23 Sachs, F., Neil, J., and Bakakati, N., The automated analysis of data from single ionic channels. Pflügers Arch. *395* (1982) 331–340
24 Schneider, M. F., and Chandler, W. K., Voltage-dependent charge movement in skeletal muscle: A possible step in excitation-contraction coupling. Nature *242* (1973) 244–246.
25 Sigworth, F. J., The patch clamp is more useful than anyone had expected. Fedn Proc. *45* (1986) 2673–2677.

0014-4754/88/030183-06$1.50 + 0.20/0

Casein, a prohormone with an immunomodulating role for the newborn?

D. Migliore-Samour and P. Jollès

Laboratoire des Protéines, Unité associée du C.N.R.S. 1188, Université de Paris V, 45 rue des Saints-Pères, F-75270 Paris Cedex 06 (France)
Received 16 October 1987; accepted 16 December 1987

Summary. Maternal colostrum and milk, the earliest food of the newborn, should not only be considered as supplying nutrients, but also as agents providing protection against aggressions from the new environment. Indeed by enzymatic digestion of the main milk proteins, the caseins, biologically active peptides are released; they may be implicated in the stimulation of the newborn's immune system. From this point of view a 'strategic active zone' has been characterized in β-casein. A possible role of casein as a 'prohormone' for the newborn is suggested.
Key words. Casein; immunomodulation; casomorphin; immunomodulators.

After birth, the newborn enters into a temporary period during which its immature immune system still depends on maternal help, particularly when host defenses are considered. Its polymorphonuclear leukocytes (PMNL) have a decreased chemotaxis function and deformability of their membranes, and show both depressed oxidative metabolic responsiveness and lower bactericidal activity, compared to adult PMNL[37,38]. The percentage of T lymphocytes is significantly lower in the first days after birth in healthy neonates compared to adult controls[51]. Even though the number of suppressor T cells has been reported to be significantly lower in cord blood compared to adult blood, with a corresponding increase in the proportion of helper T cells[19,57], global helper function manifested by newborn T cells is low compared to their suppressive function[18]; newborn B cells differentiate into plasmocytes in response to pokeweed mitogen stimulation less well than adult B cells and synthesize exclusively IgM[18]. Nevertheless, the newborn must protect himself from the aggressions of its new environment, and breast-feeding facilitates transmission of a passive immunity.

Multifunctional factors contained in the maternal colostrum and milk have a direct effect on the newborn's resistance to bacterial and viral infections and on the harmonious development of the bacterial flora of the gut. High levels of immunoglobulins are present, mainly secretory IgA (sIgA) but also IgM and IgG[14]. Enzymes such as lysozyme (EC 3.2.1.17)[1,24] and peroxidase (EC 1.11.1.7)[15] and iron-binding proteins such as lactoferrin and transferrin[23] play important bactericidal and bacteriostatic roles. Other factors are of cellular nature, such as macrophages, granulocytes, T and B lymphocytes[21,42]; they promote humoral and cellular immunity against enteric bacteria (like *E. coli*) and favor indirectly growth of *Bifidobacterium bifidum* type IV which is able to transform lactose into glucose and lactic acid.

Meanwhile the newborn immune system must try to establish its autonomy. We suggest that its evolution may also be influenced by milk components or their degradation products. The most abundant milk protein, casein, has been found to generate, during enzymatic digestion, short peptides endowed with biological activities. We wish to discuss their possible involvement with the immune system.

Casein consists of several proteins, αs_1-, αs_2-, β- and κ-caseins which are associated into micelles in milk; its coagulation is triggered by the action of chymosin on κ-casein. Bovine casein contains 45% α-casein, 30% β-casein and 15% κ-casein; human casein which contains 20% κ-casein differs by its high level of β-casein (50%) in contrast with its low content of α-casein (10%)[10].

Immunostimulating casein peptides

Assuming that the first food of the newborn might contribute to its natural immunostimulation thanks to peptides from milk proteins, we decided to submit tryptic or chymotryptic fragments of human caseins to some tests of biological activity. We chose two in vitro screening tests: phagocytosis of opsonized sheep red blood cells (SRBC) by resident peritoneal mouse macrophages and secretion of hemolytic antibodies by spleen cells from mice which have been immunized in vivo by SRBC, and also an in vivo test: protection of mice against *Klebsiella pneumoniae* infection. Several fractions were found to be active in these tests[25] and we purified two peptides, an hexapeptide Val-Glu-Pro-Ile-Pro-Tyr (residues 54–59 of β-casein)[44] and a tripeptide Gly-Leu-Phe, not yet located in the known sequences of human caseins[5]. These peptides stimulated phagocytosis of mouse macrophages at a concentration as low as 0.1 μM (table 1) and exerted in mice a protective effect against *Kl. pneumoniae* infection when injected intravenously at 0.3 and 1 mg/kg, 24 h before lethal infectious challenge (table 2). An analogue of the tripeptide, Gly-Phe-Leu (residues 60–63 of human β-casein, just following the hexapeptide) exhibited weaker but significant activities. We also demonstrated that these peptides stimulated human macrophages to phagocytize senescent human red blood cells[12].

