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Research Article

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The concentration of bradykinin in the cord blood of 56 newborn infants at the time of birth was significantly higher than the blood level in adult subjects (12.8 ± 4.3 ng/ml compared with 2.0 ng/ml or less). Cord arterial blood contained inactive kinin precursor (kininogen) and inactive kinin-releasing enzyme (kallikrein). Plasma kallikrein was activated, with subsequent kinin formation and kininogen depletion, by exposure to neonatal granulocytes or by a decrease in the temperature of cord blood from 37 to 27°C. A comparable decrease in the temperature of umbilical arterial blood occurs at the time of birth.

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Thus, bradykinin [...]

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Kinins: Possible Mediators of Neonatal Circulatory Changes in Man

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ABSTRACT Bradykinin is a potent constrictor of the human umbilical artery and vein and the ductus arteriosus of the lamb in vitro at oxygen tensions above 40 mm Hg (comparable to those in the newborn infant). Bradykinin is also capable of producing remarkable dilatation of the pulmonary vasculature of the lamb. Theoretically, kinins are capable of effecting some of the rapid circulatory changes required of the neonate. The present study was undertaken to investigate the role of kinins as mediators of such changes.

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Thus, bradykinin can constrict and dilate vessels as required for the transition of fetal to neonatal circulation. Bradykinin can be produced in plasma of the newborn by decreases in temperature, such as occur in the umbilical blood at birth, and by exposure to granulocytes which are present in the circulation in increased numbers shortly after birth. We propose that bradykinin is produced at birth and may be a mediator of neonatal circulatory changes.

INTRODUCTION

A rise in partial pressures of oxygen and mechanical alteration of vessels are thought to be the primary events leading to constriction of the ductus arteriosus and umbilical arteries and veins at birth. The same events, together with physical expansion of the lungs, are postulated as being responsible for dilatation of the pulmonary vascular bed (1). Some evidence, however, indicates that these factors do not adequately account for all the circulatory changes at birth, e.g. in the lamb, the umbilical cord vessels are constricted poorly by oxygen (2). Knowledge of the mechanisms of these events is of obvious importance in understanding normal and abnormal circulatory physiology in neonates. The chemical mediators of such changes have not been defined. For example, sympathetic nerve endings are not seen in the umbilical cord beyond the first few centimeters proximal to the umbilicus (3). Norepinephrine has not been found in human umbilical blood vessels¹ (4). With the

¹ Goldfien, A. Personal communication.

possible exception of histamine, the vasoactive substances found in man (e.g. acetylcholine, epinephrine, norepinephrine, and serotonin) do not have pharmacologic effects that would produce all the vascular changes seen at birth (5-7). Finally, the release or production of vasoactive amines and peptides has not been correlated with either mechanical events at birth or the increase in oxygen pressure (P_{O_2}).

Recently, the peptide bradykinin, the most potent endogenous vasodilator known in man, has also proved to be a potent dilator of pulmonary vessels in the fetal lamb and a constrictor of the ductus arteriosus in the fetal lamb, calf, and guinea pig (8, 9), and of human umbilical vessels (4, 8). Constriction in vitro of the umbilical vessels by kinin is minimum at a P_{O_2} corresponding to fetal oxygen concentrations and near maximum at oxygen concentrations found in the newborn² (8). The kinin receptors are independent of histamine, serotonin, and classical alpha and beta adrenergic receptors but cannot be distinguished from oxygen receptors. These data served as the basis for the present studies, which have characterized the kinin-generating system in the human neonate, established the presence of significantly increased concentrations of kinin in umbilical venous and arterial and placental venous blood samples taken at birth, and defined two physiologic changes in the newborn infant (release of granulocytes from the lung and cooling of the blood in umbilical vessels) that may possibly be responsible for initiating kinin generation. These observations strongly suggest that kinins may be mediators of neonatal circulatory changes.

