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The action of pharmacologically active substances on the flow and composition of cat hind limb lymph

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Summary

1. Lymph flow, blood flow and lymph protein concentrations have been measured in cat hind limb.

2. When naturally occurring pharmacologically active compounds were infused close arterially to the hind limb it was found that histamine, bradykinin and acetylcholine were the most potent in increasing lymph flow.

3. Dilatation of blood vessels *per se* is not sufficient to cause an increase in lymph flow since potent vasodilator agents such as isoprenaline and prostaglandins were not very active in increasing lymph flow.

4. The effect on blood flow was rapid in onset and in return to basal flow, whereas the effect on lymph flow was delayed and prolonged. The duration of the effect on lymph flow was considerably greater with histamine and bradykinin than with acetylcholine.

5. An increase in the protein concentration in the interstitial fluid is not a pre-requisite of an increase in lymph flow because (*a*) in some experiments an increase in lymph flow was not accompanied by an increase in lymph protein concentration after infusion of vasoactive substance and (*b*) the glucofuranoside, Glyvenol, was found to inhibit the increase in protein concentration but not the increase in lymph flow caused by infusion of histamine or bradykinin.

6. In contrast the specific antihistamine, mepyramine, abolished the effect of histamine on lymph flow and lymph protein concentration and considerably reduced the effect on blood flow.

7. An increase in venous pressure alone caused a small increase of lymph flow in about 50% of experiments, but when the venous pressure was increased at a time when vascular permeability was high, there was an increase in lymph flow in all experiments.

8. When the venous pressure was low only histamine caused a significant increase in the lymph protein concentration, whereas bradykinin sometimes caused a small increase and acetylcholine mostly caused a decrease in the concentration of lymph protein.

9. When the venous pressure was high, bradykinin and histamine and occasionally acetylcholine caused an increase in protein concentration in the lymph.

10. Infusions of histamine, bradykinin or prostaglandin E_1 did not increase the leakage of intracellular enzymes into the lymph.

Introduction

One of the common features of the inflammatory response or reactions to local tissue injury is an increase in lymph flow. It is therefore important to know how the flow of this drainage fluid is affected by the pharmacologically active substances which might be formed or released during local reactions. Haynes (1932) showed that histamine and acetylcholine both cause an increase in subcutaneous lymph flow in dogs. However, the doses given were very high ; 0·39 to 0·88 mg/kg of histamine injected intravenously increased flow and protein concentration of lymph draining the foot, and 40 mg acetylcholine infused into the femoral artery had a similar effect. Haynes found the effect of acetylcholine to be of shorter duration than that of histamine and suggested that the action of acetylcholine might be merely the result of vasodilatation although no blood flow measurements were made. Edery & Lewis (1963) making single injections into the femoral artery, found that only histamine caused an increase in femoral lymph flow in dogs. More recently Stürmer (1966) made arterial infusions into the femoral artery of dogs and found that bradykinin but not histamine produced an increase in hind limb lymph flow.

The present experiments were designed to measure not only lymph flow but also blood flow and lymph protein concentration as well, with the particular object of examining the interrelationship between these three parameters.

Methods

Experiments were performed on cats anaesthetized with pentobarbitone sodium 40 mg/kg intraperitoneally.

The femoral lymph vessels were dissected out and cannulated as described by Lewis & Winsey (1969). The propulsion of lymph was maintained by attaching the foot to a motor driven eccentric wheel, a modification of a method described by Edery & Lewis (1963) and Stürmer (1966).

The lymph flow was recorded with a photo-electric drop recorder and the lymph collected for estimation of protein concentration. Venous outflow from the femoral vein was measured with a second photo-electric drop recorder, the blood being returned to the animal via the contralateral femoral vein. In some experiments a ligature was tied around the whole limb excluding the femoral artery and vein.

Arterial infusions were made into the limb, via a polythene cannula placed in the central stump of the pudendal artery. Infusions were made with a Palmer continuous infusion pump at a rate of 0·4 ml/min. In experiments where venous pressure was measured, a side branch of the femoral vein was cannulated and the cannula passed centrally until the tip lay at the junction with the femoral vein.

Both venous and arterial blood pressures were measured with Statham strain gauges.

Biochemical methods. Protein and the intracellular enzymes, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and acid phosphatase (acid phos.) were determined by the methods previously described by Lewis (1967).

Lactate dehydrogenase (LDH) activity was measured by the Biochemica Test Combination (Boehringer & Soehne GMBH).