Other biological active casein peptides implicated in immunomodulation

Inhibitors of angiotensin I – converting enzyme

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) catalyzes the production of the vasoconstrictor angiotensin II as well as the inactivation of the vasodilatator bradykinin and of the enkephalins in the guinea pig ileum[3]. Inhibitors of this enzyme might increase bradykinin and enkephalin activities. Bradykinin, known as a mediator of the acute inflammatory process, is able to stimulate macrophages, to enhance lymphocyte migration and to induce the secretion of lymphokines from lymphocyte cultures[43]. Furthermore, the

Table 1. Stimulation by casein peptides of phagocytosis of sheep red blood cells (SRBC) by murine peritoneal macrophages. Figures indicate % increase in phagocytosis over controls (controls: 100). Between brackets are the values of % phagocytosis in control cultures.

Peptides		Concentration (μM)	% of stimulation		
			Assay 1	Assay 2	Assay 3
	inosiplex*		177 (43.5)	164 (45)	112 (33)
Human hexapeptide Val-Glu-Pro-Ile-Pro-Tyr[44]		30	131	142	153
		3	124	133	134
		0.3	135	133	166
		0.1	127	133	
	inosiplex		133 (37)	122 (37.5)	
Bovine hexapeptide Pro-Gly-Pro-Ile-Pro-Asn		10	137	130	
		2	139	133	
	inosiplex		115 (28.6)	114 (28)	112 (33)
Gly-Leu-Phe[5]		30	146	151	130
		3	160	157	166
		0.3	127	121	133
		0.03	121		
	inosiplex		115 (28.6)	114 (28)	112 (33)
Gly-Phe-Leu		30	139	132	160
		3	143	153	130
		0.3	122	121	160

Underlined figures are significantly different from the control (Student t-test). * inosiplex = p-Acetamidobenzoic acid salt of dimethylamino isopropanol: inosinate complex (3:1 molar ratio). Concentration 100 μg/ml.

Table 2. Activity in vivo of synthetic peptides. Enhancement of resistance of mice against infection with *Klebsiella pneumoniae*. Treatment was performed 24 h before i.v. injection of the infective agent.

Peptides	Way of injection	Concentration (mg/kg)	Survival on day + 10	ts/tc w 100 (stimulation)
Assay 1	i.v.	Controls	2/15	100
		2	7/15	228
Val-Glu-Pro-Ile-Pro-Tyr[44]		0.4	4/15	144
Assay 2	i.v.	Controls	3/29	100
		0.5	5/15	195
		0.25	2/15	134
Gly-Leu-Phe[5]	i.v.	Controls	4/30	100
	i.v.	5	6/15	176
		1	4/15	172
	s.c.	5	3/15	127
		1	7/15	206
Gly-Phe-Leu	i.v.	Controls	4/30	100
	i.v.	5	1/15	90
		1	4/15	180

Underlined figures are significantly different from the controls.

relation between neuropeptides such as endorphins and enkephalins and the immune system is now well established[39,40,45,54]. The presence of high affinity receptors for enkephalins and β-endorphin has been demonstrated on T lymphocytes[46].

Maruyama et al.[32] found in the tryptic digest of bovine casein an ACE inhibitor dodecapeptide Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys (CE I 12, residues 23–34 of bovine αs1-casein); more recently[33] they obtained a more potent ACE inhibitor heptapeptide Ala-Val-Pro-Tyr-Pro-Gln-Arg (CEI β7, residues 177–183 of bovine β-casein). These peptides potentiate bradykinin action on the rat uterus and ileum contraction. Their implication in the potentiation of bradykinin action in the gut of the newborn, and thus a stimulation of immunocompetent gut cells, might be suggested.

Opioid peptides

Opioid neuropeptides, endorphins and enkephalins, have been demonstrated to exert in vitro and in vivo immunomod-

Figure 1. Location of immunostimulating peptides and casomorphins of human and bovine β-caseins: characterization of a 'strategic zone'.