METHODS

Clinical material. The subjects consisted of 56 newborn infants. Their mothers, ranging in age from 14 to 38 yr, were hospitalized at the University of California Medical Center, San Francisco, or Cook County Hospital, Chicago. When possible, anesthetic and analgesic agents were withheld from the mothers for 15-60 min preceding delivery (20 patients); in the remaining patients local anesthesia was used. Samples of blood were drawn from the umbilical veins and arteries of the infants immediately after spontaneous birth (53 cases) or delivery by caesarian section (3 cases). Blood samples were not taken un-

² Eltherington, L. G., T. Hughes, J. Stoff, and K. L. Melmon. Oxygen and bradykinin interaction on pharmacologic receptors of human umbilical cord blood vessels. In preparation.

til the infant had breathed, and most were obtained at the time the pulsations of the cord vessels were decreasing. The temperature of blood in the umbilical veins was measured with a thermistor probe. Control specimens of venous blood were obtained from the mothers.

Chemical assays. Blood for bradykininogen and kininase determinations was drawn through siliconized needles into warmed polyethylene syringes containing sufficient heparin to make a final concentration of 1 U/ml. The specimens were centrifuged in a warmed centrifuge, and the plasma was removed with siliconized or polyethylene pipettes. Plasma bradykininogen was determined by the method of Diniz and Carvalho (10). Plasma kininase activity was assayed by following the curve of disappearance of 10 μ g of synthetic bradykinin incubated with 0.1 ml of the test plasma and 1.8 ml of 0.1 M Tris buffer, pH 7.4. 0.2-ml samples of the incubation mixture were removed at 30 sec, 10 min, and 20 min and inactivated with 1.8 ml of 0.2% acetic acid and 5 ml of boiling ethanol. The inactivated samples were then concentrated to dryness in a flash evaporator at 37°C. The residue was reconstituted with saline to a volume of 2 ml, and the remaining bradykinin was assayed on the estrus rat uterus. The reliability of the kininase assay was determined by making eight separate kininase measurements on a sample of normal plasma. The mean of the eight measurements was 99%, with a standard deviation of 5%.

Whole blood kinin was measured by a modification of the method of Webster and Gilmore (11), which allows separation of kinin peptides from basic amines. Samples of blood, 10 ml each, were drawn into syringes containing 20 ml of 0.5 M perchloric acid. The resulting mixtures were centrifuged at 3500 g at 0°C for 20 min. The supernatant of each was adjusted to pH 7.5 with 2 N KOH and re-centrifuged at 3500 g at 0°C for 10 min. The supernatant was then added to 10 ml of deionized water, adjusted to pH 5.4 with HCl, and applied to an ion exchange resin (IRC-50 H⁺) column (0.6 × 1.5 cm). The resin had been allowed to settle overnight with 0.05 M ammonium acetate, pH 5.4, at 4°C. The column was washed with 15 ml of 0.1 M ammonium phosphate buffer, pH 6.6; the peptide was eluted first with 3 ml of a solution of 0.5 M sodium chloride-0.5 M ammonium formate, pH 9, then with 3 ml of a solution of 0.25 M sodium chloride-0.25 M ammonium formate, pH 8.8. The eluate was adjusted to pH 7.5 and assayed for kinin on the estrus rat uterus (11). In each assay a known amount of synthetic bradykinin³ was added to one sample for calculation of the percentage recovery of kinin peptide. Recovery of added bradykinin from different experimental batches varied from 40 to 75%, and the results of assays on duplicate blood samples varied by less than $\pm 8\%$. Other uterine-contracting peptides were not present in the eluate as determined by chymotrypsin and trypsin incubation.

Studies with granulocytes. The isolation of leukocytes and the conditions of in vitro incubation have been de-

³ Synthetic bradykinin-SBR 640 supplied by Sandoz Pharmaceuticals, Hanover, N. J.

scribed in detail (12). As used here, the term leukocytes refers to populations of cells consisting of at least 80% granulocytes and metamyelocytes. In leukocyte counts on umbilical arterial blood the mean value was $18,000 \pm 2300$ cells/ml, with $56 \pm 4\%$ granulocytes. Eagle's minimal essential medium containing human serum albumin (25 mg/ml) was used as the suspending medium. Because kinins are simultaneously released from substrate and destroyed by the kininase activities of plasma and granulocytes, their concentration at any time is a function of these two opposing processes (13). Therefore, the decrease in the concentration of kininogen was used as an index of the total generation of kinin.