TABLE 1. *Effect of bradykinin, histamine and acetylcholine on blood flow, lymph flow and protein concentration of lymph at high venous pressures**

Infusion ((μg/0·4 ml)/min)		No. of Expts.	Blood flow (ml/min)			Lymph flow (μl/min)			Protein (mg/ml)		
			Before	During	% Increase	Before	During	% Increase	Before	During	% Increase
Bradykinin	0·05 μg	1	0·41	0·62	51	5	5	0	—	—	—
	0·1 μg	4	0·70	0·88	26	5	6·5	30	43	38	−10
	0·3 μg	6	0·81	1·58	95	5·5	22	233	30	41	32
	1·0 μg	10	0·88	1·41	60	6·5	16·5	154	27	43	63
	10·0 μg	1	0·59	1·15	95	6·5	26	300	28·5	56	96
Histamine	0·1 μg	1	0·49	0·86	75	3·5	3·5	0	—	—	—
	1·0 μg	2	0·79	1·88	147	8·5	11·5	35	28	32	14
	3·0 μg	6	0·42	1·71	307	6·5	14	114	29·5	42·5	44
	10·0 μg	2	0·49	1·28	161	11	31	182	30	48	60
	100 μg	1	0·22	4·72	2,000	5·5	20	260	33	54·5	65
Acetylcholine	1 μg	1	0·36	1·52	323	6	13	117	17	18	5
	3 μg	4	3·03	6·18	105	19	45	137	26·1	26·6	−2
	10 μg	1	0·13	0·4	208	0·3	3	900	47	76	62

* Venous pressure at 70–80 mmHg (1 mmHg≡1·333 mbar).

TABLE 2. *Effect of pharmacologically active substances on blood flow, lymph flow and protein concentration at high venous pressures**

Infusion ((μg/0·4 ml)/min)		Blood flow (ml/min)			Lymph flow (μl/min)			Protein (mg/ml)		
		Before	During	% Increase	Before	During	% Increase	Before	During	% Increase
5-Hydroxytryptamine	1 μg	0·36	0·54	50	10	15	50	29	30	3
	3 μg	3·0	1·27	−58	5	6	20	50	43	−13
	10 μg	1·63	2·27	40	20	18	−10	—	—	—
Prostaglandin E_1	1 μg	0·45	2·7	500	7·5	10	33	33·5	41·6	24
	3 μg	0·6	3·22	440	6	10	66	37	37	0
Prostaglandin $F_{1\alpha}$	3 μg	1·27	2·54	100	10	12	20	31·5	28·5	−10
Isoprenaline	3 μg	0·63	0·91	44	10	12·5	25	—	—	—
	3 μg	1·1	2·2	100	10	7·5	−25	32	28·5	−11
Angiotensin	1 μg	2·0	2·9	45	8	6	−25	—	—	—

* Venous pressures at 50–80 mmHg.

TABLE 3. *Effect of active substances on blood flow, lymph flow and protein concentration of lymph at normal venous pressure*

Infusion ((μg/0·4 ml)/min)		No. of Expts.	Blood flow (ml/min)			Lymph flow (μl/min)			Protein (mg/ml)		
			Before	During	% Increase	Before	During	% Increase	Before	During	% Increase
Bradykinin	1 μg	8	8·1	16·6	103 (4·5)	12·75	19·75	55 (3·75)	29·7	32·1	8 (2·8)
Histamine	3 μg	7	4·3	12·4	190 (4·0)	7·5	20·0	165 (9·7)	29·5	44·9	53 (8·0)
Acetylcholine	1 μg	2	4·25	12·2	200	4·5	13·5	200	32·6	27·8	−14
	3 μg	5	4·1	21·4	420 (8·9)	7·5	31·6	320 (12·4)	38·1	34	−11 (4·3)
5-Hydroxy-tryptamine	3 μg	1	14	11	−31	2	2	0	—	—	—
Prostaglandin E_1	1 μg	1	8·5	13·5	59	18	17	−5·5	29·2	29·8	2·4
	3 μg	2	5·25	5·8	10	12	12·5	4	19·9	19·5	−2
	20 μg	2	4·3	9·1	112	4	5	25	22	20	−9
Isoprenaline	1 μg	1	7	13	86	18	18	0	36·1	34·9	−3·3
Adrenaline	3 μg	3	5·8	1·8	−68·8	10·5	9	−14	22·2	21·2	−4·5

Figures in brackets are mean deviations of the differences.

Results

Several pharmacologically active substances were infused intra-arterially into the hind limbs of cats anaesthetized with pentobarbitone. The effects of these infusions on blood flow, lymph flow and protein concentration in the lymph are shown in Tables 1, 2 and 3. Bradykinin, histamine and acetylcholine were the most effective agents in causing an increase in all three parameters. The experiment of Fig. 1 shows the increase in blood flow and lymph flow during infusion of histamine 3 μg/min, that of Fig. 2 the increase during infusion of bradykinin 1 μg/min and that of Fig. 3 during the infusion of acetylcholine 3 μg/min. With all three substances,