	Bovine β-casein	Human β-casein
	60 70	51 63
Strategic zone	Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu	Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro-Tyr-Gly-Phe-Leu
Immunostimulating peptides		
Hexapeptide	Pro-Gly-Pro-Ile-Pro-Asn	Val-Glu-Pro-Ile-Pro-Tyr
Tripeptide		Gly-Phe-Leu
β-casomorphins		
1 → 4	Tyr-Pro-Phe-Pro	Tyr-Pro-Phe-Val
	Tyr-Pro-Phe-Pro-$CONH_2$ (morphiceptin)	Tyr-Pro-Phe-Val-$CONH_2$ (valmuceptin)
		Tyr-Pro-Phe-$_D$Val-$CONH_2$ (devalmuceptin)
1 → 5	Tyr-Pro-Phe-Pro-Gly	Tyr-Pro-Phe-Val-Glu
1 → 7	Tyr-Pro-Phe-Pro-Gly-Pro-Ile (β-casomorphin)	Tyr-Pro-Phe-Val-Glu-Pro-Ile
1 → 8	Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro	Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro

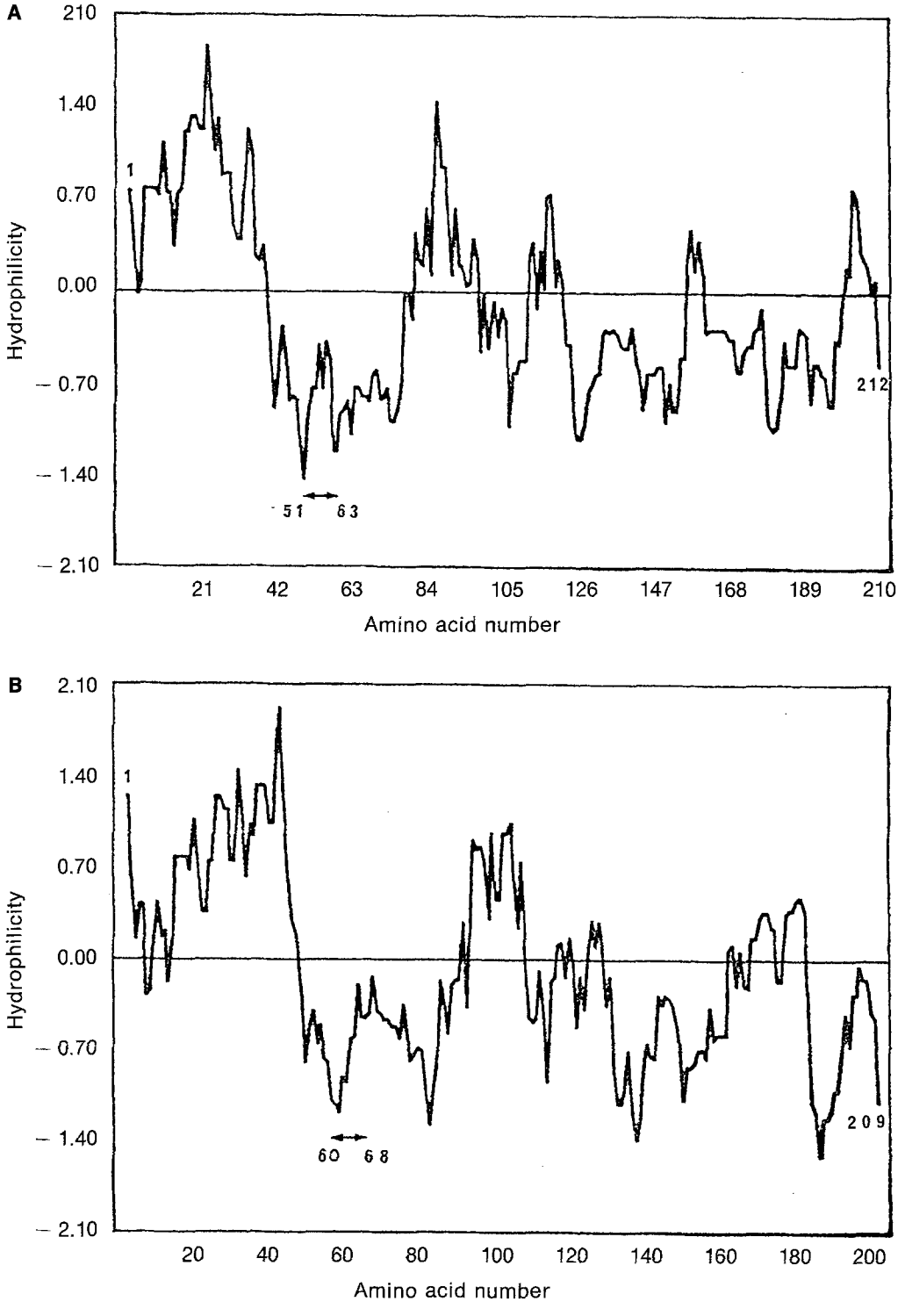

Figure 2. Hydrophilicity of β-caseins and location of the 'strategic zone' (↔).
A Human β-casein; *B* Bovine β-casein.

ulating activities[49,50]; they enhance lymphocyte proliferative responses[13,48]. In vivo, enkephalins increase the size of mouse and rat thymus and decrease the size of the spleen: they enhance the active T cell rosettes from human volunteers[36,49] and natural human killer cell cytotoxicity[35].

