Immunology 1984 **52** 511

Modification of lymphocyte traffic by vasoactive neurotransmitter substances

T. C. MOORE *MRC Mechanisms in Tumour Immunity Unit, MRC Centre, and the Department of Pathology, University of Cambridge, Cambridge*

Accepted for publication 14 December 1983

Summary. The effects of a number of vasoactive and neurotransmitter substances on lymphocyte traffic were studied by assessing their effects on the release of lymphocytes into primary peripheral (popliteal) nodal efferent lymph of sheep following acute infusion into cannulated afferent nodal lymphatics. In a total of 23 experiments, the output of lymphocytes, small and blast, was increased by serotonin, substance P, bombesin, [met]enkephalin, isoprenaline and phenylephrine and was decreased by vasoactive intestinal peptide (VIP), neurotensin and carbachol. Substances whose actions are modulated by prostaglandins and enhanced by prostaglandin synthesis inhibitors and which elevate blood monocyte and nervous tissue levels of cyclic GMP tended to increase lymphocyte traffic through peripheral lymph nodes in sheep *in vivo*. The opposite effect tended to be produced by substances whose actions require or are associated with prostaglandins or histamine, and which affect blood monocytic cyclic nucleotide levels by elevation of cyclic AMP or depression of cyclic GMP. Pain and inflammation tended to increase lymphocyte traffic, while analgesics and immunomodulators tended to decrease it.

Correspondence: Dr Thomas C. Moore, Dept. of Surgery, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509, U.S.A.

INTRODUCTION

Recent studies have indicated that potent vasoactive substances such as prostaglandin E_2 (PGE_2), histamine and bradykinin may have significant stimulatory or inhibitory effects on the traffic of lymphocytes through peripheral primary lymph nodes of sheep *in vivo*, traffic being stimulated by bradykinin and decreased by PGE_2 and histamine (Moore *et al.*, 1980; Moore, 1981, 1982; Moore & Lachmann, 1984). There is increasing evidence that many of these substances also have important neurotransmitter or neuromodulator roles (Hedqvist, 1970; Schwartz, Pollard & Tam Quoch, 1980; Correa *et al.*, 1979).

It has been found in rats that unmyelinated nerves are in close proximity to arterio-venous fistulae and to post-capillary venous sphincters of lymph nodes (Anderson & Anderson, 1975). These two structures play important roles in the function of the high endothelial post-capillary venules which regulate recirculating lymphocyte traffic in these animals (Gowans & Knight, 1964).

In recent years, the number of known and putative neurotransmitter and neuromodulator substances has increased considerably. Neuropharmacologists and gastroenterologists have postulated the existence of a 'brain–gut' axis. Although the existence of a 'brain–immune response' axis must be considered highly speculative at the present time, it is quite possible that the peripheral and central nervous systems and their transmitter, effector and modulator substances may

play important roles in lymphoid and reticuloendothelial function, with resulting influences of significance on the activities of the immune response system.

The present study was carried out to evaluate the influence of a number of older and newer vasoactive substances on the output of lymphocytes into the efferent lymph of popliteal nodes of sheep following their acute infusion into cannulated nodal afferent lymphatics.

