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KININS PRODUCED FROM BOVINE COLOSTRUM BY KALLIKREIN AND SALIVA

BY

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Substances capable of stimulating smooth muscle are produced on the incubation of bovine colostrum with urinary kallikrein or calf saliva. These substances, called urine- and saliva-colostrokinin, have been differentiated from kallidin, substance A and similar smooth muscle activating agents. Saliva-colostrokinin is likely to be formed in the suckling calf. Further, as colostrum became milk, the ability to form colostrokinin diminished. A function for saliva-colostrokinin in the newborn is suggested.

Kallikrein is a substance occurring in the urine, pancreatic juice, salivary glands, and saliva of many animals (Frey, Kraut, and Werle, 1950). Its properties indicate that it is an enzyme or mixture of enzymes similar to but not identical with trypsin (Frey *et al.*, 1950 ; Werle, 1955 ; Berg and Beeler, 1955). Kallikrein causes a fall in blood pressure and an inhibition of induced spasticity in the stomach, bronchi, and heart. Selected smooth muscle preparations are stimulated by kallikrein, but most of the more commonly employed smooth muscle organs remain unaffected (Frey *et al.*, 1950 ; Werle, 1955).

When kallikrein is incubated with whole plasma or with suitable plasma protein fractions such as the α_2 globulins or Cohn fraction IV-4, smooth muscle stimulating activity is developed. The substance causing this activity has been called kallidin (Frey *et al.*, 1950 ; Werle, 1955).

Evidence has accumulated indicating that kallidin belongs to the group of polypeptides called the kinins, such as wasp kinin, bradykinin, glass-activated kinin, and urinary kinin (Werle, 1953 ; Gaddum and Horton, 1959 ; Holdstock, Mathias and Schachter, 1957). In accordance with recent practice (Gaddum and Horton, 1959 ; Horton, 1958 ; Horton, 1959 ; Margolis, 1958 ; Lewis, 1958 ; Hilton and Lewis, 1958) any smooth muscle stimulating substance produced by the action of kallikrein on plasma protein will be called a plasma kinin.

Colostrum proteins appear to be derived from the proteins of plasma (Crowther and Raistrick, 1916 ; Askonas, Campbell, Humphrey, and Work, 1954 ; Larson and Gillespie, 1957). When kallikrein is incubated with colostrum a smooth muscle stimulating substance is produced (Werle, 1959 ; Guth, 1959). This substance has been called colostrokinin by Werle (1959). It will be called urine-colostrokinin in this report, to indicate the source of the kallikrein and to differentiate it from another kinin formed from colostrum protein.

Saliva or saliva constituents are capable of producing plasma kinins on incubation with plasma protein (Frey *et al.*, 1950 ; Hilton and Lewis, 1957). Saliva is physiologically incubated with colostrum by the newborn in the act of suckling. The kinin formed by the reaction of saliva and colostrum will be called saliva-colostrokinin. The formation of kinins by the reaction of colostrum and saliva is likely to be a common physiological occurrence. It is conceivable that the kinin formed in the saliva-colostrum mixture subserves some physiological function in the newborn.

METHODS

Rat Uterus.—The uteri from virgin rats (150 to 250 g.) were suspended in De Jalon solution in a 3 ml. bath at 30°.

Guinea-pig Ileum.—The terminal segments of ileum from guinea-pigs weighing 180 to 300 g. were suspended in Tyrode solution in a 3 ml. bath at 37°. Atropine sulphate (1 mg./l.) was added when spontaneous movements were not otherwise controllable.

Rat Duodenum.—The proximal segments of the duodenum from rats weighing 150 to 250 g. were suspended in De Jalon solution in a 3 ml. bath at

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30 to 37°. The tissue was stored at 4° for 2 to 3 hr. before use.

The dose cycle was usually repeated at 3 min. intervals. Using a slow continuous flow of fluid from below, the preparations seemed most responsive after a 30 sec. equilibration period during which the inflow was stopped. The substances under investigation were injected at the end of this equilibration period. The inflow was started 15 sec. after injection with the duodenum and 1 min. after injection with the ileum and uterus preparations.

