Salivain, but not glandulain and the kalliktrein–like peptidase, was found by us to be present in saliva at high concentrations. Since salivain is a secretory enzyme[12] it is apparently located in the secretory (zymogen) granules. Bhoola and Ogle (1965)[13] have, in fact, demonstrated that the kallidin, releasing activity of the guinea-pig submaxillary gland homogenate is located in the fraction containing the zymogen granules. The activity was taken as kallikrein even while the distribution of the protease activity was not tested. It seems likely, that the activity tested by these authors has been the proteinase called salivain by us. Werle *et al.* (1960)[14] have stated that rat submandibular gland contains 3000 kallikrein units/g wet tissue, while e.g. human gland contains 1–2 units. In the light of the present study it seems clear that both salivain and kallikrein-like peptidase are included in the kallikrein activity measured by Werle *et al.* Whether both of these enzymes are real kallikreins depends on the definition of kallikrein.

Since its discovery, kallikrein has been taken as a circulatory hormone. It has been suggested that salivary kallikrein might be the mediator of the cholinergic nerve-induced vasodilatation in the submandibular gland. Hilton and Lewis[15] concluded that both cholinergic and adrenergic vasodilatation in this organ is mediated by plasma kinins liberated by kallikrein. It remains to be explained whether the submandibular kallikrein (producing plasma kinin) in their experiments has been salivain, the kallikrein-like peptidase or some additonal enzyme. It could perhaps be suggested that salivain, being a secretory enzyme, has a function in the digestive processes while the non-secretory kallikrein-like peptidase would be responsible for the possible circulatory control mechanism.

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Histamine and spermidine in tissues of the guinea pig*

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THE *n*-BUTANOL extraction of tissues with low histamine content is inadequate for the fluorometric determination of histamine[1] because of the presence of the interfering substance.[2–6] Spermidine,[5,6] one of the major contaminants in the extract made by organic solvents, can be separated from

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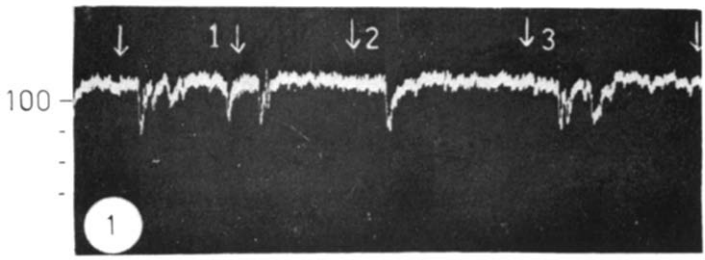

FIG. 1. Effect of (1) 2·0 ml Tris-HCl buffer (pH 7·0, 0·1 M) (2) 0·4 ml trypsin-solution and (3) of 1·6 ml trypsin solution (70 μg/kg) on the blood pressure of the rabbit. Numbers on the left give blood pressure in mmHg.

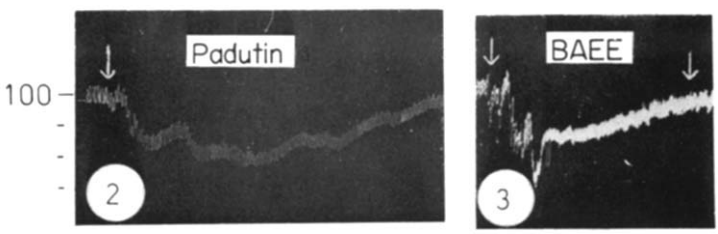

FIG. 2. Effect of one unit (60 μg/kg) of Depot-Padutin® in 1 ml of distilled water.

FIG. 3. Effect of 1 μg/kg of the BAEE-splitting fraction of Depot-Padutin® separated by chromatography on DEAE-cellulose.