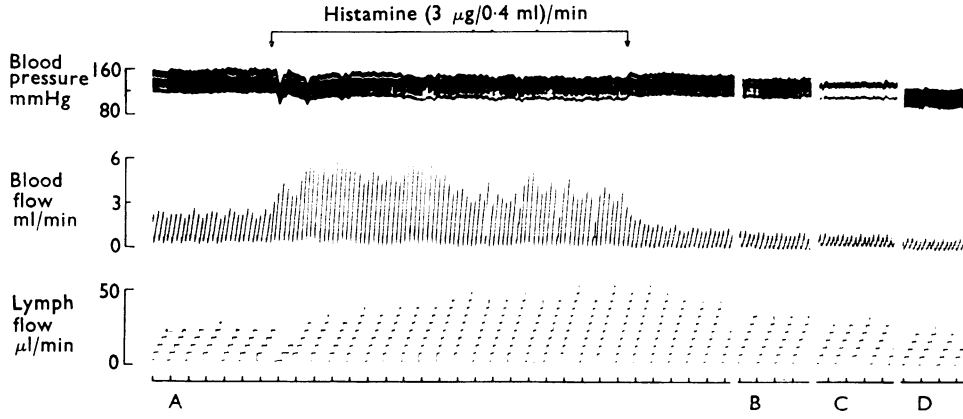

FIG. 1. Female cat 2·8 kg. Upper record, arterial blood pressure ; middle record, venous outflow from right hind limb ; lower record, lymph flow from right hind limb. Between the arrows histamine (3 μg/0·4 ml)/min was infused into the hind limb arterially. 30 min intervals were allowed between A and B, B and C, and C and D. Time scale, 1 min.

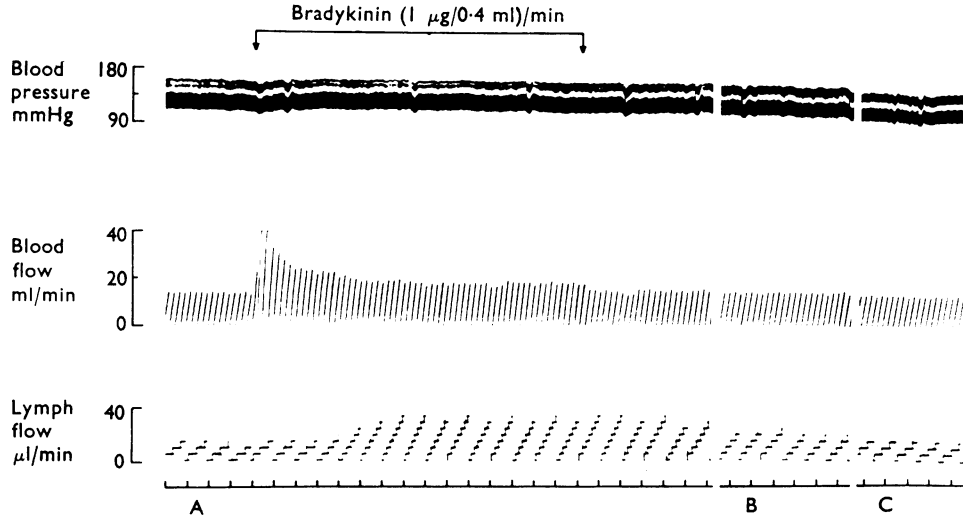

FIG. 2. Male cat 3·0 kg. Upper record, arterial blood pressure ; middle record, venous outflow from right hind limb ; lower record, lymph flow from right hind limb. Between the arrows bradykinin (1 μg/0·4 ml)/min was infused into the hind limb arterially. 30 min intervals were allowed between A and B, and B and C. Time scale, 1 min.

the increase in blood flow occurs almost immediately the infusion was started. There was a consistent difference, however, in the duration of the response to the substances. During histamine and acetylcholine infusions, the blood flow remained at an increased level throughout the infusion, whereas during infusions of bradykinin the blood flow began to return to the resting level even 1 to 2 min after the beginning of the infusion. The rates of flow given in Tables 1, 2 and 3 are those taken when the rate had reached a steady level. The lymph flow increased in response to the three substances in much the same way. There was always a delay of 3–7 min after the start of the infusion before the onset of increase in the lymph flow. The flow was usually sustained and sometimes continued to increase throughout the infusion.

Unlike the blood flow which returned to the resting level as soon as the infusion was stopped, the lymph flow returned gradually to the basal flow. After infusions of bradykinin and histamine as seen from Figs. 1 and 2 the lymph flow remained elevated over the resting level for 1·5–2 h. With acetylcholine, however, as seen from Fig. 3, the lymph flow returned to normal in 15–30 min.

One of the ways in which the pharmacologically active substances increase lymph flow might be by altering venous pressure. When partial venous occlusion was used to increase venous pressure, the effect was found to be variable. In about 50% of the experiments in which the venous pressure was elevated to 70–80 mmHg (1 mmHg≡1·333 mbar) there was an increase in lymph flow, and one of these experiments is illustrated in Fig. 4. In other experiments, the lymph flow either remained constant or decreased. When the venous pressure was raised during infusion of one of the vasoactive substances which increased lymph flow, the flow was further increased.