MATERIALS AND METHODS

The sheep primary peripheral lymph node model of Hall & Morris (1962) was used. Both the afferent and efferent lymphatics of popliteal lymph nodes may be cannulated for chronic use in sampling and infusion. Xylazine (Bayer) and ketamine (Parke Davis) were employed for anaesthesia and the sheep were supported in sternal recumbancy (upright) during operation and subsequent studies. Both Finnish Landrace sheep and Clun Forest sheep crossed with Blue Face Leicester sheep were used. The maintenance of the supported upright position by girth supports in metabolic cages during these studies minimized lymph flow and lymphocytes output alterations associated with positional changes (Moore, 1982). Efferent lymph collections were precisely timed to the minute, and lymph flow and cell outputs were calculated. Lymphocyte outputs were expressed as cell outputs per hour for each sample. Samples were collected every 20 min post-acute infusion for 2 hr, and at $\frac{1}{2}$ and 1 hr intervals thereafter, until the times of maximum changes had been observed and outputs had returned or largely returned to pre-infusion levels. Differential counts for both small and blast lymphocytes were made on all collections. Blast lymphocytes did not appear in efferent lymph until after 48 hr post-anaesthesia and post-operation, and were not present in all studies. Acute infusions of all substances were into cannulated popliteal afferent lymphatics in 100–200 μl of sterile saline solution and in amounts of 50 μg (except one substance P infusion and both phenylephrine infusions). Serotonin, carbachol, phenylephrine and isoprenoline were obtained from Sigma. Vasoactive intestinal peptide (VIP), substance P, neurotensin, [met]enkephalin and bombesin were obtained from Peninsula Laboratories, San Carlos, CA.

RESULTS

Substances which increased lymphocyte output

Lymphocyte outputs into popliteal node efferent lymph were increased promptly and sharply after the acute infusions into cannulated afferent lymphatics of bombesin, serotonin, substance P, [met]enkephalin, isoprenaline and phenylephrine.

The largest number of studies was with bombesin (Table 1). The output of both small and blast lymphocytes was increased in all five acute infusions. The period of maximum increase in small lymphocyte outputs into efferent lymph was at or beyond 100 min post-acute infusion in four of five studies. Maximum

Table 1. Lymphocyte output elevations following acute infusion of 50 μg of bombesin into cannulated popliteal nodal afferent lymphatics of sheep

	Output of lymphocytes into efferent lymph ($\times 10^6$/hr)			
	Small cells		Blast cells	
Study No.	Maximum output change	Time of maximum change (min)	Maximum output change	Time of maximum change (min)
1	65→95	100	3·0→5·2	40
2	78→122	100	14·0→18·0	80
3	74→360	100	31→75	60
4	38→74	20	1·5→8·5	100
5	120→219	160	15·7→40·8	160

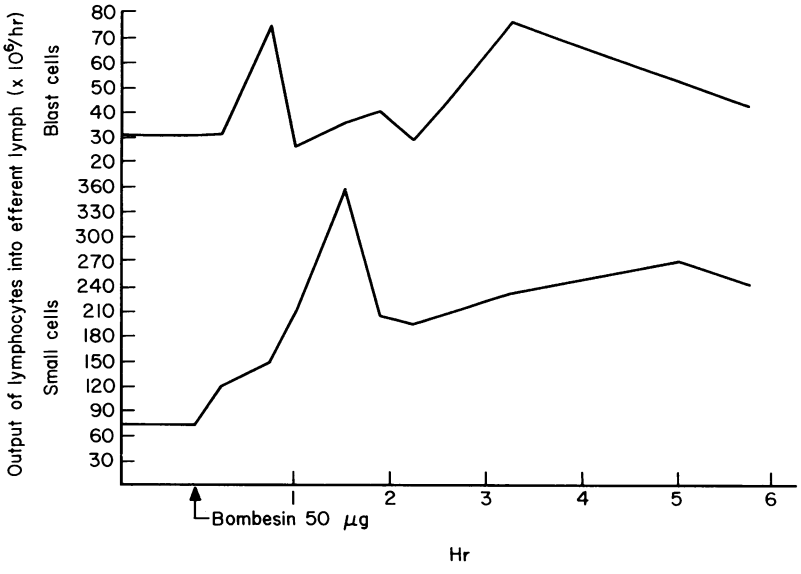

Figure 1. Time course of elevations in the output of small and blast lymphocytes into popliteal nodal efferent lymph following the acute infusion of bombesin (50 μg) into a cannulated afferent lymphatic of the node (study No. 3).