Various peptides with opiate activities have been isolated from enzymatic hydrolysates of caseins. Zioudrou et al.[61] characterized in peptic digests of bovine casein the presence of opioid peptides, called 'exorphins', such as Arg-Tyr-Leu-Gly-Tyr-Leu-Glu and Arg-Tyr-Leu-Gly-Tyr-Leu (residues 90–96 and 90–95 of bovine αs_1-casein, respectively)[31]. These peptides displaced $[^3H]_D$Ala^2-Met^5-enkephalin and [^{3}H] dihydromorphin from rat brain membrane receptors and exerted in vitro and in vivo a reversible inhibition of naloxone activity.

Simultaneously, Brantl et al.[6] isolated from the peptone of bovine casein a heptapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile (residues 60–66 of β-casein) named 'β-casomorphin' (fig. 1)

which inhibited electrically stimulated muscle contraction of isolated guinea pig ileum. A more potent opiate-like activity was displayed by the pentapeptide analogue Tyr-Pro-Phe-Pro-Gly (β-casomorphin 1–5)[20] which may influence the peristaltic activity of the ileum[28].

In 1981, Chang et al.[8] reported that a synthetic tetrapeptide analogue of β-casomorphin, Tyr-Pro-Phe-Pro $CONH_2$, named 'morphiceptin', possessed a high opioid activity both in vitro and in vivo and bound with a high selective affinity to μ opiate receptors when compared to δ receptors. Later[9] these authors established by radioimmunoassay and mass spectrometry, from a bovine casein digest (Sigma), the existence of the amidated peptide as well as of β-casomorphin and of the octapeptide analogue β-casomorphin 1–8 (fig. 1).

Opioid activity in a peptic digest of human whole casein was found by a radio receptor assay[58]. After sequence determination of human β-casein[16,17], β-casomorphin analogues, with the common N-terminal sequence Tyr-Pro-Phe, were synthesized and their opioid potency evaluated. Brantl established that human β-casomorphins 1–4 and 1–5 were less potent in the inhibition of electrically induced contraction of guinea pig ileum than the corresponding bovine analogues[7].

Yashikawa et al.[59] synthesized human analogues of β-casomorphin and morphiceptin, named valmuceptin and devalmuceptin (fig. 1), and tested their binding affinity to rat brain opiate receptors in the presence of naloxone; devalmuceptin had the highest affinity for opiate receptors and valmuceptin was also a potent peptide with a higher affinity than the bovine analogue morphiceptin. Longer peptides had weaker affinity.

As opiate receptors have been demonstrated on T lymphocytes[56] and human phagocytic leukocytes[30], it is conceivable that opioid casein peptides, β-casomorphins or exorphins, with a great affinity for μ opiate receptors have an endorphin-like activity on neonate immune cells, particularly with respect to the development of T cell functions and cellular immunity.

Characterization of a 'strategic zone' of β-casein

It is noteworthy that in human β-casein (fig. 1) the hexapeptide (residues 54–59) is situated at the C-terminal part of human β-casomorphin (residues 51–57) and is followed by the tripeptide Gly-Phe-Leu (residues 60–63), a weak but significant immunostimulant. The bovine analogue of the human hexapeptide, Pro-Gly-Pro-Ile-Pro-Asn, also stimulated phagocytosis of SRBC by murine macrophages (table 1). This part of the molecule, residues 51–63 of human and residues 60–70 of bovine β-caseins, seem to play a biological role, and this led us to consider it as a 'strategic zone' of β-casein.

As shown by hydrophobicity patterns of human and bovine β-caseins, determined according to Hopp et al.[22] (fig. 2), these peptides are situated in a hydrophobic part of the molecule: thus their accessibility to enzymatic digestion might be low.

The physiological occurrence and consequently the role of these peptides, particularly in the newborn, remains an open question. In the case of human neonates for at least three weeks after birth, both gastric secretion and pepsin activity are weak[2]; the stomach enzymes are almost completely inactivated by the high pH levels, 5.1 to 6.4, 2 h after the start of breast-feeding[34]. The major part of the food taken by the newborn leaves the stomach after minimal protein digestion[4]. Hydrolysis of the human milk proteins occurs mainly in the proximal small intestine, through the action of trypsin and to a lesser extent to chymotrypsin and carboxypeptidase B. Newborns have roughly the same concentration of trypsin in the duodenal lumen as adults, whereas chymotrypsin and carboxypeptidase B are present in lower concentrations[29]. These physiological data are not incompatible with the possible appearance of the β-casein 'strategic zone' peptides since we obtained immunostimulating peptides after tryptic/chymotryptic digestion[25]. Their uptake into the blood is possible owing to the permeability of the newborn gut for proteins and antigens[41,52]: their 'viability' in the plasma is conceivable, since the presence of human β-casomorphin 1–8 material was observed in the plasma of nursing mothers by immunoenzymatic assays[27].