Venous pressure *per se* did not consistently alter the protein concentration of the lymph. However, increased venous pressure significantly altered the change in protein concentration which occurred during infusion of vasoactive substances.

In view of this finding the effect of infusions of vasoactive substances on the protein concentration of lymph must be considered in two separate parts. In the

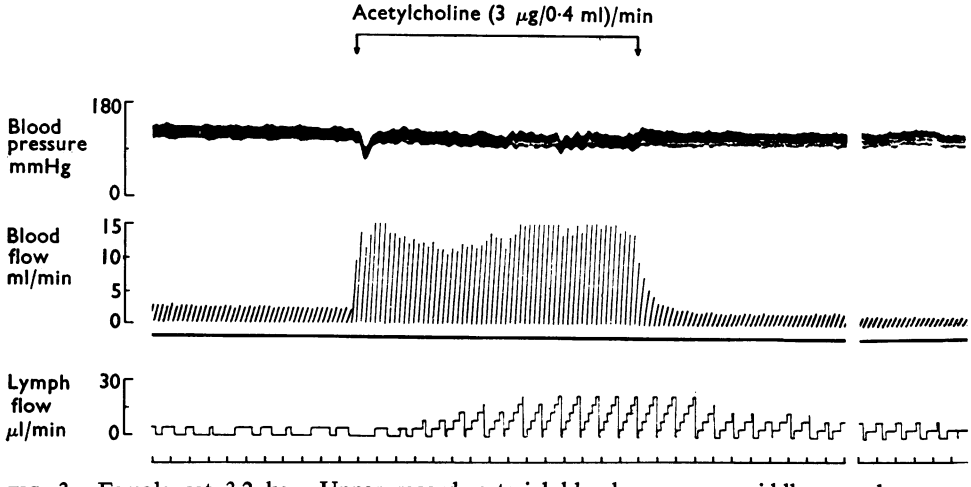

FIG. 3. Female cat 3·2 kg. Upper record, arterial blood pressure; middle record, venous outflow from right hind limb; lower record, lymph flow from right hind limb. Between the arrows acetylcholine (3 μg/0·4 ml)/min was infused into the limb arterially. 20 min were allowed between A and B. Time scale, 1 min.

experiments reported in Tables 1 and 2, narrow bore tubing (Portex PP 100), was used in the flow chamber for the measurement of venous outflow. This tubing was eventually found to restrict the outflow producing high pressure, the large vein pressure being as much as 50–80 mmHg. Tables 1 and 2 show the effects of infusions of bradykinin, histamine and acetylcholine. In most of these experiments bradykinin and histamine produced an increase in protein concentration whereas only occassionally was this observed with acetylcholine. The result of a typical experiment is shown in Fig. 5 in which infusion of histamine 3 μg/min caused an increase in venous outflow, lymph flow, and lymph protein concentration. In

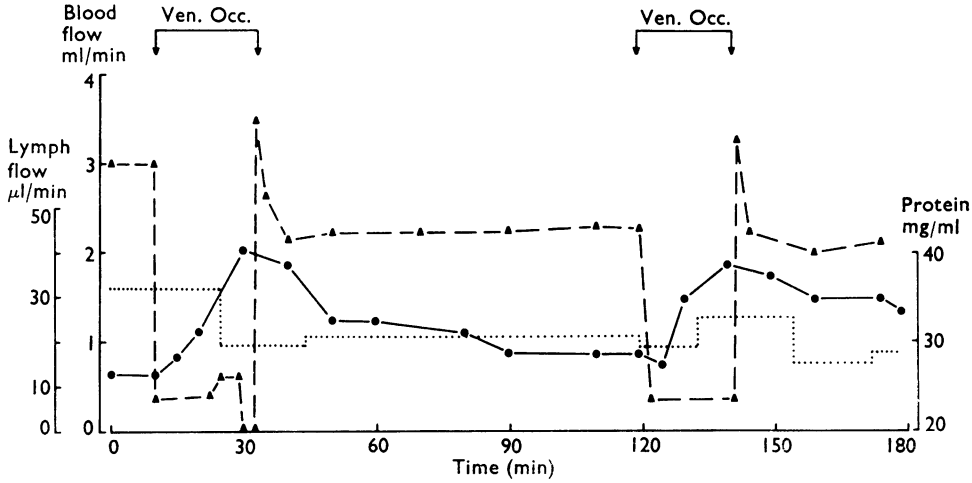

FIG. 4. Female cat, 2·5 kg. Record of venous outflow (▲——▲) and lymph flow (●——●) from the right hind limb and protein concentration of the lymph plotted as a histogram (.....). Between the arrows the venous pressure was raised by partial occlusion of the femoral vein.