Preparation of Human and Sheep Urinary Kallikreins.—Urine from men and Clun Forest wether sheep was put under toluene and frozen until used. The procedure for preparing kallikrein was that described by Gaddum and Horton (1959), with the exception that the urine was concentrated by placing it in dialysis bags and hanging them before a fan and heat source rather than by using a climbing film evaporator.

Plasma Kinin.—Human urinary kallikrein (1 mg./10 ml. mixture) was allowed to stand with acid-treated dialysed dog plasma for 2 hr. at room temperature. The mixture was then poured into twice its volume of boiling absolute alcohol. When boiling resumed, it was allowed to continue for 5 min. The supernatant fluid and the precipitate were separated by centrifugation for 15 min. at 2,500 revs./min. The precipitate was washed once by centrifugation with 66% (v/v) ethanol. The supernatant fluids were combined and their volume reduced by vacuum distillation at 50 to 60°. The final evaporation was performed in a lyophilizer. The dried mass was powdered in a mortar and passed through a No. 60 sieve.

Urine-colostrokinin. — Sheep urinary kallikrein (1 mg./10 ml. mixture) was allowed to stand with acid-treated dialysed bovine colostrum for 2 hr. at room temperature. The procedure for the preparation of plasma kinin was then employed.

Saliva-colostrokinin.—Dialysed calf saliva (1 ml./10 ml. mixture) was allowed to stand with acid-treated dialysed bovine colostrum for 2 hr. at room temperature. The procedure for the preparation of plasma kinin was then employed.

Acid treatment of plasma was found by Horton (1958) to inhibit kallikreinase and kininase without affecting kinin formation. Preliminary experiments indicated that acid treatment might not be necessary with colostrum. It was nevertheless used to make the kinins of plasma and colostrum more strictly comparable. The kinin produced by reaction of sheep urinary kallikrein and colostrum is called urine-colostrokinin. That produced by reaction of saliva and colostrum is called saliva-colostrokinin.

The saliva used in these experiments was always obtained from the calf whose dam kindly donated the colostrum.

Kallikrein Substrate Content of Colostrum.—The procedure outlined for the preparation of kinins was designed to promote maximum kinin formation. Excess kallikrein was used and the incubation period was about twice as long as necessary for the reaction to go to completion. The limiting factor of the reaction was therefore the concentration of substrate in the colostrum. The relative content of kallikrein substrate was estimated by assaying the kinins so produced against a standard plasma kinin.

Enzyme Destruction Studies.—Two different trypsin preparations were used (Armour and Co. and Light and Co.). The trypsin was incubated at a concentration of 100 μg./ml. with solutions of plasma kinin, urine-colostrokinin, and saliva-colostrokinin containing 1 mg./ml. Samples were taken and tested on the isolated rat uterus. Chymotrypsin (Sigma Chemical Corp.) was tested in the same manner. Appropriate controls were employed.

RESULTS

The production of a smooth muscle stimulating substance (saliva-colostrokinin) when dialysed calf saliva was incubated with acid-treated