To determine the oxygen dependence of leukocyte kinin production, isolated leukocytes at a concentration of 2×10^7 cells/ml were preincubated with mixtures of nitrogen or CO_2 and 95, 20, 10, or 0% oxygen in a Dubnoff metabolic shaking incubator. The cells were then incubated with autologous plasma (33% by volume) containing 2 U of heparin per ml. In certain experiments isologous plasma of compatible major blood group was used.

Kininase activity was assayed by incubating well-washed granulocytes, 3×10^6 cells/ml, with synthetic bradykinin, 2 ng/ml, for 10–60 min. Residual kinin was then determined as described previously (14).

Temperature studies. The effects of decreasing temperatures on the conversion of kininogen to kinin was determined as follows. 10-ml samples of blood were drawn into plastic or siliconized syringes, which had been prewarmed to 37°C and contained heparin, 1 U/ml. The blood was immediately transferred to polypropylene test tubes in a 37°C water bath. A sample from each tube was then placed in a 27°C water bath and reached that temperature within 10 min. Samples from the tubes at 37°C were taken at 0 and 10 min and from the tubes at 27°C at 10 min. The plasma was rapidly separated from each sample and assayed for kininogen. In some experiments neutralized sodium ethylenediaminetetraacetate (EDTA) was added to the blood samples (final concentration, 2×10^{-3} mole/liter) to prevent destruction of formed kinin. Aliquots were taken before and after cooling to 27°C and assayed for kinin concentration. In three additional experiments, plasma was separated from whole blood at

37°C . Samples were cooled quickly to 36°C and then assayed for kininogen concentration.

Plasma kallikrein activation. 4-ml samples of plasma from the newborn infants and their mothers were shaken with glass beads as described previously (13), and kallikrein activation was assessed by kininogen depletion, the appearance of free kinin, or both. Additional samples were rapidly acidified to pH 3 by the addition of 2 M HCl and assayed for kinin activity.

Assays for Hageman factor were performed by a standard technique (15).

RESULTS

Kallikrein-kinin-kininase system in plasma and granulocytes of neonates. As shown in Table I, the mean concentration of kininogen in neonatal plasma (1753 ± 189 [SEM] ng/ml) was significantly less ($P < 0.001$) than that in adult plasma (4717 ± 323 [SEM] ng/ml), but if completely converted to plasma kinin, the concentration would be about 1000 times the physiologic level. Despite a normal concentration of Hageman factor (neonate, 48–96%; adult, 50–150%), neonatal plasma kallikrein, unlike adult plasma kallikrein, could not be activated by exposure to glass surfaces. The kallikrein of both neonatal and adult plasma, however, was activated by acidification. Kininase activity (percentage destroyed in 30 min) was not significantly lower in neonatal plasma, $38 \pm 10\%$, than in adult venous plasma, $54 \pm 19\%$, $P > 0.5$ (Table I).

Neonatal granulocytes in concentrations of 0.5×10^6 cells/ml or greater produced kinin and consumed kininogen (Table II). The ability of neonatal cells to consume kininogen was dependent on cell concentration and was equal to that of adult cells. Kininogen consumption by granulocytes also was dependent on the oxygen content of the incubation mixture. At partial pressures produced by

TABLE I
*Kinin System in Neonatal and Adult Plasma**

	No. of experiments	Neonatal plasma	Adult plasma	Significance of difference
Kininogen, ng/ml	12	1753 ± 189	4717 ± 323	$P < 0.001$
Kallikrein, % residual kininogen				
After glass activation	9	97 ± 2	39 ± 11	$P < 0.01$
After acidification	10	47 ± 6	42 ± 4	$P > 0.05$
Kininase, % control value‡	10	38 ± 10	54 ± 19	$P > 0.5$

* Values are expressed as the mean \pm standard error of the mean.

