unlike most plain muscle, the rat duodenum relaxed in the presence of kinin (Fig. 1). That this inhibitory action and the stimulation of other types of plain muscle were produced by the same active principle has been confirmed by parallel assays using kinin preparations of different potency and of different origin. The inhibitory action on the duodenum like the stimulant action on the uterus was abolished by incubation with chymotrypsin (Fig. 1).

The Action of Other Oxytocic Polypeptides on the Rat Duodenum

Vasopressin and oxytocin had no action on the rat duodenum in concentrations comparable to those which stimulate the rat uterus. In very high concentrations (100 mU./ml.) both these polypeptides had a qualitatively similar effect to plasma kinin (Fig. 2). Parallel assays of vasopressin and oxytocin with plasma kinin were performed on the rat uterus and rat duodenum;

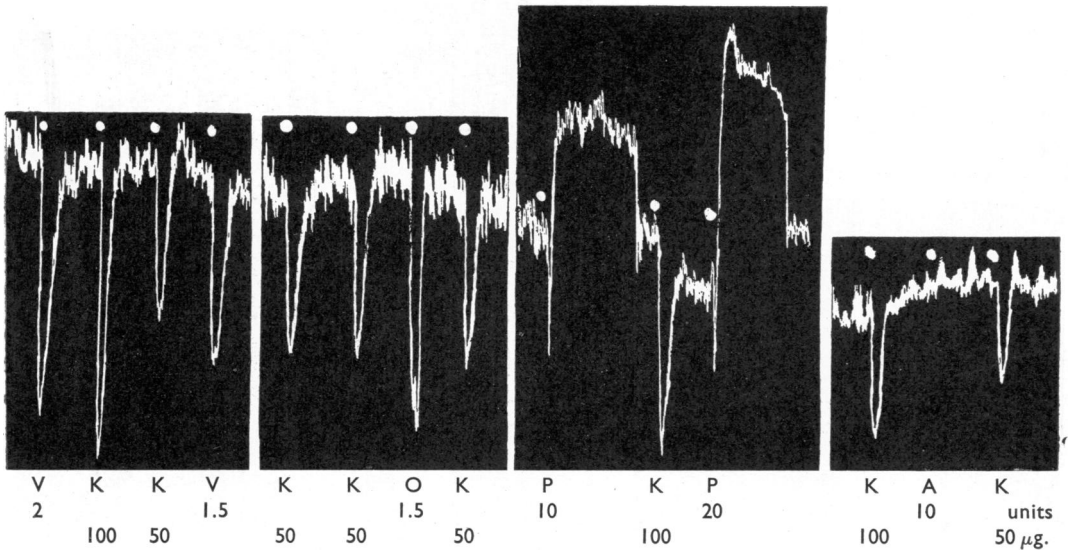

FIG. 2.—Isolated rat duodenum suspended in a 15 ml. bath containing atropinized (10^{-6}) de Jalon solution at 30°. *V*=vasopressin (units). *O*=oxytocin (units). *P*=substance P (units). *A*=angiotensin (units). *K*=standard plasma kinin extract (μg.).

the results are shown in Table I. Clearly there is a great discrepancy between the results on the two tissues, and the index of discrimination (Gaddum, 1955) is very high. Parallel assays using these two tissues will certainly distinguish vasopressin and oxytocin from plasma kinin in a tissue extract.

TABLE I

UNITS OF OXYTOCIC POLYPEPTIDES EQUIACTIVE WITH 1 MG. STANDARD PLASMA KININ

	Rat Uterus	Rat Duodenum	Index of Discrimination
Vasopressin	0·15	15	100
Oxytocin	0·015	22·5	1,500
Angiotensin	12·5	>200	>16
Substance P	243	Contracts	—

Substance P stimulated the rat duodenum, although some extracts had a very transient initial inhibitory effect (Fig. 2). The stimulant action was not due to contamination with histamine because the effect was not inhibited by mepyramine maleate (10^{-6}).

Angiotensin [hypertensin, angiotonin] (Braun-Menéndez and Page, 1958) had no action on the duodenum in the largest doses used (Fig. 2), whereas it was active on the rat uterus. The index of discrimination is over 16 (Table I), showing that these two tissues will easily distinguish between angiotensin and plasma kinin.