From this observation it might be inferred that caseins are partly degraded in the mammary gland and released peptides might pass into the mother's plasma.

Human colostrum and milk contain a large number of leukocytes and about 80 to 90% of these cells are monocytes or macrophages[11] which are active phagocytic cells. If they are released in the mammary gland, immunostimulating peptides of the 'strategic zone' may have an influence on the phagocytic activity of maternal milk macrophages. These cells have been shown to contribute to the intestinal defense of the newborn[47].

In the case of bovine milk, Yvon et al.[60] demonstrated that in the calf abomasum, gastric proteolysis released large and small peptides but neither β-casomorphin nor larger fragments after a 7-h digestion. They may be produced after the intestinal digestive process as suggested by the existence of β-casomorphin in the plasma of newborn calves after milk intake, demonstrated by radioimmunoassay[55], and even in the juice of the small intestine of adult humans after ingestion of bovine milk[53].

Conclusion

Caseins represent an important protein supply for the newborn. Moreover, their enzymatic digestion is the source of numerous short peptides, endowed with a sufficiently by long physiological existence to exhibit biological activities.

In this paper we focused our attention on some of the peptides released during casein digestion which might be implicated in the immune defense system of the newborn.

β-casein was particularly generous in the production of active peptides, such as opiate-like β-casomorphins, morphiceptin and immunostimulating peptides, located in a 'strategic zone' in a hydrophobic part of the molecule. These peptides stimulated phagocytic activity of macrophages and might be able to play a role in the proliferation and maturation of T cells and natural killer cells for the defense of the neonate against a large range of bacteria, particularly enteric bacteria.

In addition, other casein peptides present additional biological properties as shown by Jollès et al.[26]: a peptide derived from bovine κ-casein by chymosin and tryptic digestion inhibited both aggregation of ADP-treated platelets and binding of fibrinogen to ADP-treated platelets.

Caseins seem thus to be able to offer to the newborn a series of active peptides favorable to its development. Are caseins prohormones for the neonate? The question is open to discussion.

Acknowledgment. We would like to express our gratitude to Dr G. H. Werner (Rhône-Poulenc Santé, Centre de Recherches de Vitry) for many helpful suggestions.

1 Adinolfi, M., Glynn, A. H., Lindsay, M. P., and Milne, C. M., Serological properties of gamma A antibodies to *Escherichia coli* present in human colostrum. Immunology *10* (1966) 517–526.

2 Agunod, M., Yamaguchi, N., Lopez, R., Luhby, A. L., and Glass, G. B., Correlative study of hydrochloric acid, pepsin and IF secretion in newborn and infants. Am. J. Dig. Dis. *14* (1969) 400–414.

3 Aoki, K., Kajiwara, M., and Oka, T., The role of bestatin-sensitive aminopeptidase, angiotensin converting enzyme and thiorphan sensitive "enkephalinase" in the potency of enkephalins in the guinea-pig ileum. Jap. J. Pharmac. *36* (1984) 59–65.

4 Berfenstam, R., Jagenburg, R., and Mellander, O., Protein hydrolysis in the stomach of premature and full-term infants. Acta paediat. *44* (1955) 348–354.

5 Berthou, J., Migliore-Samour, D., Lifchitz, A., Delettré, J., Floc'h, F., and Jollès, P., Immunostimulating properties and three-dimensional structure of tripeptides from human and cow caseins. FEBS Lett. *218* (1987) 55–58.

6 Brantl, V., Teschemacher, H., Henschen, A., and Lottspeich, F., Novel opioid peptides derived from casein (β-casomorphins) I. Isolation from bovine peptone. Hoppe-Seyler's Z. physiol. Chem. *360* (1979) 1211–1216.

7 Brantl, V., Novel opioid peptides derived from human β-casein: human β-casomorphins. Eur. J. Pharmac. *106* (1984) 213–214.

8 Chang, K. J., Killian, A., Hazum, E., Cuatrecasas, P., and Chang, J. K., Morphiceptin (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$): a potent and specific agonist for morphine (μ) receptors. Science *212* (1981) 75–77.

9 Chang, K. J., Su, Y. F., Brent, D. A., and Chang, J. K., Isolation of a specifiqu -opiate receptor peptide, morphiceptin, from an enzymatic digest of milk proteins. J. biol. Chem. *260* (1985) 9706–9712.

10 Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Harwalkar, V. R., Jenness, R., and Whitney, R. McL., Nomenclature of proteins of cow's milk: fifth revision. J. Dairy Sci. *67* (1984) 1599–1631.

11 Faden, H., and Ogra, P. L., Breast milk as an immunologic vehicle for transport of immunocompetence, in: Textbook of Gastroenterology and Nutrition in Infancy, pp. 355–361. Ed. E. Lebenthal. Raven Press, New York 1981.