‡ After 10 min incubation.

TABLE II
Kinin System in Neonatal and Adult Granulocytes*

	No. of experiments	Cell concentration per ml	Neonatal granulocytes, at birth	Adult granulocytes	Significance of difference†
Kallikrein, % residual kininogen after activation	5	0	100 ± 3	97 ± 3	<i>P</i> > 0.5
	5	0.5 × 10 ⁶	81 ± 4	80 ± 5	<i>P</i> > 0.5
	5	2.5 × 10 ⁶	61 ± 8	59 ± 3	<i>P</i> > 0.5
	5	5.0 × 10 ⁶	52 ± 12	55 ± 7	<i>P</i> > 0.5
	5	25.0 × 10 ⁶	36 ± 4	23 ± 9	<i>P</i> > 0.5
Kallikrein, at oxygen environments of		3 × 10 ⁶			
0	4		88 ± 5	98 ± 3	<i>P</i> > 0.5
5%	3		84 ± 2	90 ± 14	<i>P</i> > 0.5
10%	3		55 ± 11	60 ± 15	<i>P</i> > 0.5
20%	4		62 ± 8	62 ± 12	<i>P</i> > 0.5
95%	4		53 ± 13	64 ± 8	<i>P</i> > 0.5
Kininase, % of control value§		6 × 10 ⁷			
	6		90 ± 10.4	} 30 ± 4	<i>P</i> > 0.7
	6		81.6 ± 22		<i>P</i> < 0.02
	10				

* Values are expressed as the mean ± standard error of the mean.

† The difference in kininogen concentration of neonatal and adult granulocytes at a given oxygen environment is not significant. The significance of difference between values at 0 or 5% oxygen and at 10, 20, and 95% is at least <0.05.

§ After 10 min incubation.

|| Granulocytes were obtained 3 days after birth.

less than 5% O₂ (producing a Po₂ of less than 35 mm Hg), kininogen consumption was negligible. A significant increase in kininogen consumption occurred when the gas mixture contained 10% or

more oxygen (producing a Po₂ greater than 65 mm Hg), but increasing O₂ concentrations above 10% did not produce a further significant increase in kininogen conversion when cell concentrations

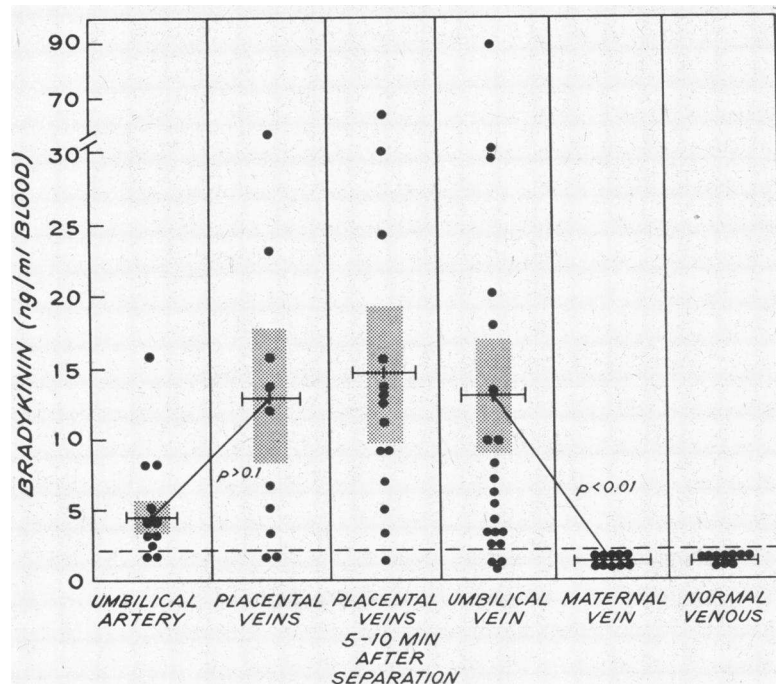


FIGURE 1 Whole blood kinin concentrations. Each point represents the average of two separate determinations. Recovery of standard ranged from 40 to 75%.