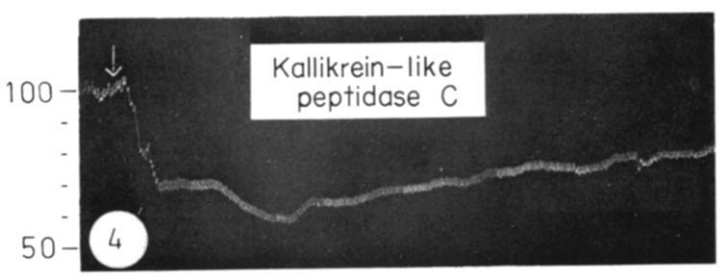

FIG. 4. Effect of 5 μg/kg of the isoenzyme C of kallikrein-like peptidase purified from rat submandibular glands. Depression lasted about 15–20 min.

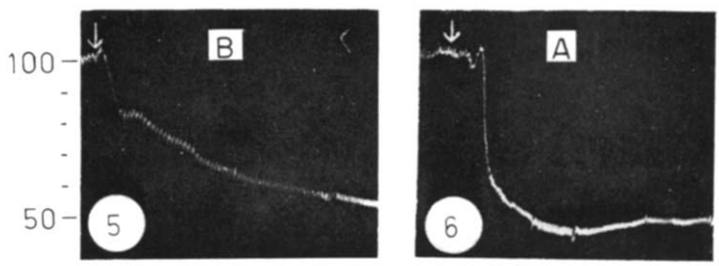

FIG. 5. Effect of isozyme B of the same peptidase as shown in Fig. 5. The dose was 25 μg/kg.

FIG. 6. Effect of isozyme A of the same peptidase as shown in Figs. 5 and 6. The dose was 25 μg/kg.

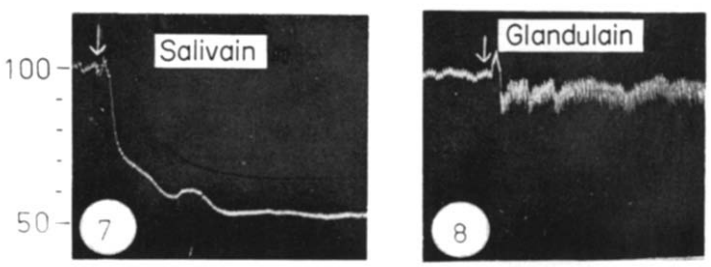

FIG. 7. Effect of salivain as a dose of 10 μg/kg. An essentially similar result was obtained with a dose of 1 μg/kg.

FIG. 8. Effect of glandulain in a dose of 300 μg/kg.

histamine on cellulose-phosphate cation-exchange resin columns (Cellex-P, BioRad). Both react with *o*-phthaldialdehyde (OPT) to give fluorescent products; these differ in spectral characteristics.[5,6]

Here we report our studies with the fluorometric method for the estimation of histamine and spermidine and the concentrations of histamine and spermidine in eleven guinea pig tissues, in comparison with published results obtained by other procedures.

Male guinea pigs (400–500 g) were decapitated; the tissues were excised and placed in beakers chilled in ice. Cerebral hemispheres, lung, liver and skeletal muscle from individual animals were used, whereas midbrain, heart, spleen, kidney, adrenal gland, testis, and pancreas were pooled to obtain at least 1 g tissue. Five vol. (w/v) of perchloric acid (ice-cold 0·4 N) were added and the mixture homogenized in an all-glass homogenizer. After standing for 10 min in ice chips, the homogenate was treated in one of the following ways: (1) *N*-Butanol extraction followed by 0·1 N HCl extraction, and assay as described by Shore *et al.*[1] (2) The 0·1 N HCl extract of (1) was neutralized to pH 6·1 as measured with a glass electrode. (3) The original 0·4 N perchloric acid supernatant was neutralized with solid K_2CO_3 and adjusted to pH 6·1. The final neutralized extracts of (2) and (3) were transferred to a cellulose phosphate resin column (80 mm × 6 mm), and eluted with water containing stepwise increases in HCl concentration; each 1-ml fraction of effluent was assayed fluorometrically for histamine[1] and spermidine.[5,6]

During the course of these experiments a new batch of Cellex-P was obtained; its adsorption and elution characteristics were different from those observed with the earlier batch. Clearly, each new batch of Cellex-P must be recalibrated in order to obtain reproducible results.