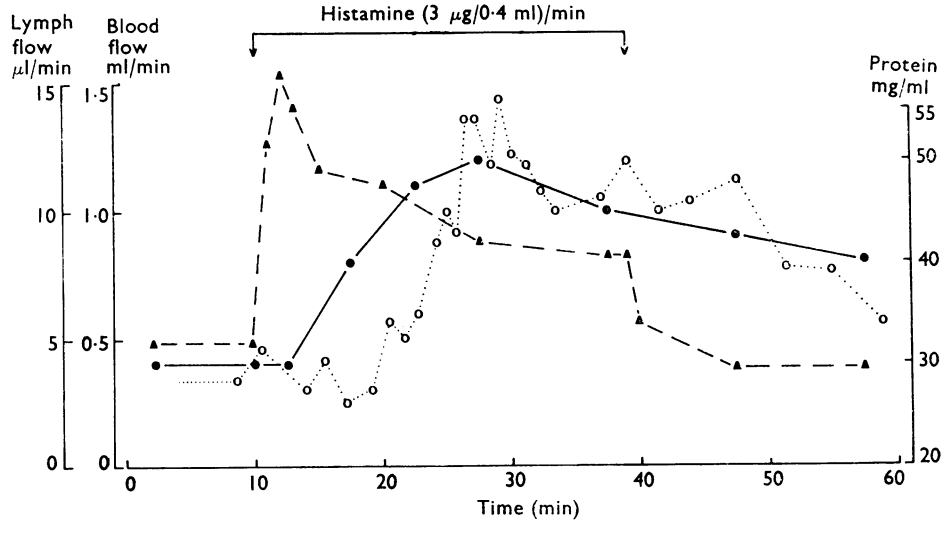

FIG. 5. Female cat, 2·7 kg. Record of venous outflow (▲——▲) and lymph flow (●——●) from the right hind limb and protein concentration of the lymph (○---○). Between the arrows histamine (3 μg/0·4 ml)/min was infused arterially into the hind limb.

TABLE 4. *Effects of venous pressure on the blood flow, lymph flow and protein concentration in lymph during infusions of bradykinin and histamine*

Infusions ((μg/0·4 ml)/min)	No. of Expts.	Blood flow (ml/min)				Lymph flow (μl/min)				Protein (mg/ml)			
		Before	During	Mean Diff.	*P* value*	Before	During	Mean Diff.	*P* value*	Before	During	Mean Diff.	*P* value*
Bradykinin 1 μg High venous pressure (70–80 mmHg)	10	0·88 ±0·61 (0·45–2·27)	1·41 ±0·77 (0·57–3·27)	0·53 ±0·25	$P<0·001$	5·6 ±3·2 (2·5–12)	22 ±12·1 (6–33)	16·4 ±9·5	$P<0·001$	30 ±5·1 (22–36)	41 ±13·3 (28–65)	16·8 ±14·6	$P<0·05$
Bradykinin 1 μg Low venous pressure (15–25 mmHg)	8	8·1 ±3·5 (2·5–14·6)	16·6 ±8·9 (7–32)	8·5 ±5·99	$P<0·01$	12·7 ±11·1 (1–37)	19·7 ±11·3 (5–39)	7 ±5·04	$P<0·01$	29·7 ±8·2 (20–43)	32·1 ±9·2 (22·5–45)	2·4 ±3·2	N.S.†
Histamine 3 μg High venous pressure (70–80 mmHg)	6	0·42 ±0·11 (0·32–0·63)	1·71 ±1·1 (1·0–3·4)	1·28 ±1·09	$P<0·05$	6·5 ±5·3 (2–17·5)	14 ±8·7 (4–26)	6·5 ±6·2	$P<0·05$	29·4 ±3·8 (25–35)	42·5 ±11 (30–56)	13·2 ±12·2	$P<0·05$
Histamine 3 μg Low venous pressure (15–25 mmHg)	7	4·3 ±2·28 (1·3–8)	12·4 ±6·53 (7·4–20·5)	8 ±5·1	$P<0·01$	7·5 ±5·94 (2·5–19)	20·0 ±14·6 (7–45)	12·3 ±13·4	$P<0·05$	29·5 ±5·26 (22·9–36·6)	44·9 ±9·56 (32·2–51·9)	15·5 ±10·4	$P<0·01$

Means are given ± standard deviation and ranges are given in brackets.
**P* value derived using *t* test for paired differences and not *t* test for difference of two means.
†N.S. denotes mean differences are not significant.

the experiment of Fig. 5, the increase in protein concentration occurred just before the increase in lymph flow. But in some of the experiments the increase in lymph flow occurred first.

In a second series of experiments in which wide bore tubing (Portex PP 220) was used in the blood flow chamber, the large vein pressure was 15–25 mmHg. It was found, as shown in Table 3, that of the substances tested under these conditions, only histamine produced a significant rise in the protein concentration in lymph. Table 4 gives a statistical analysis of the results comparing the effect of histamine and bradykinin on blood flow, lymph flow, and protein concentration in experiments using narrow bore tubing giving a high venous pressure on the one hand and wide bore tubing resulting in a low venous pressure on the other. Both bradykinin and histamine caused an increase in blood flow and lymph flow with the wide bore tubing, but although a small rise in protein concentration was observed during a number of infusions of bradykinin, the mean increase was not statistically significant. Infusion of acetylcholine, although increasing lymph flow, caused a fall in protein concentration under the conditions of low venous pressure.