Other kinins have been compared with the standard plasma kinin on these two preparations (Fig. 3). Neither bradykinin, the plasma kinin formed by the action of trypsin on plasma, nor the human urinary kinin (substance Z) nor wasp kinin could be distinguished from the standard plasma kinin (kallidin), the index of discrimination in each case not differing significantly from 1.0 (Table II).

TABLE II

AMOUNTS OF KININS EQUIACTIVE WITH 1 MG. STANDARD PLASMA KININ

Amounts are given with standard errors.

	Rat Uterus	Rat Duodenum	Index of Discrimination
Bradykinin (units) ..	1·3±0·3	1·5±0·5	1·2
Urinary kinin (units) ..	8·1±0·5	9·2±1·2	1·1
Wasp kinin (μg.) ..	1·2±0·4	1·2±0·4	1·0

The Action of Non-peptide Substances on the Rat Duodenum

Acetylcholine and 5 - hydroxytryptamine stimulate the rat uterus and both were found to stimulate the rat duodenum (Fig. 4). Histamine in large doses also stimulated the rat duodenum. Adrenaline and noradrenaline inhibited the duodenum, concentrations of 10^{-6} being required. Adenosine and its monophosphate both inhibited the rat duodenum, but the maximum relaxation which they could produce was less than half the maximum which could be reached with plasma kinin (Fig. 4). Thus none of these substances behaved like plasma kinin on both the rat uterus and the rat duodenum.

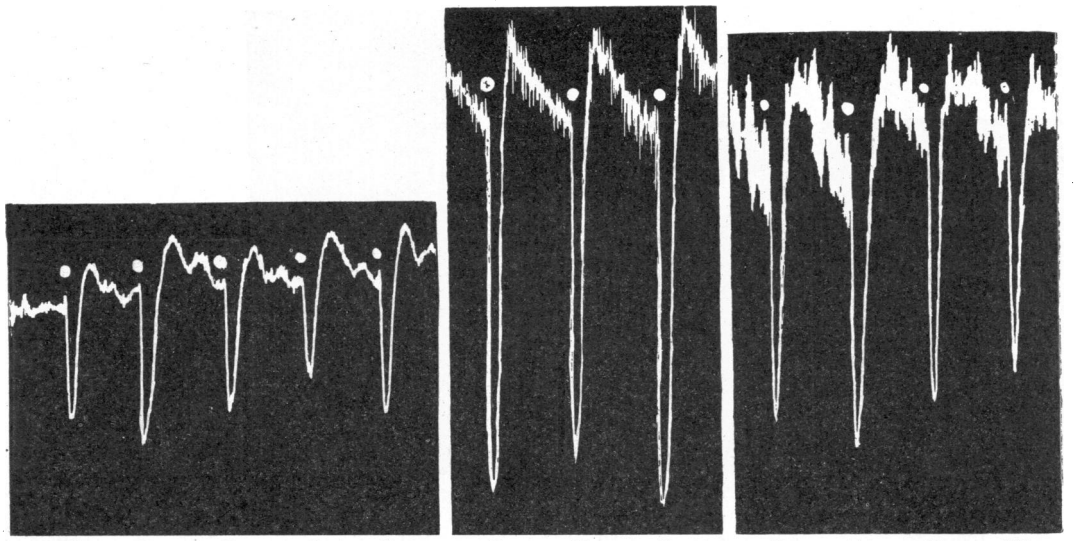

FIG. 3.—Isolated rat duodenum suspended in a 15 ml. bath containing atropinized (10^{-6}) de Jalon solution at 30°. ***K***=standard plasma kinin extract (μg.). ***B***=ox bradykinin (units). ***U***=urinary kinin (units). ***W***=wasp kinin (μg.).