As previously reported,[5,6] the extract prepared by method 1, when reacted with OPT, yields a fluorophore different from that of histamine. By means of Cellex-P (method 2) one can separate from histamine a second OPT-reactive amine, spermidine.[5–7]

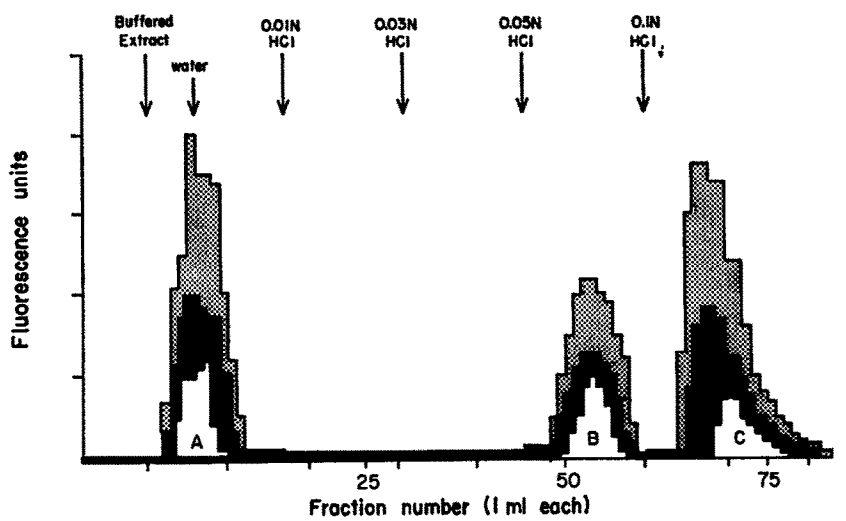

FIG. 1. Composite resinogram showing the elution pattern of (A) histidine, 4·6 μg; (B) histamine, 111 mμg; and (C) spermidine, 4·4 μg (white areas) compared to perchloric acid extract (method 3) of 2 g guinea pig midbrain (black areas) and to the same amount of brain plus (A), (B), and (C) (shaded areas).

One can circumvent *n*-butanol extraction by using method 3 and still separately measure histamine and spermidine (Fig. 1). Recovery from the resin is 87 per cent, and as little as 55 mμg histamine and 2·1 μg spermidine present in 1 g tissue may be measured accurately. Histidine coupled with OPT gives a fluorophore like that of histamine, with 1/30 of its fluorescence intensity; at pH 6·1, histidine is not adsorbed onto the resin and is completely washed out by the first water wash of the column.

In Table 1 are the values of histamine and spermidine in guinea pig tissues determined by method 3. The values are based on the sum of the area under the curve (cf. Fig. 1) after correction for base-line fluorescence relative to standard curves plotted in each experiment and for recovery as cited. The concentration of histamine in brain, liver, heart, spleen, and kidney is consistently lower than that reported for the same guinea pig organs by other workers using the OPT method and *n*-butanol extraction.[1,8]

TABLE 1. HISTAMINE AND SPERMIDINE IN GUINEA PIG TISSUES, DETERMINED BY METHOD 3

	Histamine			Spermidine			Ratio
Organ	OPT	OPT*	Ref.	DNFB	OPT*	Ref.	S/H
Midbrain		**0·067 ± 0·014** (4)			**39·44 ± 6·24** (5)		588
		0·057	6		57	6	
	0·2, 0·3		1		35	7	
	0·245		11	38		14	
Cerebral cortex		**0·058 ± 0·009** (3)			**20·3 ± 3·63** (4)		357
Lung		**12·88 ± 2·78** (4)			**24·80 ± 7·69** (4)		1·93
	24·6–48		8				
	19–35		1				
Liver		**0·564 ± 0·115** (5)			**20·74 ± 7·11** (6)		36·76
	1·8–2·8		8		34	7	
	1·2–2·2		1	28		13	
				32		12	
Heart		**2·31 ± 0·5** (3)			**7·11 ± 2·76** (3)		3·08
	7·8–10		8				
	5·8–6·4		1				
Spleen		**2·83 ± 1·02** (4)			**48·72 ± 13·78** (5)		17·21
	12·6		8				
Kidney		**0·932 ± 0·366** (4)			**16·82 ± 2·61** (4)		18·05
	2–2·8		8		27·8	7	
	2·4–4·5		1	42		13	
				38		12	
Adrenal gland		**2·89 ± 1·11** (4)			**20·74 ± 0·58** (3)		7·19
Testis		**0·061 ± 0·027** (3)			**34·51 ± 5·37** (3)		56·57
Skeletal muscle		**0·471 ± 0·1** (4)			**5·22 ± 0·29** (3)		11·08
Pancreas		**2·087 ± 0·27** (3)			**126·88 ± 34·08** (5)		60·79
				296		13	
				312		12	