Table 2. Lymphocyte output elevations following acute infusions of serotonin, substance P, [met]enkephalin, isoproterenol and phenyleprine into cannulated popliteal nodal afferent lymphatics

	Output of lymphocytes into efferent lymph ($\times 10^6$/hr)			
	Small cells		Blast cells	
Substance	Maximum output change	Time of maximum change (min)	Maximum output change	Time of maximum change (min)
Serotonin	50→104	60		
Serotonin	75→152	100	1·2→5·8	100
Serotonin	131→185	40		
Substance P	92→165	60	1·0→19·5	60
Substance P	151→241	40	7·2→12·8	240
Substance P*	165→191	120		
[Met]enkephalin	108→221	100	3·2→13·7	120
[Met]enkephalin	105→147	120	2·8→6·0	200
Isoprenaline*	92→163	20		
Isoprenaline*	135→200	30		
Phenylephrine	98→165	80		
Phenylephrine	132→220	50	7·8→14·1	15

* These acute infusions were of 15 μg. All others were of 50 μg.

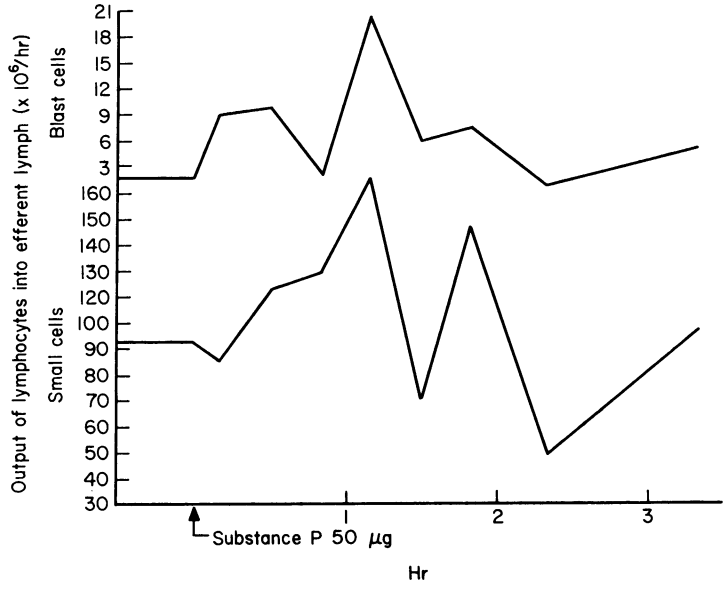

Figure 2. Increase in the output of small and blast lymphocytes into popliteal efferent lymph following acute intra-afferent lymphatic infusion of substance P (50 μg). The initial small dip in the output of small lymphocytes was not encountered with blast lymphocytes.

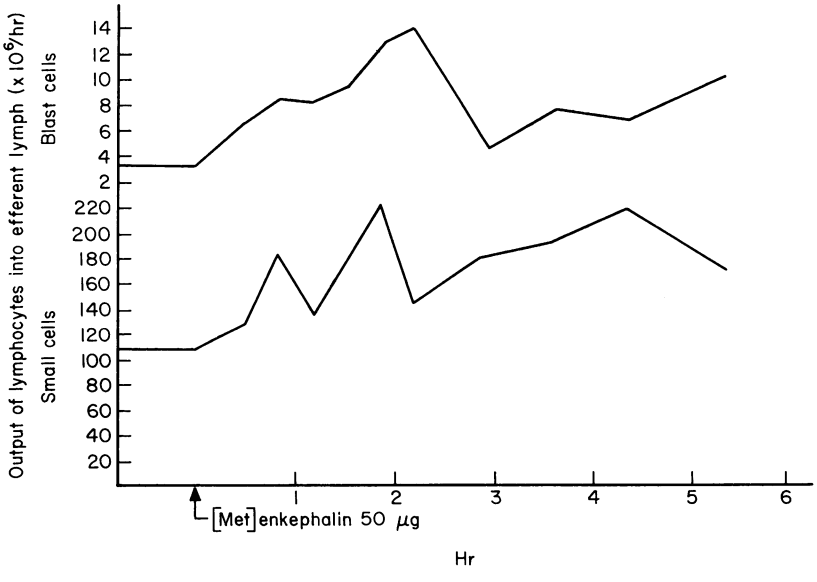

Figure 3. Time course of the increase in the output into efferent lymph of small and blast lymphocytes after acute infusion of [met]enkephalin (50 μg) into a connulated afferent popliteal lymphatic.