12 Gattegno, L., Migliore-Samour, D., Saffar, L., and Jollès, P., Influence sur l'immunité de quelques éléments nutritionnels. Les Entretiens de Bobigny (1987) 141–144.

13 Gilman, S. C., Schwartz, J. M., Milner, R. J., Bloom, F. E., and Feldman, J. D., Beta-endorphin enhances lymphocyte proliferative responses. Proc. natl Acad. Sci. USA *79* (1982) 4226–4230.

14 Goldblum, R. M., Ahbstedt, S., Carlsson, B., Hanson, L. Å., Jodal, U., Lidin-Janson, G., and Sohl, A., Antibody-forming cells in human colostrum after oral immunization. Nature *257* (1975) 797–799.

15 Gothefors, L., and Marklund, S., Lactoperoxidase activity in human milk and in saliva of newborn infants. Infect. Immun. *11* (1975) 1201–1215.

16 Greenberg, R., Groves, M. L., and Dower, H. J., Human β-casein, amino acid sequence and identification of phosphorylation sites. J. biol. Chem. *259* (1984) 5132–5138.

17 Groves, M. L., and Gordon, W. G., The major component of human casein: a protein phosphorylated at different levels. Archs Biochem. Biophys. *140* (1970) 47–51.

18 Hayward, A. R., Development and maturation of immunity in the newborn. Immunological aspects of infection in the fetus and newborn, pp. 107–114. Eds H. P. Lambert, and C. B. S.. Wood. Academic Press, New York 1981.

19 Hayward, A. R., and Kurnick, J., Newborn T cell suppression: early appearance, maintenance in culture and lack of growth factor suppression. J. Immun. *126* (1981) 50–53.

20 Henschen, A., Lottspeich, F., Brantl, V., and Teschemacher, H., Novel opioid peptides derived from casein (β-casomorphins) II. Structure of active components of bovine casein peptone. Hoppe-Seyler's Z. Physiol. Chem. *360* (1979) 1217–1224.

21 Ho, P. C., and Lawton, J. W. M., The immunologic role of viable leukocytic cells in mammary exosecretions, in: Lactation IV, pp. 332–335. Eds B. L. Larson and V. R. Smith. Academic Press, New York 1978.

22 Hopp, T. P., and Woods, K. R., Prediction of protein antigenic determinants from amino acid sequences. Proc. natl Acad. Sci. USA *78* (1981) 3824–3828.

23 Jenness, R., Composition and characteristics of goat milk: review 1968–1979. J. Dairy Sci. *63* (1980) 1605–1630.

24 Jollès, P., and Jollès, J., Lysozyme from human milk. Nature *192* (1961) 1187–1188.

25 Jollès, P., Parker, F., Floc'h, F., Migliore, D., Alliel, P., Zerial, A., and Werner, G. H., Immunostimulating substances from human casein. J. Immunopharmac. *3* (1982) 363–369.

26 Jollès, P., Lévy-Toledano, S., Fiat, A. M., Soria, C., Gillessen, D., Thomaidis, A., Dunn, F. W., and Caen, J. P., Analogy between fibrinogen and casein. Effect of an undecapeptide isolated from κ-casein on platelet function. Eur. J. Biochem. *158* (1986) 379–382.

27 Koch, G., Wiedemann, K., and Zimmermann, W., Human β-casomorphin (1–8) immunoreactive materials in the plasma of nursing mothers. Poster/Frühjahrstagung der Deutschen Pharmakologischen Gesellschaft, Mainz 1986.

28 Kromer, W., Pretzlaff, W., and Woinoff, F., Opioids modulate periodicity rather than efficacy of peristaltic waves in the guinea-pig ileum in vitro. Life Sci. *26* (1980) 1857–1865.

29 Lebenthal, E., Lee, P. C., and Heitlingert, L. A., Medical progress. Impact of development of the gastrointestinal tract on infant feeding. J. Pediatr. *102* (1983) 1–9.

30 Lopker, A., Abood, L. G., Hoss, W., and Lionetti, F. J., Stereoselective muscarinic, acetylcholine and opiate receptors in human phagocytic leukocytes. Biochem. Pharmac. *29* (1980) 1361–1365.

31 Loukas, S., Varoucha, D., Zioudrou, C., Streaty, R. A., and Klee, W. A., Opioid activities and structures of α-casein-derived exorphins. Biochemistry *22* (1983) 4567–4573.

32 Maruyama, S., and Suzuki, H., A peptide inhibitor of angiotensin I-converting enzyme in the tryptic hydrolysate of casein. Agric. Biol. Chem. *46* (1982) 1393–1394.

