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THE RELATIONSHIP BETWEEN THE PERMEABILITY-INCREASING AND THE HYPOTENSIVE PROTEASES OF PLASMA

BY

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Guinea-pig plasma contains at least two proteases that increase the permeability of small blood vessels—the globulin factor designated PF/Dil by Miles & Wilhelm (1955) and a kininogenase, analogous to the kallikrein of human plasma described by Werle (1959). When diluted guinea-pig plasma comes into contact with glass, the PF/Dil is activated from pro-PF, and the kininogenase appears to be activated from prokininogenase by the PF/Dil (Mason & Miles, 1962). Both substances are inhibited by dyflos and by soya bean trypsin inhibitor. They differ significantly in that PF/Dil, unlike the kininogenase, does not release kinins from ox globulin substrates or from the kininogens of guinea-pig plasma, and is much more heat-stable. Preparations of kininogenases (see Lewis, 1960) and plasma fractions rich in PF/Dil (Wilhelm, Miles & Mackay, 1955) are hypotensive on intravenous injection; and kininogenase-like PF/Dil increases vascular permeability. Fractions of rat and rabbit plasma rich in globulin permeability factors are likewise hypotensive.

The PF/Dil and kininogenases of human plasma have properties analogous to those of guinea-pig plasma, and appear to occupy analogous places in the human kinin-producing system (Mason & Miles, unpublished). Human PF/Dil has been identified as an S5.7 β-globulin, and the kininogenase as an S11.3 γ-globulin (Kagen, Leddy & Becker, 1963).

We record here a survey of a variety of fractions of guinea-pig and rat sera, in terms of their permeability increasing and hypotensive activities. Our primary object was to determine whether, in these crude preparations, the ratio of permeability increasing to hypotensive potency was constant, as evidence that both effects were attributable to one substance. To this end, we measured these effects in three test animals, the guinea-pig, rat and rabbit. We also compared, in the guinea-pig alone, fractions of human plasma rich in PF/Dil and in kallikrein.

METHODS

Serum and plasma fractions

The sera were fractionated as described for the guinea-pig (Wilhelm, Mill, Sparrow, Mackay & Miles, 1958), rat (Wilhelm *et al.*, 1958) and man (Mill, Elder, Miles & Wilhelm, 1958). In human serum most of the PF/Dil was in the fraction designated B1/R, and permeability factor (PF) activity was associated with a predominance of β-globulins; reprecipitated B1/R, designated B1/R1, was more potent. From guinea-pig

and rat serum, three principal fractions were separated: G2, mainly α- and β-globulins; G3, mainly γ-globulin (G4, a reprecipitated G3, consisting of 98% γ-globulin, was sometimes used); and AP, crude albumin (see Mackay, 1955). Most of the PF/Dil was in the G2 fraction, which was further fractionated into G2/1 and G2/2, of which G2/1 was the more potent. An even more potent fraction, G2/1R, was made from G2/1 by reprecipitation. A fraction G1S/P, rich in inhibitor of PF/Dil, was isolated from the supernatant fluid left after precipitation of the material that yields the G2 and G3 fractions. Fractions from several batches of rat and guinea-pig sera were examined; and the remains of a number of potent guinea-pig batches of G2/1R, G2/1 and G2/2 fractions were pooled, in the hope of making a particularly heterogenous preparation for test, G2(Pool). Fractions are referred to in the text by their type designation, followed by the number of the batch of serum or plasma fractionated. Thus the G2/1R precipitate from serum batch 33=G2/1R(33).

Assay of hypotensive factor

Rats of about 500 g and rabbits and guinea-pigs of about 1 kg were used, with anaesthesia, induced by 4 ml./kg of 25% urethane given intraperitoneally and maintained when necessary by ether. All animals received 5 mg/kg of heparin intravenously at the start of the assay. Pressures were recorded by a mercury manometer connected to a carotid artery, with 1% sodium citrate as the intervening fluid.