blast lymphocyte output increases occurred prior to 100 min in three of five studies. The time course of the increases in lymphocyte outputs following acute bombesin infusion are shown in Fig. 1. The volume of efferent lymph flow was unchanged in one bombesin acute infusion and increased in the other 4 (5·9 ml/hr to 6·8; 2·9 ml/hr to 4·8; 9·4/hr to 13·5; and 8·5 ml/hr to 13·9).

The results with acute infusions of the other four lymphocyte output-increasing substances are presented in Table 2. Output of small lymphocytes was increased in all 12 studies. Data on blast lymphocytes outputs were available in six studies and were increased in all. The period of maximum increase in output with the standard 50 μg dose was before 100 min in four of five acute infusions with serotonin or substance P and before 100 min in all four studies with adrenergic agonists. Peak elevations were later following enkephalin infusions.

Two of the substance P acute infusions produced first 20 min sample dips (decreases) in lymphocyte outputs followed by sustained elevations. In one other study with substances P, a slight decrease in output was found in the first collection with respect to small lymphocytes but not blast lymphocytes, with later increases and subsequent falls (Fig. 2). In this study, a rather massive increase in blast lymphocyte output into efferent lymph occurred within a relatively short time after infusion of substance P.

Acute infusion of [met]enkephalin produced a steady and more prolonged increase in the output of both small and blast lymphocytes into efferent lymph (Fig. 3).

Substances decreasing lymphocyte output

Lymphocyte outputs into efferent lymph were reduced following acute infusions into cannulated popliteal afferent lymphatics of neurotensin, VIP and carbachol (Table 3). The reductions in small lymphocyte output following both neurotensin and VIP were prompt and marked (Fig. 4). Sharp and prompt decreases in blast lymphocyte outputs post-acute infusion also were found with neurotensin. Blast cell outputs after VIP were unchanged in one with a late increase and intermittently increased and decreased in the other with the increase coming first. Output decreases following carbachol were less prompt than encoun-

Table 3. Lymphocyte output depressions following acute infusions of neurotensin, vasoactive intestinal peptide (VIP) and carbamylcholine into cannulated popliteal nodal afferent lymphatics

	Output of lymphocytes into efferent lymph ($\times 10^6$/hr)			
	Small cells		Blast cells	
Substance	Maximum output change	Time of maximum change (min)	Maximum output change	Time of maximum change (min)
Neurotensin	335→90	100	79→12	100
Neurotensin	122→50	20	8·7→0·5	40
VIP	224→42	60	12→58*	180
VIP	282→43	120	21→31→10†	20 & 100
Carbachol	114→23	100		
Carbachol	97→22	135		

* There was no change in blast lymphocyte output in the initial 80 min post-acute infusion. Blast cell output was elevated sharply at 100 min to 30×10^6/hr, returned to 14 at 120 min, before the maximum elevation at 180 min, followed by returns to 18 and 20 in the 220 and 300 min collections (Fig. 4).

† Final sharp increase to 54×10^6 cells/hr at 160 min, before return to pre-infusion levels at 200 min post-acute infusion.