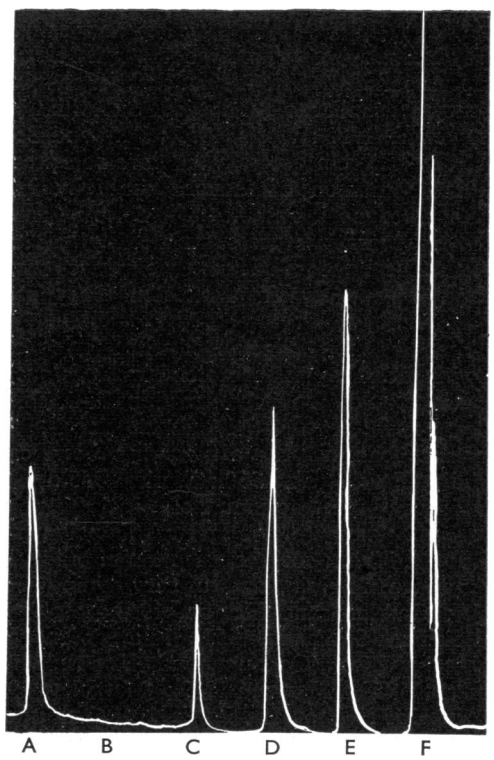

FIG. 1.—Production of smooth muscle stimulating substance (saliva-colostrokinin) in a mixture of saliva and colostrum. The isolated rat uterus in De Jalon solution is the test organ. At **A**, 100 μg. of standard plasma kinin was injected into bath; **B**, 0.2 ml. of dialysed colostrum was added; **C**, 0.1 ml. calf saliva. After C, 3 ml. calf saliva was mixed with 22 ml. colostrum. **D**, **E**, and **F** are the effects of 0.1 ml. of the mixture taken 1, 4, and 7 min. after mixing. Bath vol., 3 ml.

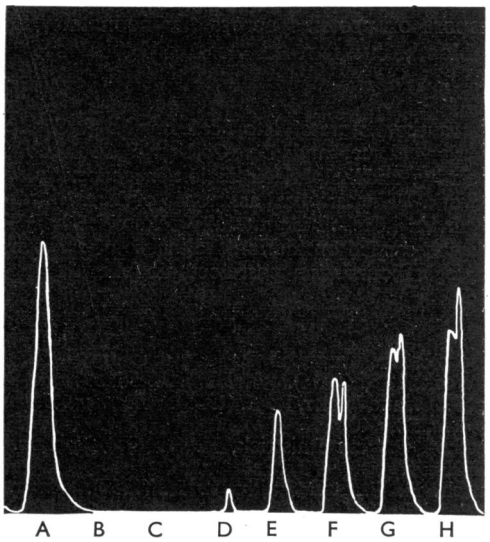

FIG. 2.—Production of smooth muscle stimulating substance (urine-colostrokinin) in a mixture of kallikrein and colostrum. The isolated rat uterus in De Jalon solution is the test organ. At A, 100 μg. of standard plasma kinin is injected into the bath; B, 0.2 ml. dialysed bovine colostrum; C, 0.1 ml. sheep urinary kallikrein (5 μg./ml.). After C, 5 ml. of sheep urinary kallikrein solution (1 mg./ml.) was added to 10 ml. of dialysed acid-treated bovine colostrum. D to H are the effects of 0.1 ml. of incubation mixture taken every 3 min.

dialysed bovine colostrum is shown in Fig. 1. Fig. 2 illustrates the production of a smooth muscle stimulating substance (urine-colostrokinin) when sheep urinary kallikinin was incubated with acid-treated dialysed bovine colostrum.

The experiments in which plasma kinin, urine-colostrokinin, and saliva-colostrokinin were incubated with proteases showed that chymotrypsin, as had been expected, destroyed the activities of all three kinins. The Armour trypsin preparation destroyed none of them. As the trypsin preparation of Light and Co. destroyed the activity of plasma kinin without affecting that of urine-colostrokinin or saliva-colostrokinin, it is suggested that there may be a basic difference between plasma kinin and the colostrokinins.

TABLE I

PARALLEL ASSAYS OF PLASMA KININ, URINE-COLOSTROKININ, AND SALIVA-COLOSTROKININ

Milligram doses equiactive with 1 mg. of plasma kinin on three smooth muscle preparations are given. These values are the means of at least four six-point assays. The numerals in parentheses indicate the range. I.D. is the Index of Discrimination obtained by dividing the value for one organ by that obtained on a different organ. An I.D. near unity indicates no detectable differences between the substances being assayed.

	Saliva-colostrokinin	No. of Assays	Urine-colostrokinin	No. of Assays
Rat uterus ..	44 (25–50)	6	6·9 (6·6–7·5)	4
,, duodenum ..	16·5 (10–25)	4	6·0 (4·0–8·0)	4
Guinea-pig ileum	8·1 (7·7–10·0)	5	4·5 (3·3–6·0)	4
I.D. (rat uterus/ rat duodenum)	2·7		1·1	
I.D. (rat uterus/ guinea-pig ileum)	5·4		1·5	

Table I summarizes the results of parallel assays. The index of discrimination (Gaddum, 1955) provides a measure of the dissimilarity between possibly-related substances. An index near unity indicates that the test preparations used are incapable of discriminating between the substances under test. These results show that plasma kinin and urine-colostrokinin are more nearly identical than are plasma kinin and saliva-colostrokinin. The differences between plasma kinin and saliva-colostrokinin are, however, relatively small and may be reconciled when purer preparations of the kinins become available.