Tachyphylaxis. A noticeable feature of these responses to bradykinin and histamine was that even after the first infusion, some degree of tachyphylaxis developed. The experiment of Fig. 6 shows the development of tachyphylaxis to bradykinin after infusions of 0·3 μg/min. There were reduced responses of blood flow, lymph flow and protein concentration during the second infusion of bradykinin, even though nearly 1·5 h were allowed for recovery after the first infusion. A further reduction in responses occurred during the third infusion.

In five experiments in which infusions of both bradykinin and histamine were made, it appeared that there was no cross-tachyphylaxis between the two substances.

Antagonists. The effect of three antagonists was examined on the responses to histamine, acetylcholine and bradykinin. Glyvenol (*ethyl*-3,5,6-tri-O-benzyl-D-glucofuranoside) has been described as a non-specific inhibitor of the responses to many pharmacologically active substances (Jaques, Huber, Neipp, Rossi, Schär &

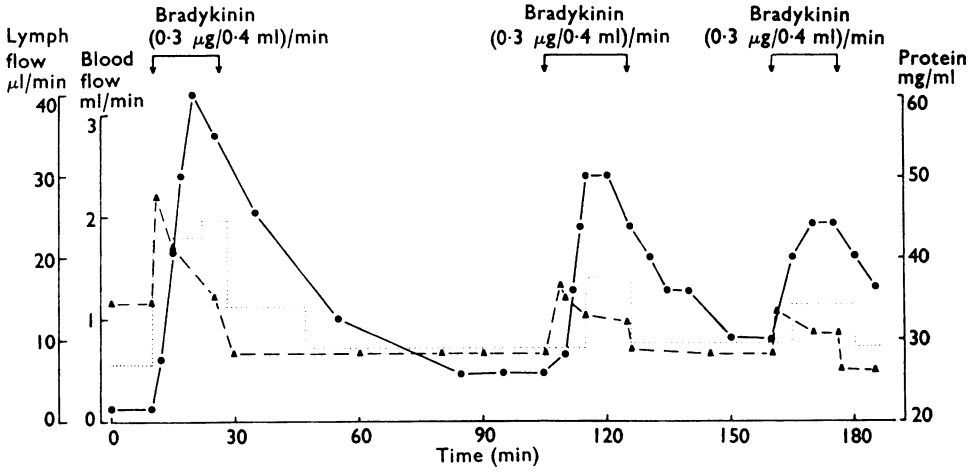

FIG. 6. Cat 3·1 kg. Record of venous outflow (▲——▲) and lymph flow (●——●) from the right hind limb and protein concentration of the lymph (○ - - - ○). Between the arrows bradykinin (0·3 μg/0·4 ml)/min was infused arterially into the hind limb.

TABLE 5. *Effect of Glyvenol on the responses to infusions of bradykinin and histamine*

Infusion	Blood flow (ml/min)			Lymph flow (μl/min)			Protein (mg/ml)		
	Before	During	% Increase	Before	During	% Increase	Before	During	% Increase
Bradykinin (4)									
(1·0 μg/0·4 ml)/min	0·94	3·04	223	8·5	18	112	28	49	75
+Glyvenol 1 mg/ml	1·12	2·89	160	8·0	20	150	26	31	19
Histamine (2)									
(3·0 μg/0·4 ml)/min	0·37	1·05	184	10	13	30	26	44	70
+Glyvenol 1 mg/ml	0·82	2·05	150	15	25	67	27	26	0
Histamine (1)									
(10 μg/0·4 ml)/min	0·45	1·77	280	20	27	35	35	43	23
+Glyvenol 1 mg/ml	1·63	2·90	78	25	90	200	39	39	0

Values in upper lines are from infusions of bradykinin or histamine alone, those in the lower lines are from infusions of mixtures with Glyvenol.
Mean values; number of experiments in brackets.

Meirer, 1967), while mepyramine and atropine are specific antagonists to histamine and acetylcholine.

In experiments using the substance Glyvenol, it was possible to show that an increase in lymph flow did not depend on an increase in the concentration of protein. When Glyvenol was infused together with either histamine or bradykinin, although the blood flow and lymph flow were still increased, there was little or no increase in protein concentration (Table 5). Figure 7 illustrates an experiment in which bradykinin (1 μg/0·4 ml)/min was infused first with Glyvenol (400 μg/0·4 ml)/min and then 1·5 h later, alone. Whilst the blood flow and lymph flow responses were normal during both infusions, the increase in protein concentrations was very much inhibited in the presence of Glyvenol.