TABLE III
Effect of Temperature on Plasma Kininogen Concentration*

Temperature of blood‡	No. of experiments	Kininogen concentration		Significance of difference
		Neonatal plasma	Adult plasma	
°C			ng/ml	
37	4	2071 ± 233	4500 ± 560	} P < 0.05 } P < 0.001
36§	3	1162 ± 180	2981 ± 162	
27	4	722 ± 421	2140 ± 29	

* Values are expressed as the mean ± standard error of the mean.

‡ All samples were assayed after 10 min at indicated temperature.

§ Samples were rapidly cooled from 37° to 36°C.

remained at about 3×10^6 /ml. The kininase activity of granulocytes obtained at birth and 3 days after birth was significantly less than that of adult cells (Table II).

Kinin concentration of umbilical venous and arterial blood and placental venous blood. Bradykinin concentrations were significantly higher in cord blood (12.8 ± 4.3 ng/ml) than in adult blood (< 2.0 ng/ml [$P < 0.01$]). In 8 of the 56 samples, the values were within normal limits; in the remainder the concentrations covered a wide range, in some instances rising as high as 45 times normal. The kinin concentrations of umbilical arterial, umbilical venous, and placental venous blood did not differ significantly (Fig. 1). No difference could be found in the kinin levels of blood obtained from infants delivered by caesarian section and those born spontaneously. Perhaps this was because we were unable to obtain blood samples before the infant had breathed. Administration of local or systemic anesthetic agents to the mother did not consistently alter the kinin concentration of

the blood samples from any source. When sampling from placental veins was delayed until 5 or 10 min after placental separation, however, kinin concentrations were still elevated.

The temperature of umbilical arterial blood (in all five infants tested) decreased from $37 \pm 0.5^\circ\text{C}$ to $26 \pm 2.1^\circ\text{C}$ within 10 min after delivery of the child and exposure of the cord to the atmosphere ($P < 0.001$). Flow through the vessels was indicated by continued pulsation of the artery. Quantitation of the flow was not attempted. The effects of decreases in temperature on the conversion of kininogen to kinin are shown in Table III. Temperature drops of 1 or 10°C in umbilical blood resulted in significant conversion of kininogen.

DISCUSSION

The kinin-generating system (Fig. 2) is present in the umbilical blood of newborn infants, but it clearly differs from the system in the plasma of adult subjects in at least two ways. First, the concentration of kininogen in the plasma of infants at

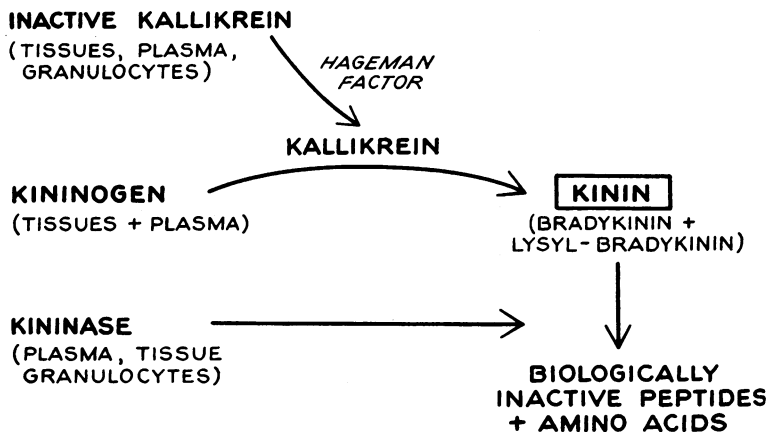


FIGURE 2 Schematic diagram of the kallikrein-kinin-kininase system in plasma and granulocytes. The system in the neonate differs from that in the adult primarily by the decreased kininogen concentration, lack of glass activation of plasma kallikrein, and lower granulocyte kininase activity.