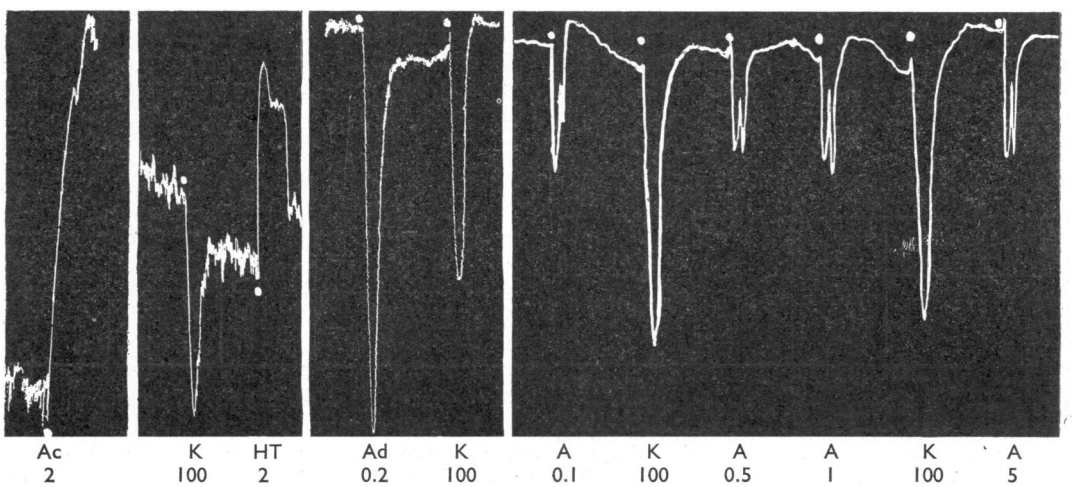

FIG. 4.—Isolated rat duodenum suspended in a 10 ml. bath containing de Jalon solution at 30°. ***Ac***=acetylcholine. ***K***=standard plasma kinin extract. ***HT***=5-hydroxytryptamine creatinine sulphate. ***Ad***=adrenaline hydrochloride. ***A***=Adenosine. Doses in μg.

The Estimation of Urinary Kinin

The kinin in human urine was originally detected by testing diluted crude human urine on the superfused rat uterus. When parallel assays were performed on the rat uterus and the rat duodenum using crude urine, the results were often markedly different, the apparent potency of the urine being up to five times greater on the duodenum. The cause for this discrepancy was found to be a substance in urine which inhibits the duodenum but does not stimulate the uterus. This inhibitory substance has not been identified, but it can be removed from urine by adsorption on alumina.

A sample of crude urine was assayed on the uterus and duodenum before and after passage

through an alumina column. The results of the assay with the rat uterus, expressed in units/ml. of kinin, before and after alumina treatment were 6 (±2 s.e.) and 15 (±5) and with the rat duodenum 30 (±6) and 15 (±5). After the alumina treatment, not only was the duodenum estimate lower but the uterus value was appreciably higher, suggesting that the inhibitory substance may also depress the stimulant action of kinin on the uterus. This result illustrated very clearly the unreliability of a single tissue for these estimations.

The recovery of urinary kinin from alumina columns was investigated by applying a standard extract dissolved in 5 ml. water and washing the column with water. 2.5 ml. samples were collected on a fractionator. Urinary kinin passed through the column and appeared in the filtrate as soon as the hold-up volume had been displaced (Fig. 5). Recoveries were always over 90%.

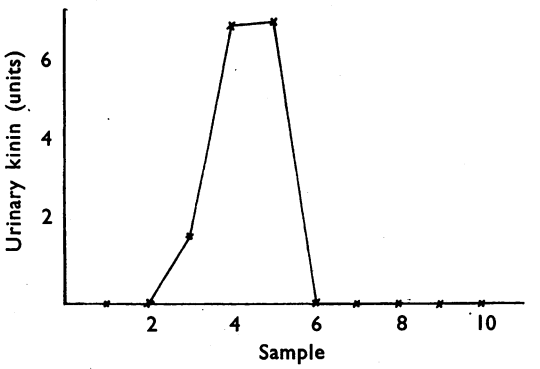

FIG. 5.—Alumina column 6·5×1·0 cm. 16 units urinary kinin were applied in 5 ml. distilled water. The column was washed with distilled water. Recovery=95%. Sample volume, 2·5 ml.

Stability of Urine Samples

Jensen (1958) showed that extracts of urinary kinin in solution were more stable than crude urine. This observation has been confirmed. Since a kinin-inactivating enzyme has been detected in urine (Frey, Kraut, and Werle, 1950), it was thought probable that this might account for the relative instability of crude urine.

The urinary kinin content of two urine specimens was estimated by the method described above. Each specimen was then divided into three portions. One portion was adjusted to *p*H 7, the second to *p*H 2 and the third was heated at *p*H 6 in a boiling water bath for 10 min. and adjusted to *p*H 7. The specimens were left for 24 hr. at room temperature, and their kinin content was then estimated (Table III). The loss of activity in the acidified and pre-heated specimens was far less than in the untreated specimens.