The infusions were made in this order—that is, the control infusion second—to avoid confusing desensitization due to tachyphylaxis with an effect of Glyvenol. However, when the control infusion was made first, the effect of Glyvenol was still clearly seen.

Table 5 shows that although Glyvenol almost completely inhibited the increase in protein concentration which occurred during infusion of bradykinin or histamine when the venous pressure is raised, the increase in blood flow was reduced to a smaller extent and the increase in lymph flow was in fact considerably enhanced.

This is in contrast to the action of the specific antagonists mepyramine in the case of histamine and atropine with acetylcholine. In such experiments, the results of which are given in Table 6, it was found that mepyramine 1 mg/kg injected intravenously completely inhibited both the increase in protein concentration and the increase in lymph flow, although the increased blood flow was only partially inhibited. Similarly atropine abolished the effect of acetylcholine on blood flow

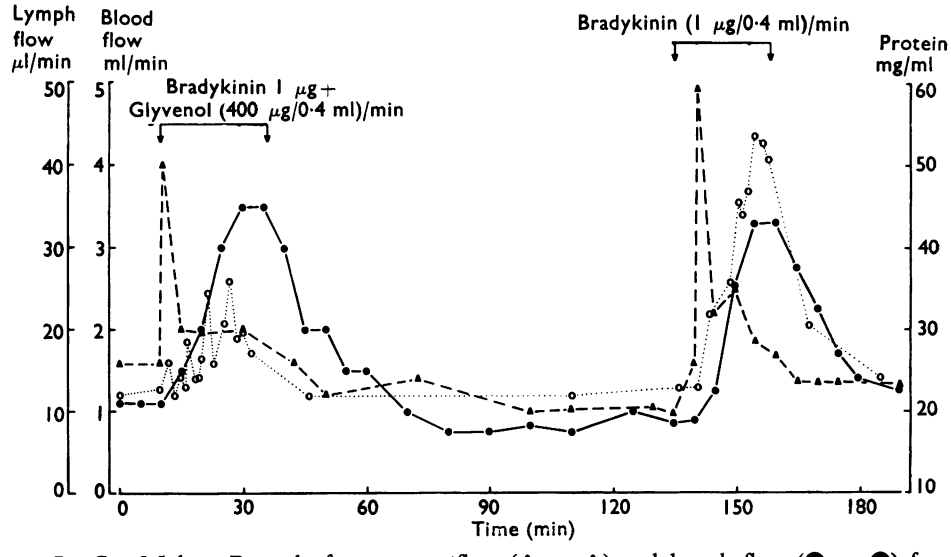

FIG. 7. Cat 2·5 kg. Record of venous outflow (▲——▲) and lymph flow (●——●) from the right hind limb and protein concentration of the lymph (○ - - - ○). Between the arrows close arterial infusions were made; during the first period a mixture of bradykinin (1 μg/0·4 ml)/min and Glyvenol (400 μg/0·4 ml)/min was infused; during the second period, 90 min later, bradykinin (1 μg/0·4 ml)/min was infused alone.

TABLE 6. *Effects of antagonists on infusions of histamine and acetylcholine*

Infusion	No. of Expts.	Blood flow (ml/min)			Lymph flow (μl/min)			Protein concentration (mg/ml)		
		Before	During	% Increase	Before	During	% Increase	Before	During	% Increase
Histamine 3 μg/min	3	4·1	11·3	263	4	23	475	24·8	34·8	40
Histamine 3 μg/min + mepyramine 12 mg/kg intravenously		4	8·6	115	8	8·5	6	25·9	27·0	4
Acetylcholine 3 μg/min	3	3·6	7·7	114	20	47	135	29·2	28·8	0
Acetylcholine 3 μg/min + atropine 1 mg/kg intravenously		2·3	2·3	0	23	21	−8·5	30	29·9	0

TABLE 7. *Effects of infusions of bradykinin and histamine on the enzyme composition of lymph*

Infusion	No. of Expts.	Protein (mg/ml)	Lymph flow (μl/min)	LDH (mu./ml)	GOT (mu./ml)	GPT (mu./ml)	Acid Phos. (u./100 ml)
Bradykinin 20 μg/min	3						
Lymph before		19·4	5·5	133	21	7	2
during		27·3	26	74	19	7	1
Plasma		60		160	12	6	2·5
Histamine 20 μg/min	3						
Lymph before		19·3	6·6	200	49	16	5·8
during		24·3	22	162	42	25	5·8
Plasma		55		250	49	36	3·7
Prostaglandin E_1 20 μg/min	2						
Lymph before		22	4	372	35	—	—
during		20	5	345	35	—	—
Plasma		69·5		91	0	—	—

and lymph flow and also reduced the increase of protein concentration where this occurred.