birth is about half the level in adult blood. Whether this low concentration results from rapid consumption of kininogen at birth or slowed formation in the fetus cannot be stated at present. Second, the kallikrein of neonatal plasma is only minimally activated by exposure to glass, a procedure that activates Hageman factor. Activation of Hageman factor has been considered requisite to kallikrein activation in adult plasma (16). Hageman factor is present in the plasma of newborn infants, and we can assume that it is activated normally. Kallikrein is also present in neonatal plasma, as evidenced by its activation when the plasma is acidified. Thus, it is possible that an additional intermediate present in adult but not in neonatal plasma is necessary for glass activation of plasma kallikrein or, alternatively, that the kininogen susceptible to the kallikrein activated by Hageman factor has already been consumed. Our data suggest either that the activation of kallikrein in the newborn's plasma produced by temperature changes is not dependent on Hageman factor, or that the Hageman factor of fetal blood has different biochemical properties from the factor in adult blood.

The kininase activity of plasma from the neonate was not significantly lower than that of adult plasma. Apparently neonatal plasma has the ability to destroy formed kinin even though the granulocyte is not able to do so. Granulocytosis occurs in fetal blood, reaches a maximum 6–12 hr after birth, and gradually decreases over a 2–4 day period, after which a relative lymphocytosis predominates (17, 18). It may be postulated that the source of granulocytes is the lung, since the lung may be a trap for granulocytes (19), and since excursion of the lung can greatly influence the concentration of circulating cells (20). The granulocytes of neonatal and adult blood were equally able to produce kinin. In previous studies we showed that kinin production by granulocytes requires oxidative activity of the cells (21). In the present study, kininogen depletion by the granulocytes was also found to be dependent on the oxygen environment. At oxygen concentrations found in the fetus (P_{O_2} of 15–35 mm Hg [22]), granulocytes were unable to release kinin. The maximum increase in kinin production took place at a P_{O_2} equivalent of 50–80 mm Hg, the level found in the newborn infant (22).

The kinin-generating system in the neonate ap-

pears to be active. Kinin concentrations in all neonatal blood samples (umbilical and placental) were above normal and were equivalent or greater than those found in patients with carcinoid syndrome during a flush (23, 24). Such concentrations of kinin would be sufficient to produce pharmacologic effects (25) and could certainly contribute to a decrease in pulmonary and systemic vascular resistance, as well as to constriction of vessels key to neonatal circulatory adaptation. The source of kinin may be the blood vessels, the fetal organs, or the plasma itself. Since the kinin concentration of umbilical arterial and umbilical venous blood did not differ to any significant extent, it seems unlikely that the placenta is a major contributor of kinin.

One mechanism for kinin production in the neonate, but not in the fetus, is the release of granulocytes into an oxygen-rich environment. Another mechanism may be the rapid decrease in the temperature of umbilical vessel blood at the time of birth, resulting in activation of plasma or tissue kallikrein and consequent kinin generation. Armstrong, Mills, and Sicuteri showed that wide changes in temperature (0–27°C and 37–0°C) resulted in depletion of plasma kininogen (16). In the present study, even a slight drop in temperature (37–36°C) produced a decrease in kininogen concentration. A 10°C temperature change, corresponding to the decrease in the temperature of cord blood shortly after birth, resulted in significant depletion of kininogen ($P < 0.05$). Whether a physiologic function of Wharton's jelly is to radiate sufficient heat to lower blood temperature, thereby encouraging kinin formation, or to transport oxygen, thereby producing a favorable environment for granulocyte production of kinin, is yet to be determined. Of interest, however, is the fact that an omphalocele is composed largely of Wharton's jelly, and that a common complication of such a congenital abnormality recognized at the University of California Medical Center is rapidly progressive hypothermia and shock.⁴ The umbilical vessels themselves contain unidentified substances that may effect their constriction; the substances so far studied, however, do not appear to be kinins (26), and kallikrein has not yet been sought in the vessel wall or Wharton's jelly.

⁴ De Lorimier, A. Personal communication.