TABLE III

RECOVERY OF KININ FROM URINE SAMPLES LEFT AT 10 TO 15° FOR 24 HR.

The recovery is given as %.

Treatment	Expt. 1	Expt. 2
*p*H 2	100	77
Boiled 10 min. then *p*H 7	80	72
*p*H 7	3	18

These results strongly suggest that in crude urine kinin was destroyed by an enzyme, which could be inactivated by heat and was inactive at a low *p*H.

Urine specimens which were frozen and stored in a deep-freeze showed no detectable loss of activity over a two-month period. The urinary kininase was evidently inactive at this temperature. All specimens were therefore frozen within 30 min. of collection unless they were to be assayed immediately.

Individual Variation in Urinary Kinin Excretion

The urinary excretion of kinin was estimated in 14 healthy adults, 12 male and 2 female. All except one (subject 9) were within the age range 16 to 40 years. The results are shown in Table IV.

TABLE IV

THE URINARY EXCRETION OF KININ IN 14 HEALTHY ADULTS, ESTIMATED ON THE RAT UTERUS AND RAT DUODENUM

The kinin values are expressed in units/min. with s.e. and the number of specimens tested is given in parentheses. An asterisk indicates a female subject.

Subject No.	Rat Uterus	Rat Duodenum
1	12·8± 5·9 (46)	8·2±4·8 (40)
2*	9·0± 5·5 (30)	5·7±6·1 (20)
3	12·3± 8·3 (6)	13·8±7·8 (4)
4	15·8± 2·1 (5)	11·2±3·3 (5)
5	6·5± 2·9 (8)	4·9±2·3 (8)
6	15·1± 6·0 (5)	11·0±4·5 (5)
7	12·1± 4·8 (5)	8·4±5·6 (5)
8	10·9± 6·3 (6)	7·4±6·4 (5)
9	12·4± 4·5 (5)	6·3±3·3 (5)
10	9·1± 4·7 (5)	7·1±3·2 (5)
11	17·5±12·5 (5)	13·8±1·0 (5)
12	14·0± 2·5 (3)	2·5±0·4 (3)
13	7·9± 4·7 (5)	3·3±1·4 (5)
14*	13·3± 4·4 (3)	3·3±1·0 (3)

The estimates of kinin on the rat duodenum were fairly consistently lower than on the uterus, though in one subject (3) the mean was higher. In three subjects (12, 13 and 14) the values were markedly lower on the duodenum. The differences suggested that there is a substance in urine which interfered with one or both assays, and that it was excreted to a much greater extent in some individuals. The differences were small compared

to those occurring with urine which had not passed through alumina. In general, variations were in the same direction on the two tissues. In further analysis of the results only those on the uterus will be discussed, though in most cases specimens were assayed on both tissues.

The estimates of kinin excretion were remarkably constant. There was a less than threefold difference between the means at the extremes of the range (6.5 and 17.5) in 14 subjects. The individual values varied from 2.6 to 38.0 units/min., but in 205 specimens tested only 3 were lower than 3 units/min. and 4 were higher than 30 units/min. The two female subjects (2 and 14) excreted kinin at similar rates to the males.

The Effect of Urinary Output on Kinin Excretion

The early experiments showed that the concentration of kinin in urine was roughly proportional to the concentration of the urine itself. Thus the urinary excretion of kinin has been expressed as units/min. The results in Table V show that the rate of kinin excretion was independent of the urinary output over the range 0.26 to 18 ml./min. In these experiments adjustments to the rate of urine formation (when required) were made by varying the fluid intake.

TABLE V

THE URINARY EXCRETION OF KININ (UNITS/MIN.) AT DIFFERENT RATES OF URINARY OUTPUT (14 SUBJECTS), ESTIMATED ON THE RAT UTERUS

The numbers of specimens studied are given within brackets.

Urinary Output (ml./min.)	Mean±s.e.
0·26 – 0·50	9·8±6·4 (36)
0·51 – 1·00	12·1±5·1 (30)
1·01 – 2·00	12·3±6·6 (47)
2·01 –18·00	10·9±5·1 (19)

The Effect of Urinary pH on Kinin Excretion

It was possible that kinin would be excreted at different rates depending upon the *p*H of the urine. The results obtained with 14 subjects were analysed and are shown in Table VI. The excretion rate of kinin was similar at each *p*H value.