From Fig. 3 it may be seen that, as bovine colostrum becomes milk, the kallikrein substrate concentration diminished. This diminution coincided in the calf with the time at which the permeability of the gut to proteins diminished (Deutsch and Smith, 1957).

Substance A (Huggins and Walaszek, 1959) is produced by incubating plasma protein fraction IV-4 with salivary α-amylase. It might seem a good candidate for comparison with saliva-colostrokinin. Three considerations militate against the identity of substance A and saliva-colostrokinin: substance A is destroyed by trypsin while saliva-colostrokinin is not (Walaszek and

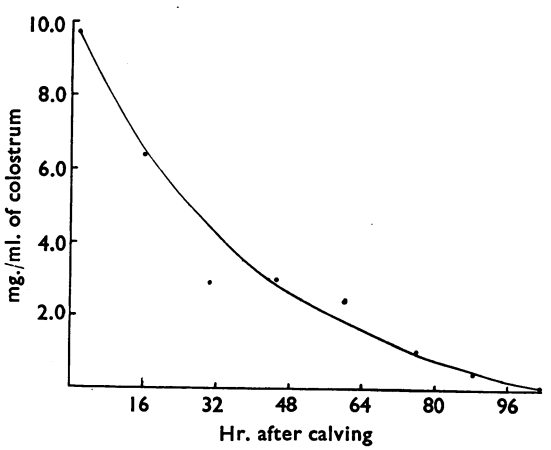

FIG. 3.—Diminution in kallikrein-substrate as bovine colostrum becomes milk. Samples of bovine colostrum taken at various intervals after calving were incubated with sheep urinary kallikrein. The urine-colostrokinin produced was assayed against plasma kinin, and the relative activities produced expressed as mg. equivalents of plasma kinin are plotted against time.

Huggins, 1959); the content of α-amylase in calf saliva is very low (Matsuoka, 1937); and an indirect comparison between substance A and the colostrokinins on the isolated rat duodenum suggested they were different. The rat duodenum preparation often responded to substance A in a biphasic manner. The colostrokinins were pure relaxants.

Discussion

The relaxation of the isolated rat duodenum by the colostrokinins permits them to be distinguished from substance P, acetylcholine, histamine, angiotensin, and 5-hydroxytryptamine. Adenosine and adenosine monophosphate both relax the isolated rat duodenum, but never to the same extent as the kinins (Horton, 1959). The doses of vasopressin and oxytocin that relax the rat duodenum are at least one hundred times greater than those necessary for contraction of the isolated rat uterus (Horton, 1959). The kinins are about equipotent on the two preparations. On this single preparation, the isolated rat duodenum, the kinins may be distinguished from many smooth muscle activating agents.

Kallikrein is probably the prime agent in saliva responsible for the production of saliva-colostrokinin. Hilton and Lewis (1957) and Holdstock *et al.* (1957) have used whole saliva or saliva extracts for the activation of plasma proteins. Werle (1955) and Frey *et al.* (1950) have demonstrated the presence of kallikrein in saliva. They have also shown that the kallikreins of saliva and urine are different (Frey *et al.*, 1950; Werle, 1955). The protein substrates of colostrum and plasma are probably not identical. Urinary kallikrein acting on plasma and on colostrum produces two kinins, one of which is destroyed by a single trypsin preparation. Considering that the substrates in colostrum and plasma may be different and that the kallikrein of urine and saliva are different, it is not surprising that small differences appeared in parallel assay experiments. It is too early to be certain that saliva-colostrokinin and urine-colostrokinin are identical.

Kinins are capable of increasing capillary permeability (Holdstock *et al.*, 1957). By this property saliva-colostrokinin might aid in the transport of proteins across the neonatal gut to the blood, but further work on this aspect is needed.

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