Concentration of histamine and spermidine in tissues of the guinea pig (μg/g).
Values given as average ± S.D. are from the present paper, and are in bold-face type.
Ratios in the right-hand column are from these values.
* After ion-exchange purification; DNFB, dinitrofluorobenzene;

$$(S/H) = \frac{\text{spermidine } \mu g/g.}{\text{histamine } \mu g/g}$$

To account for the 2- to 5-fold difference between our present findings and previous ones, we performed the following experiment. Tissues were prepared by Method 2, the neutral butanol extract placed on a Cellex-P column, and each milliliter of effluent reacted with OPT. The fluorescence was measured at 450 mμ on activation at 350 mμ; these are the activation and fluorescence maxima of histamine. The ion-exchange chromatographic separation of the *n*-butanol extract of liver, by stepwise elution, as judged by fluorescence resulting from its interaction with OPT, is shown in Fig. 2. The per cent distribution of fluorescence intensity (corrected) in all the effluents from similar experiments in several tissues is given in Table 2. Histamine recovered in the 0·05 N HCl effluent (column E) accounts for 15 per cent (midbrain), 35 per cent (liver), and 19 per cent (kidney) of the total fluorescent activity found in extracts by method 1. When the concentrations of tissue histamine (column H) are recalculated on the basis of OPT-reactive materials other than histamine (column I) the values for apparent histamine in brain, liver, and kidney are in good agreement with the values published by Beall[8] and Shore *et al.*[1] (columns J, K), but this was not true for heart, spleen, and lung.

Until recently the only acceptable method for the estimation of low histamine concentrations in tissues and body fluids was biological assay. The fluorometric method of Shore *et al.*[1] approaches the sensitivity of bioassay, but its specificity has been questioned.[2–6] The critics suggested that *o*-phthaldialdehyde, reacting with other compounds, produces fluorophores that lead to high values for "histamine." The major contaminant is spermidine.[5,6] After separating histamine and spermidine on an ion-exchange column, we find the OPT reaction to be a rapid and simple method, specific and sensitive for histamine and spermidine whose distinct spectra allow their assay in fractions of the same extract.

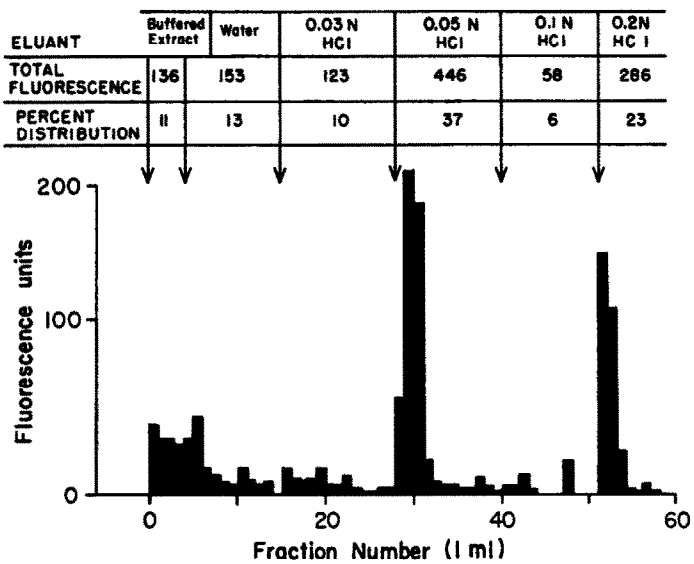

FIG. 2. Ion exchange chromatographic separation of *n*-butanol extract of guinea pig liver, adjusted to pH 6·1 (method 2). Each 1-ml fraction of effluent was reacted with OPT. Fluorescence intensity was measured at 450 mμ on activation at 350 mμ (the fluorescence and activation maxima of histamine).