TABLE VI

THE URINARY EXCRETION OF KININ (UNITS/MIN. AT DIFFERENT URINARY *p*H VALUES IN 14 SUBJECTS ESTIMATED ON THE RAT UTERUS

The numbers of specimens studied are given within brackets.

*p*H	Mean±s.e.
5·5	11·9±8·9 (16)
5·8	11·4±6·5 (42)
6·1	13·9±3·7 (13)
6·4	13·3±6·5 (8)
6·7	11·3±4·4 (8)
7·0	12·2±6·0 (6)
7·3	10·9±4·1 (12)

Diurnal Variation of Kinin Excretion

The mean urinary excretion of kinin in 13 overnight specimens was 10.3 (±4.2 s.e.) and in 13 control specimens collected during the day 12.0 (±4.6) units/min. All specimens were obtained from the same subject. These results provide no evidence of any diurnal variation.

It was observed that 3 overnight specimens, collected while the subject was taking sodium bicarbonate orally (urine *p*H 7.3), all contained small amounts of kinin (4.4, 2.6 and 5.3 units/min.). Day specimens which were *p*H 7.3 but were in the bladder for shorter periods did not have these low values.

The Effect of Food on Kinin Excretion

Since kinin is released during functional vasodilatation of the salivary glands (Hilton and Lewis, 1955, 1956), it was possible that the salivation occurring at meals might be associated with increased kinin excretion in the urine. In these experiments meals (usually 2 or 3 course) were not designed specially to promote excessive salivation. The bladder was emptied before the meal and urine was collected 1 to 2 hr. later. Urine specimens were collected over similar (fasting) periods 4 to 6 hr. after a full meal. The mean excretion of kinin in 10 urine specimens collected after meals was 10.9 (±4.2 s.e.) and in the 10 control specimens 13.4 (±8.5) units/min. All specimens were obtained from the same subject. These results showed that kinin was excreted at similar rates during the meal and control (fasting) periods.

The Effect of Sweating on Kinin Excretion

Fox and Hilton (1958) demonstrated the formation of plasma kinin during functional vasodilatation of the sweat glands. In view of this, the effect of sweating on urinary kinin excretion has been investigated. Sweating has been induced by pilocarpine and by heat.

Pilocarpine nitrate (9 mg.) was injected subcutaneously. After 2 min. facial flushing occurred and was quickly followed by profuse salivation and considerable sweating. The symptoms lasted 30 to 40 min. Urine was collected over the 1 to 2 hr. period before the injection and for a similar period after the injection. Three experiments were carried out on one subject. The mean urinary excretion of kinin was 12.1 (±6.0) units/min. before pilocarpine and 16.4 (±7.2) units/min. after a subcutaneous injection of 9 mg. of pilocarpine nitrate. Pilocarpine thus did not produce much change in the kinin excretion rate, although in

each experiment the value during the test period was slightly higher.

One experiment was performed in which sweating was induced by heat. The lower half of the subject was immersed in a hot bath (40 to 43°) for 1 hr. His oral temperature was 38°. To maintain the urinary output 150 ml. warm tea was drunk during the experiment. The excretion of kinin during the experimental period (21 units/min.) was little greater than the rate during the preceding control period (20 units/min.).

Discussion

The Sensitivity and Specificity of the Method

Since the rat duodenum and the rat uterus are so sensitive, only small quantities of kinin are necessary for adequate parallel assays. This is of particular importance for urinary estimations where the kinin concentration is relatively low.

A second advantage of this method is its specificity for kinins. Vasopressin and oxytocin were the only other substances found to be qualitatively similar on the two tissues, but their action on the duodenum required very high doses. In practice these peptides would be distinguished very easily by their much more powerful oxytocic action, and therefore by the lack of agreement between assays on the two tissues.

Biologically, the kinins are more closely related to substance P than to any other known peptide. Both are vasodilators and both stimulate plain muscle. However, the two peptides have opposite actions on the rat duodenum. Substance P produced a slow contraction, an observation which confirms that of Pernow (1953). The rat duodenum may prove to be a useful qualitative test object for distinguishing between these two peptides, but its sensitivity to substance P is low.