33 Maruyama, S., Nakagomi, K., Tomizuka, N., and Suzuki, H., Angiotensin I-converting enzyme inhibitor derived from a enzymatic hydrolysate of casein. II. Isolation of bradykinin-potentiating activity on the uterus and the ileum of rats. Agric. Biol. Chem. *49* (1985) 1405–1409.

34 Mason, S., Some aspects of gastric function in the newborn. Archs Dis. Childh. *37* (1962) 387–391.

35 Mathews, P. M., Froelich, C. J., Sibbitt, W. L. Jr, and Bankhurst, A. D., Enhancement of natural cytotoxicity of beta-endorphin. J. Immun. *130* (1983) 1658–1662.

36 Miller, G. C., Murgo, A. J., and Plotnikoff, N. P., Enkephalins-enhancement of active T cell rosettes from normal volunteers. Clin. Immun. Immunopath. *31* (1984) 132–137.

37 Miller, M. E., Phagocyte function in the Neonate: Selected aspects. Pediatrics *64* (1979) 709–712.

38 Mills, E., Thompson, T., Bjorksten, B., Filipovich, B., and Quie, P., The chemiluminescence response and bactericidal activity of polymorphonuclear neutrophils from newborns and their mothers. Pediatrics *63* (1979) 429–434.

39 Modigliani, E., and Gattegno, L., Le lymphocyte est une cellule endocrine. Medecine/Science *3* (1987) 275–281.

40 Neuroimmune interactions: proceedings of the second international workshop on neuroimmunomodulation. Eds B. D. Jankovic, B. M. Markovic, and N. H. Spector. Ann. N.Y. Acad. Sci. *496* (1987).

41 Ogra, S. S., Weintraub, D., and Ogra, P. L., Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. J. Immun. *119* (1977) 245–248.

42 Packard, V. S., Human milk and infant formula 4. Immune factors. Food Science and Technology, pp. 81–83. Eds G. F. Steward, B. S. Schweizert and J. Hawthorn. Academic Press, New York 1982.

43 Paegelow, I., and Werner, H., Immunomodulation by some oligopeptides. Meth. Find. expl. clin. Pharmac. *8* (1986) 91–95.

44 Parker, F., Migliore-Samour, D., Floc'h, F., Zerial, A., Werner, G. H., Jollès, J., Casaretto, M., Zahn, H., and Jollès, P., Immunostimulating hexapeptide from human casein: amino acid sequence, synthesis and biological properties. Eur. J. Biochem. *145* (1984) 677–682.

45 Payan, D. G., and Goetzl, E. J., Modulation of lymphocyte function by sensori neuropeptides. J. Immun. *135* (1985) 782–784.

46 Payan, D. G., McGillis, J. P., Renold, F. K., Mitsuhashi, M., and Goetzl, E., Neuropeptide modulation of leukocyte function. Eds B. D. Jankovic, B. M. Markovic and N. H. Spector. Ann. N.Y. Acad. Sci. *496* (1987) 182–191.

47 Pitt, J., Barlow, D., Heird, W. C., and Snatulli, T. V., Macrophages and the protective action of breast milk on necrotizing enterocolitis. Pediatr. Res. *8* (1974) 384.

48 Plotnikoff, N. P., Kastin, A. J., Coy, D. H., Christensen, C. W., Schally, S. V., and Spirtes, M. A., Neuropharmacological actions of enkephalins after systemic administration. Life Sci. *19* (1976) 1283–1288.

49 Plotnikoff, N. P., Murgo, A. J., Miller, G. C., Corder, C. N., and Faith, R. E., Enkephalins: immunomodulators. Fedn Proc. *44* (1985) 118–122.

50 Plotnikoff, N. P., Miller, G. C., Solomon, S. K. T., Faith, R. E., Edwards, L. D., and Murgo, A. J., Methionine-enkephalin: immunomodulation in normal volunteers (in vivo). Psychopharmac. Bull. *22* (1986) 1097–1100.