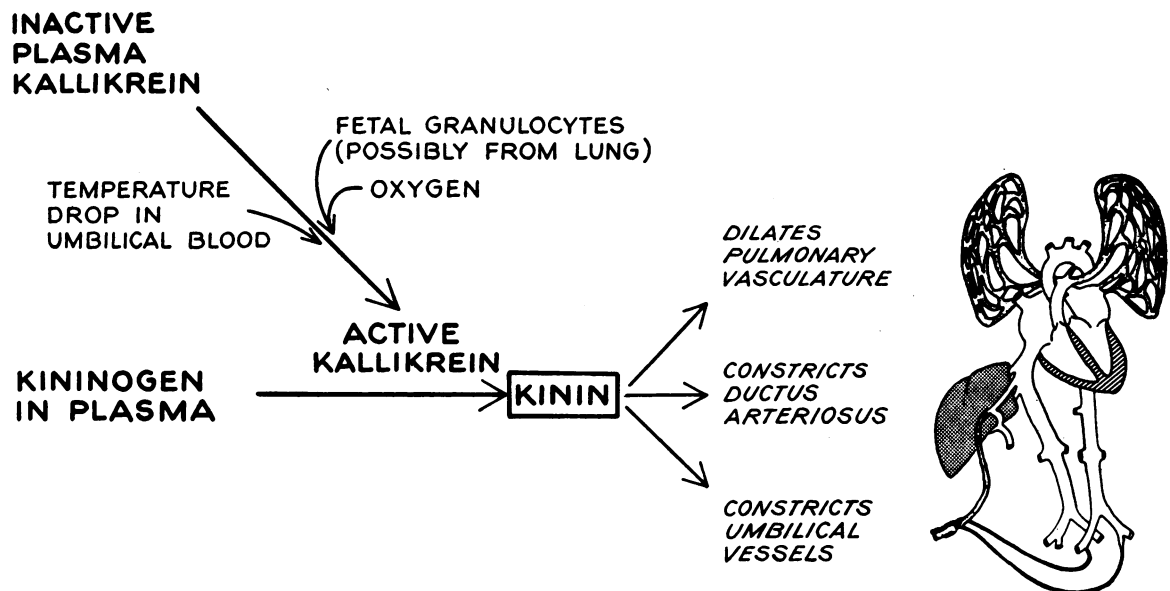


FIGURE 3 Schematic diagram of the mechanisms involved in kinin generation and possible effects of kinins on neonatal circulatory adjustments.

Evidence that kinins play an important role in neonatal circulatory adjustment is as follows: (a) The pharmacologic effects of kinin on the cardiovascular system reproduce some of the physiologic events of birth. Kinins dilate the pulmonary arterioles in the lamb and in vitro constrict human umbilical vessels and the ductus arteriosus of the calf, lamb, and guinea pig. (b) The kinin-generating system, although differing in some respects from the system in adult plasma, does exist in umbilical blood plasma. (c) Kinin concentration in the umbilical vessels, and presumably in other fetal vessels, is sufficient to produce pharmacologic effects. The kinins may be generated by at least two physiologic events unique to the neonate: temperature decreases in the umbilical circulation and granulocytosis (Fig. 3). We propose that plasma kallikrein is activated by a drop in the temperature of umbilical blood at or shortly after birth, that kinins may also be generated by neonatal granulocytes (possibly released from the lungs) on exposure to adequate oxygen, and that, in turn, kininogen is consumed and active kinin produced in sufficient quantities to contribute to dilatation of the pulmonary vasculature and constriction of the ductus arteriosus and umbilical vessels. Kinins may not be solely responsible for neonatal adaptation at birth because there was no increase in

kinin concentration in the umbilical blood of some infants who had normal neonatal periods and in whom the ductus arteriosus eventually closed. Whether raising the partial pressure of oxygen or expanding the lung alone could increase kinin generation is not known. Nor is it known whether prevention of heat loss from the cord, administration of enzymes that destroy kinins, (e.g., carboxypeptidase B [27]) or drugs that interfere with the peripheral effects of kinins would alter adaptive changes in the newborn. Whether the kinin-generating or kinin-destroying system is abnormal in newborn infants with respiratory distress, congestive heart failure, or a persistent patent ductus arteriosus also remains to be investigated.

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