Angiotensin may have an inhibitory action on the duodenum in higher doses than those used in this investigation, but the index of discrimination is sufficiently great for it to be distinguished easily from kinin.

The results with bradykinin and urinary kinin are in agreement with more extensive parallel assays and it has been suggested that all these kinins may contain the same active principle (Gaddum and Horton, 1959). However, if the active principles are different, this method will not discriminate between them.

Wasp kinin (Jacques and Schachter, 1954; Schachter and Thain, 1954) and the plasma kinins are very similar; but wasp kinin is inactivated by trypsin and can be separated from the plasma kinins by paper chromatography (Holdstock *et al.*, 1957). They could not be distinguished by parallel assays on the rat uterus and the rat duodenum in the present investigation. If the active principles in wasp kinin and plasma kinin are different, this failure to discriminate is most significant. It suggests that the method described is unable to distinguish between closely allied but chemically different kinins.

Substances which act upon the rat uterus and the rat duodenum may be divided into four groups according to their effects: (1) stimulation of both tissues (namely, substance P), (2) inhibition of both tissues (such as adrenaline), (3) inhibition of the uterus and stimulation of the duodenum (for example, histamine), and (4) stimulation of the uterus and inhibition of the duodenum (like the kinins). Of the substances investigated, only vasopressin and oxytocin fall into the same group as the kinins, and they are easily distinguished. It follows therefore that if an active tissue extract is assayed in parallel on the rat uterus and the rat duodenum using plasma kinin as the standard, and the results agree, it is good evidence that the active principle is really a kinin.

Since it is conceivable, though improbable, that such agreement would occur if the unknown contained a mixture of say 5-hydroxytryptamine and adrenaline, the specificity can be confirmed by showing that the activity is destroyed by chymotrypsin incubation.

Interfering Substances

Biological estimates of the potency of an extract may be modified by the presence of impurities. These may have either an additive or an antagonistic action on the active principle being estimated. The qualitative difference in the responses of the rat uterus and the rat duodenum to kinin is particularly valuable in the detection of such interfering substances.

If the kinin extract contains an inhibitory substance such as adrenaline, its potency measured by the rat uterus will be low, because the kinin in the extract will be partially inhibited. On the other hand, the potency measured by the rat duodenum will be high, because both kinin and the impurity will inhibit that tissue and their effects will be additive. Thus, the discrepancy between results on these two tissues will tend to be more marked than with a pair of tissues which responded in a qualitatively similar manner, and smaller amounts of impurity will be detected.

One such impurity was soon detected when crude urine was used for the assays. This inhibitory substance could be removed by

alumina adsorption. However, there was still a slight discrepancy between the results on the two tissues, indicating that some other interfering substance was present.

Stability of Kinin in Urine

The loss of activity of urine specimens at room temperature is of great significance from a practical viewpoint. If a true figure for kinin excretion is to be obtained the urine must either be assayed within a short time of collection, or steps must be taken to prevent the inactivation. This generally precludes the possibility of estimating it in 24 hr. samples as collected clinically. The experiments in which urine specimens were protected from inactivation by *p*H adjustment or by previous boiling strongly suggest that an enzyme is responsible for much of the loss of activity in crude urine, confirming the findings of previous workers (Frey *et al.*, 1950). The action of the enzyme was prevented most conveniently by freezing the urine samples as soon as possible after collection.

The Excretion of Kinin in Human Urine

The results show that the rate of urinary kinin excretion is fairly constant. Conditions under which kinin is known to be formed in increased amounts were not associated with much change in the urinary kinin output. This suggests that the kinin released during glandular activity must be destroyed rapidly, before the blood levels rise sufficiently to alter the urinary content. Such inactivation by plasma occurs very readily *in vitro*, and is thus to be excreted *in vivo*. In addition, since a kininase is present in urine, some kinin destruction may occur in the bladder.

The question of the origin of urinary kinin remains unsettled. It may represent the renal clearance of kinin from the plasma. Human plasma which has been collected with precautions to prevent glass activation (pre-active plasma) does contain small amounts of kinin (Armstrong *et al.*, 1957) and this may represent the normal plasma level. On the other hand, it is possible that the kinin in urine is formed locally in the kidney and that it is a mediator of renal vasodilatation similar to its function in the salivary and sweat glands.

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