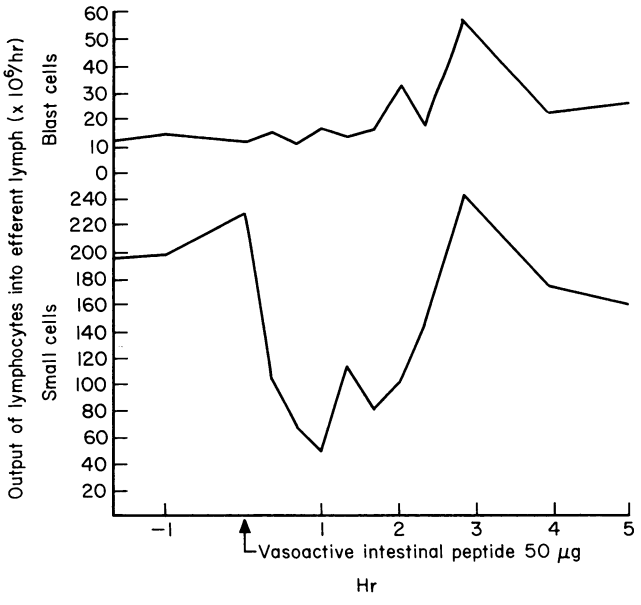

Figure 4. Time course of the decrease in the output of small lymphocytes into popliteal efferent lymph following acute infusion of VIP (50 μg) into a cannulated nodal afferent lymphatic. Blast lymphocyte output was little changed until late in the response when two brief spikes of elevation in output occurred.

tered with neurotensin and VIP and took longer to reach their maximum depressions.

DISCUSSION

Regarding lymphocyte traffic-increasing substances, serotonin, an established neurotransmitter with involvements in inflammation, has been reported to increase monocyte levels of cyclic GMP (Sandler, Gallin & Vaughn, 1975). A cyclic GMP analogue has been found to increase lymphocyte traffic through peripheral lymph nodes of sheep *in vivo* (Moore & Lachman, 1982).

Substance P also is a neurotransmitter-type substance which also appears to be involved in a stimulatory way in pain and inflammation. It has been found in nervous tissue (Iversen, Jessell & Kanazawa, 1976) and may play a mediator role in neurogenic inflammation and plasma extravasation (Gamse, Holzer & Lembeck, 1980). It has been suggested that substance P, as a neurotransmitter for primary nociceptor afferents, may also be the mediator for 'axon reflex' (Piercey *et al.*, 1981).

The most potent effect of bombesin is on temperature regulation. It is of interest that its effect on thermoregulation (hypothermia) is inhibited by PGE_2 (Brown, Rivier & Vale, 1977).

[Met]enkephalin is an endogenous opiate peptide with wide anatomic distribution including brain, spinal cord, peripheral nerves and gut. Multiple opiate receptors have been identified in primary afferent sensory fibres (Fields *et al.*, 1980). Enkephalins are widely distributed in the brain, are concentrated in synaptasomes and released in a Ca^{++}-dependent manner (Iversen *et al.*, 1978; Henderson, Hughes & Kosterlitz, 1978). They have been found *in vitro* to increase cyclic GMP levels of corpus striatum (Minneman & Iversen, 1976). Halothane, a general anaesthetic agent which profoundly decreases lymphocyte outputs into efferent lymph of sheep, has been reported to decrease brain cerebellar levels of cyclic GMP (Triner *et al.*, 1981). Increased potency of naloxone (opiate μ receptor inhibitor) has been suggested to be mediated through a PGE-linked process (Wong & Wai, 1981).

Phenylphrine and isoprenaline are adrenergic agonists and both increased the output of lymphocytes

into efferent lymph. In studies on mammalian spleen, nor-adrenalin (like bradykinin), evoked simulatory responses which were antagonized by prostaglandins (Ferreira, Moncada & Vane, 1973a, b).

With respect to lymphocyte traffic decreasing substances, neurotensin, a 13 amino acid peptide originally isolated bovine hypothalmus (Carraway & Leeman, 1973), has been found to have a high affinity binding sites on brain membranes and synaptosomes and on mast cells. Prostaglandins have reported to facilitate some of the vascular actions of neurotensin (Rioux *et al.*, 1980).

Vasoactive intestinal peptide (VIP), a 28 amino acid peptide that was first isolated from bovine duodenum (Said & Mutt, 1970), has been found to have wide distribution of significance throughout the nervous system (Said, Giachetti & Nicosia, 1980). Indomethacin (an inhibitor of prostaglandin synthesis) has been found to inhibit VIP-stimulated intestinal secretion (Albuquerque, Owens & Bloom, 1979). VIP also has been reported to increase cyclic AMP levels of blood mononuclear cells (Guerrero *et al.*, 1981). It has been found to be concentrated in mast cells where it appears to be associated with histamine and is released by histamine releasers (Cutz *et al.*, 1978).