Earlier workers using bioassay found a range of values for histamine in lungs of different animals of the same strain (5–95 μg/g[9] and 3·3–50 μg/g[10]) wider than the range we find (Table 2: 12·88 $\pm$ 2·78 μg/g). The concentration of histamine found in guinea pig brain by bioassay by Carlini and Green[3,4] (53 mμg/g) resembles our value (68 mμg/g) and that of Kremzner and Pfeiffer[6] (56 mμg/g).

The spermidine fluorophore has about 1/30 of the fluorescence intensity of that of histamine.[5] In extracts made with organic solvents, and reacted with OPT, spermidine would contribute to apparent histamine according to the ratio of their concentrations (Table 1: from lung, 1·94, to midbrain, 588). The values for histamine we have found after purification (Table 1) are 1/2 to 1/5 of those reported from unpurified extracts.[1,8] OPT fluorophores in effluents not containing histamine can account to varying degrees in different tissues (Table 2, column I) for what can be misconstrued as histamine when fluorescence intensity is read at 450 mμ when activated at 350 mμ; our values calculated in this way agree with those obtained without purification by Beall[8] and Shore *et al.*[1] (Table 2, columns J, K). Up to 30 per cent of the total fluorescence intensity is due to OPT fluorophores other than those of histamine and spermidine (Table 2, columns A–D, G). The other OPT-reactive substances remain to be characterized.

Little is known about the functional significance of spermidine or its distribution in mammalian organs.[12–14] Its study has been hindered by the lack of specific and sensitive methods. Method 3 for spermidine is simpler and quicker than existing procedures[12,14,15] and about 80 times more sensitive than that of Dubin.[15] Tissue values found in the present study (Table 1) approximate previous findings based on OPT[7] or dinitrofluorobenzene.[12,14]

TABLE 2. PER CENT DISTRIBUTION OF FLUORESCENCE INTENSITY OF OPT-REACTIVITY MATERIALS IN EFFLUENTS OF TISSUE EXTRACTS WITH PURIFIED CELLEX-P: FLUORESCENCE AT 450 mμ RESULTING FROM ACTIVATION AT 350 mμ

	A	B	C	D	E	F	G	H	I	J	K
		Distribution (%)						Histamine (μg/g)		Reported values	
Buffered extract		H_2O	0·01 N HCl	0·03 N HCl	0·05 N HCl	0·1 N HCl	0·2 N HCl	Present values	Adjusted values*	Beall[8]	Shore *et al.*[1]
Brain	15·3	4·4	2·5	15·8	**15·4**	46·5	0	0·062	0·39	0·245	0·2, 0·3
Liver	5·5	11·0	2·0	4·0	**33·4**	36	8	0·564	1·69	1·2–2·2	1·8–2·8
Kidney	16	12	0	0	**19**	48	5	0·932	4·94	2·4–4·5	2–2·8
Spleen	11·5	7·8	1·8	2·2	**49·7**	25·3	11·6	2·83	5·66	12·6	
Heart	3·1	2·9	0	0·7	**83·6**	9·7	0	2·31	2·77	7·8–10	5·8–6·4
Lung	0·15	0·23	0·27	0·31	**97·2**	1·5	0·3	12·88	13·27	24·6–48	19–35
	1·5	1·0		0·76	**91·4**	2·5	2·7	12·88	14·04		

* Adjusted values are recalculated on the basis of OPT-reactive fluorescence not due to histamine (compare Table 1).
Values for histamine-containing effluent are in bold-face type.

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