51 Puri, P., and Reen, D. J., Host defenses in the newborn. Mod. Probl. Paediat. *23* (1985) 13–33.

52 Robertson, D. H., Paganelli, R., Dinwiddie, R., and Levinsky, R. J., Milk antigen absorption in the preterm and term neonate. Archs. Dis. Childh. *57* (1982) 369–372.
53 Svedberg, J., Dettaas, J., Leimenstoll, G., Paul, F., and Teschemacher, H., Demonstration of β-casomorphin immunoreactive materials in in vitro digests of bovine milk and in small intestine contents after bovine milk ingestion in adult human. Peptides *6* (1985) 825–830.
54 Smith, E. M., Menamin, D. H., and Blalock, J. E., Lymphocyte production of endorphins and endorphin-mediated immunoregulatory activity. J. Immun. *135* (1985) 779–781.
55 Umbach, M., Teschemacher, H., Praetorius, K., Hirshhäusser, R., and Bostedt, H., Demonstration of a β-casomorphin immunoreactive material in the plasma of newborn calves after milk intake. Reg. Pept. *12* (1985) 223–230.
56 Wybran, J., Appelboom, T., Famacy, J. P., and Govaerts, A., Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human T lymphocytes. J. Immun. *123* (1979) 1068–1070.
57 Yachie, A., Miyawaki, T., Nagaoki, T., Yokoi, T., Mikio, M., Uwadana, N., and Taniguchi, N., Regulation of B cell differentiation by T cell subsets defined with monoclonal OK T4 and OK T8 antibodies in human cord blood. J. Immun. *127* (1981) 1314–1317.
58 Yoshikawa, M., and Chiba, H., Abstracts of the Annual Meeting of Agric. chem. Soc. Jap., Sendai (1983) 574.
59 Yoshikawa, M., Yoshimura, T., and Chiba, H., Opioid peptides from human β-casein. Agric. Biol. Chem. *48* (1984) 3185–3187.
60 Yvon, M., and Pelissier, J. P., Characterization and kinetics of evacuation of peptides resulting from casein hydrolysis in the stomach of the calf. J. Agric. Food Chem. *35* (1987) 148–156.
61 Zioudrou, C., Streaty, R. A., and Klee, W. A., Opioid peptides derived from food proteins. The exorphins. J. biol. Chem. *254* (1979) 2446–2449.

0014-4754/88/030188-06$1.50 + 0.20/0

Full Papers

Cadmium-induced changes in avian renal morphology

C. J. Whitehead, D. N. Prashad and R. O. Blackburn[a]

School of Biological Sciences and Environmental Health, Thames Polytechnic, Wellington Street, London SE18 6PF (England), and [a] Life Sciences Department, University of London, Goldsmiths' College, Rachel McMillan Building, Creek Road, Deptford, London SE8 3BU (England)
Received 5 May 1987; accepted 12 November 1987

Summary. The effects of i.m. administered cadmium on growth rate and nephromorphology were studied in young pullets. The growth rate of pullets treated with 0.6 mg Cd^{2+}/kg at 48-h intervals was severely retarded, reaching only 50% of normal growth by 21 days. Such a decrease in growth rate was prevented when cadmium was given with either ferric or magnesium EDTA chelate. Electron micrographs of kidney tissue from cadmium intoxicated birds revealed massive intracellular disorganisation of proximal tubular cells, showing increased vacuolation and dilated endoplasmic reticulum. Mitochondria were few and swollen with reduced cristae. Some disorganisation was noted in the group treated with MgEDTA in conjunction with cadmium, with normal morphology observed in the group treated with FeEDTA plus cadmium.
In general, glomerular morphology of intoxicated pullets appeared normal, except that a 25% increase in thickness of the glomerular basement membrane was evident. No such membrane thickening was observed in any of the chelate treated groups.
These findings indicate that both chelates can provide certain levels of protection, in terms of growth rate and morphology, from cadmium intoxication. The possible mechanisms by which chelates offer protection have been discussed, but many questions remain unanswered.
Key words. Cadmium treatment; avian nephromorphology; growth rate; chelate.

Introduction

Several reports have described the pathological, physiological and biochemical effects of cadmium intoxication in humans and other mammals[1–6]. In general, the intoxication has revealed some common features, such as demineralisation of bone and hypercalcaemia, leading ultimately to bone fragility, massive retention of the body burden of cadmium, mainly in liver and kidney tissue, and interference in mitochondrial activity and membrane bound enzymes.
It has been shown in long-term studies that rats treated with low levels of cadmium (2.1 μg/day, orally) accumulate approximately 80% of the metal in the cytosol of renal cells with 7, 4 and 3% appearing in the mitochondria, nuclei and lysosomes respectively[7]. It is possible that this large retention of cadmium, either in the bound thionein complex or in the free form, could lead to changes in renal ultrastructure. Indeed, it has been stated[4] that biochemical and ultrastructural alterations in proximal tubules appear to parallel each other.
Samarawickrama[4] has suggested that in the kidney, pathological changes resulting from cadmium intoxication are essentially the same irrespective of the route of administration. When cadmium is administered orally, however, no valid conclusion can be made about its absorption. Estimates of absorption of ingested cadmium have been suggested to be approximately 2% in laboratory animals, though values of about 6% have been recorded in humans[8].
On administration, cadmium is initially stored in the liver, and subsequently transported to other organs, predominantly the kidney[9]. In rats, a single i.v. injection (2.5 mg/Cd^{2+}/kg) resulted in dilatation of ER, mitochondrial swelling and areas of degenerated cytosol in liver cells. Over this short period, no such disruption was observed in renal tissue, except that there was occasional pyknosis of nuclei in proximal tubular cells[10]. These findings suggest that kidney changes follow those seen in the liver.