Carbachol is a long-acting, stable, non-hydrolyzable ester of acetylcholine. It has both muscarinic and nicotinic actions, with the muscarinic predominating in most areas.

Pain and inflammation have been found to increase lymphocyte traffic, as mirrored in the output of both small and blast lymphocytes into the efferent lymph of primary peripheral lymph nodes of sheep *in vivo*, while pain control through local and general anaesthetic agents and immunoinflammatory modulation have tended to decrease it (Moore, 1982).

The studies as carried out here do not eliminate entirely the possibility that some of the observed alterations in lymphocyte traffic may have been related to systemic effects. This, however, is unlikely due to the small doses of the agents used, the generally short biological half-lives of the infused drugs and the intra-afferent lymphatic route of infusion which may have delayed their entry into the systemic blood circulation. In previous studies, with similar drugs given on one side only with bilateral monitoring of peripheral node efferent lymph, alterations in lymphocyte traffic were found only on the side of drug administration (Moore, 1982; Moore & Lachmann, 1984).

ACKNOWLEDGMENTS

The author is indebted to Dr Leslie L. Iversen for his helpful suggestions during the course of these studies and for his constructive review of this manuscript.

This work was supported by a grant from the Office of the Chancellor of the University of California, Los Angeles.

REFERENCES

ALBUQUERQUE R.H. OWENS C.W.I. & BLOOM S.R. (1979) A study of vasoactive intestinal peptide (VIP) stimulated intestinal fluid secretion in rat and its inhibition by indomethacin. *Experentia*, **35**, 1496.

ANDERSON A.O. & ANDERSON N.D. (1975) Studies on the structure and permeability of the microvasculature in normal rat lymph nodes. *Am. J. Path.* **80**, 387.

BROWN M., RIVIER J. & VALE W. (1977) Actions of bombsein, thryotropin releasing factor, prostaglandin E_2 and naloxone on thermoregulation in the rat. *Life Sci.* **20**, 1681.

CARRAWAY R. & LEEMAN S.E. (1973) The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J. biol. Chem.* **248**, 6854.

CORREA F.M.A., INNIS R.B., UHL G.R. & SNYDER S.H. (1979) Bradykinin-like immunoreactive neuronal systems located in rat brain. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1489.

CUTZ E., CHAN W., TRACK N.S., GOTH A. & SAID S.I. (1978) Release of vasoactive intestinal peptide in mast cells by histamine liberators. *Nature (Lond.)*, **275**, 661.

FERREIRA S.H., MONCADA S. & VANE J.R. (1973a) Some effects of inhibiting endogenous prostaglandin formation on the response of the cat spleen. *Br. J. Pharmacol.* **47**, 48.

FERREIRA S.H., MONCADA S. & VANE J.R. (1973b) Prostaglandin and the mechanism of analgesia produced by aspirin-like drugs. *Br. J. Pharmacol.* **49**, 86.

FIELDS H.L., EMSON P.C., LEIGH B.K., GILBERT R.F.T. & IVERSEN L.L. (1980) Multiple opiate receptor sites on primary afferent fibers. *Nature (Lond.)*, **284**, 351.

GAMSE R., HOLZER P. & LEMBECK F. (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br. J. Pharmacol.* **68**, 207.

GOWANS J.L. & KNIGHT E.J. (1964) The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. (B), Biol. Sci.* **159**, 257.

GUERRERO J.M., PRIETO J.C., ELORZA F.L., RAMIREZ R. & GOBERNA R. (1981) Interaction of vasoactive intestinal peptide with human blood mono-nuclear cells. *Mol. cell Endocrinol.* **21**, 151.