Effect of infusions of bradykinin and histamine on the enzyme composition of lymph

Recently it has been shown that during tissue injury, whether the injury be caused by burning, freezing or injection of noxious chemicals (Lewis, 1967, 1969 ; Boyles, Lewis & Westcott, 1970) the degree of cellular damage could be assessed by estimation of intracellular enzymes appearing in the lymph. Since it is possible that histamine, bradykinin or even a prostaglandin play a role in the vascular reactions during tissue injury, it was relevant to investigate the possibility that they not only increased the permeability of the blood vessels but perhaps the permeability of cell membranes as well. Four intracellular enzymes representing different intracellular compartments were estimated in the lymph collected before and during infusions of high concentrations (20 μg/min) of histamine, bradykinin or prostaglandin E_1. The results are given in Table 7 and show that there was no significant increase in the concentration of any of the intracellular enzymes. The concentrations of most of the enzymes in the lymph remained constant during the infusion in spite of an increased lymph flow. However, this is not surprising for in most experiments the resting concentrations of the enzymes in plasma were about the same as those in lymph, and during infusions when there was an increased vascular permeability it seems likely that the plasma enzymes would pass into the lymph just like the other plasma proteins.

Discussion

The substances which have been found most potent in increasing lymph flow are histamine, bradykinin and acetylcholine. These are also active in dilating blood vessels, although such an effect does not appear to be essential in producing increased lymph flow since some vasodilator substances such as isoprenaline and prostaglandin E_1 do not consistently cause such an effect.

In spite of a considerable amount of variability, there is generally an increase in the response of lymph flow, blood flow and protein concentration with increasing doses of vasoactive substances. Histamine appears to produce the steepest dose/response curve and bradykinin the flattest. It seems from individual experiments, however, that there is not an interdependence between the three parameters. The lowest doses of both bradykinin and histamine, for instance, caused an increase in blood flow but had no effect on lymph flow or protein concentration. Somewhat higher doses usually caused increases of blood flow and lymph flow but little or no increase in protein concentration.

The action of the inhibitor Glyvenol also leads us to the conclusion that there is not a direct relationship between the protein concentration in the interstitial fluid and lymph flow. When Glyvenol was infused with either histamine or bradykinin, it appeared specifically to inhibit their effect in increasing the protein concentration in the lymph. On the other hand a true antagonist of histamine, for instance, such as mepyramine prevented not only the increase of lymph protein but also the effect of histamine on lymph flow and blood flow. It is not clear from these experiments the mechanism of action of Glyvenol but it seems to act at some common point in the pathway of events leading to increased vascular permeability

and not at any specific receptors of any pharmacological substances. However, Helfer & Jacques (1967), using an isolated vein preparation, have shown that Glyvenol inhibited the contractions elicited by histamine and bradykinin although they did not inhibit the contractions caused by adrenaline.

The effect of the vasoactive substances on the lymph protein concentration appeared to depend partly on venous pressure. Increasing venous resistance caused an increased lymph flow in about 50% of experiments but did not lead to an increased protein concentration on the lymph. These results agree with those of Drinker (1937) who could not consistently observe an increased lymph flow with increased venous pressure in dogs and with the finding of Irisawa & Rushmer (1959) also made in dogs, that there was no correlation between lymph pressure and venous pressure. However, in the present experiments, when the venous resistance was increased in combination with an increased vascular permeability, as is obtained during infusion of vasoactive substances, the result was an increase in lymph flow and protein concentration in most experiments.

One consistent feature of the action of the vasoactive substances on the lymph flow was a considerable delay in the onset of the effect. Although the increase in blood flow occurred almost immediately the infusion of a vasoactive substance started, there was a delay of 2 to 7 min before the flow of lymph started to increase. Usually the flow then continued to increase, reaching a maximum after 10 to 15 min. This finding explains the failure of Edery & Lewis (1963) to show an increase in lymph flow following injections of bradykinin. It seems likely that after a single injection, the bradykinin disappears before it has time to produce the effect. It was not until Stürmer (1966) made a continuous infusion of bradykinin that it became clear that the peptide increased the flow of lymph.

When the lymph flow had increased, the time taken for it to return to the resting level was considerable—sometimes several hours in the case of histamine and bradykinin. On the other hand, after an infusion of acetylcholine the recovery occurred much more rapidly. The reason for this difference is not clear but one possibility is that as acetylcholine is a most potent vasodilator, the increased lymph flow might be related more to the haemodynamic changes than changes of vascular permeability. This view is consistent with our finding that, whereas histamine and bradykinin generally cause an increase in lymph protein, acetylcholine rarely produces such an increase.

A further finding in the present investigation, which is difficult to explain, is the development of tachyphylaxis. Here again, there is some difference between the behaviour of histamine and bradykinin on the one hand and acetylcholine on the other. With histamine and bradykinin, tachyphylaxis develops more rapidly than with acetylcholine. It is possible that this phenomenon is also related to the increase of vascular permeability brought about by bradykinin and histamine. However, the finding that there is no cross-tachyphylaxis between histamine and bradykinin suggests that it might be a more specific phenomenon.

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