HALL J.G. & MORRIS B. (1962) The output of cells in lymph from the popliteal nodes of sheep. *Q. J. exp. Physiol.* **47**, 360.

HEDQVIST P. (1970) Control by prostaglandin E_2 of sympathetic neuro-transmission in the spleen. *Life Sci.* **9**, 269.

HENDERSON G., HUGHES J. & KOSTERLITZ H.W. (1978) *In vitro* release of [Leu]- and [Met]-enkephalin from the corpus striatum *Nature (Lond.)*, **271**, 677.

IVERSEN L.L., IVERSEN S.D., BLOOM F.E., VIRAGO T. & GUILLEMIN R. (1978) Release of enkephalin from rat globus pallidus *in vitro*. *Nature (Lond.)*, **271,** 679.

IVERSEN L.L., JESSELL T. & KANANZAWA I. (1976) Release and metabolism of substance P in rat hypothalmus. *Nature (Lond.)*, **264,** 81.

MINNEMAN K.P. & IVERSEN L.L. (1976) Enkephalin and opiate narcotics increase cyclic GMP accumulation in slices in rat neostriatum. *Nature (Lond.)*, **262,** 313.

MOORE T.C. (1981) Reduction in efferent lymph output of lymphocytes following infusion of histamine into cannulated afferent lymphatics in sheep. *IRCS Med. Sci.* **9,** 588.

MOORE T.C. (1982) The effects of inflammatory mediators and anaesthetic agents upon lymphocyte traffic of sheep *in vivo*. *Ph.D. Thesis*, University of Cambridge.

MOORE T.C., HOPKINS J., MCCONNELL I. & LACHMANN P.J. (1980) Efferent lymph output of lymphocytes following infusions of prostaglandin E_2 and other vasoactive mediators into cannulated afferent popliteal lymphatics in sheep. *IRCS Med. Sci.* **8,** 272.

MOORE T.C. & LACHMANN P.J. (1982) Cyclic AMP reduces and cyclic GMP increases the traffic of lymphocytes through peripheral lymph nodes of sheep *in vivo*. *Immunology*, **47,** 423.

MOORE T.C. & LACHMANN P.J. (1984) Chronic depression of lymphocyte traffic by long-term administration of prostagladins to peripheral lymph nodes in sheep. *Surgery*, **95,** 45.

PIERCEY M.F., SCHROEDER L.A., FOLKERS K., XU J-C. & HORIG J. (1981) Sensory and motor functions of spinal cord substance P. *Science*, **214,** 1361.

RIOUX F., QUIRION R., LEBLANC M.A., REGOLI D. & ST. PIERRE S. (1980) Possible interactions between neurotensin and prostaglandins in the isolated rat portal vein. *Life Sci.* **27,** 259.

SAID S.I., GIACHETTI A. & NICOSIA S. (1980) VIP: possible functions as a neural peptide. In: *Neural Peptides and Neuronal Communication* (eds E. Costa and M. Trabucchi), p. 75. Raven Press, New York.

SAID S.I. & MUTT V. (1970) Potent peripheal and splancnic vasodilator peptide from normal gut. *Nature (Lond.)*, **225,** 863.

SANDLER J.A., GALLIN J.I. & VAUGHN M. (1975) Effects of serotonin, carbamylcholine and ascorbic acid on leukocyte cyclic GMP and chemotaxis. *J. Cell Biol.* **67,** 480.

SCHWARTZ J-C., POLLARD H. & TAM QUOCH T. (1980) Histamine as a neurotransmitter in mammalian brain: neurochemical evidence. *J. Neurochem.* **35,** 26.

TRINER L., VULLIEMOZ Y., VEROSKY M. & ALPERT M. (1981) Halothane effect on cGMP and control of motor-activity in the mouse cerebellum. *Anesthesiology*, **54,** 193.

WONG G-L. & WAI M-K. (1981) The effect of prostaglandin E_2 on increased nalaxone potency induced by morphine pretreatment. *Eur. J. Pharmacol.* **74,** 365.