The test substances, in Locke solution for rats and in 0.85% saline for guinea-pigs and rabbits, were injected into an external jugular vein and followed by 0.5 to 1.0 ml. of the carrier solution. All doses were recorded as weight of protein per kg body weight. Injections were given at intervals of 5 to 10 min into rabbits and guinea-pigs and at 10 to 15 min into rats. In guinea-pigs, large doses at shorter intervals induced tachyphylaxis (Miles, 1961).

In none of the animals did duration of the hypotensive response vary with dose, in the range of low doses tested. The degree of hypotension was not related simply to dose for any given preparation; the proportionate increase in magnitude of the response rapidly diminished with increasing dose (Fig. 1). All the results therefore, except those recorded in Table 1, are of assays in terms of the smallest dose of the standard batch that consistently decreased the blood pressure by a fixed amount. In the guinea-pig, rat and rabbit,

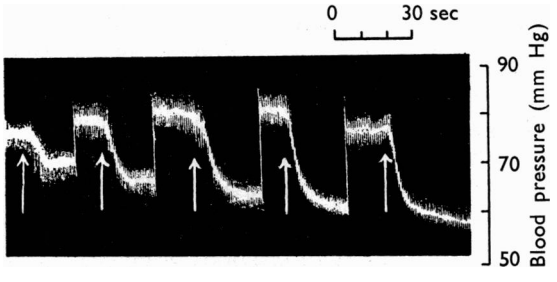

Fig. 1. Hypotensive effect on the guinea-pig of graded intravenous doses of guinea-pig serum fraction G2/1. The five responses are, successively, to 50, 100, 200, 400 and 1,000 μg/kg body weight.

decreases of 10, 8 to 14 and 12 to 16 mm Hg respectively were chosen. For each species of animal, the amount of a standard preparation inducing a decrease within the appropriate range was determined and, from the results of testing graded amounts of the unknown preparation in alternation with the standard, the amount of unknown that matched the standard was estimated. By this method it was possible to match several preparations during the course of an assay.

The doses of the fractions inducing the chosen response in guinea-pigs and rats ranged from 10 to 50 μg/kg, and in rabbits from 1 to 6 mg/kg, according to the individual animal. The amount of a fraction inducing the chosen response is defined as the " effective hypotensive dose " (EHD), and potencies of the hypotensive factor (HF) are expressed as EHD/mg of protein in the fraction.

The results in all instances are the means of at least three assays. Most of the individual values for EHD/mg were within 20% of the mean.

Assay of permeability factors

Permeability-increasing potency was measured by the technique of Miles & Wilhelm (1955; 1960). Estimates were made of the mean diameters of the lesions induced by 0.1-ml. intracutaneous injections of graded concentrations of the fractions into the depilated skin of animals with pontamine blue in their circulation. Plotted against log dose, the values fall approximately on a straight line; from this can be determined the amount of injected substance in 0.1 ml. which, on the average, induces a lesion that at maximum development is 6 mm in diameter. This amount is recorded as the " effective blueing dose " (EBD) (Wilhelm *et al.*, 1958); and permeability-increasing potency is expressed as EBD/mg. Since the effective systemic doses were consistently much larger than the intracutaneous doses, the resulting low values for the ratio EHD/mg : EBD/mg are, for convenience, expressed as 100-times this value (see, for example, Table 2).

In what follows, all hypotensive factors are for the sake of brevity referred to as " HF," and the potency of fractions as " HF potency "; all permeability factors are referred to as " PF," and the potency of fractions as " PF potency." This latter usage does not imply that the PF so designated is necessarily PF/Dil.

RESULTS

Guinea-pig serum fractions tested in guinea-pig, rat and rabbit. In a preliminary survey hypotensive activity was assessed crudely in terms of the degree of response to fixed doses of the various fractions. These were 200 μg/kg in the guinea-pig and rat, except with rabbit fractions tested in the guinea-pig, when 400 μg/kg was used; for assays in the rabbit the dose was 2 mg/kg. The G2/1 fractions from the serum of all three species have the highest content of both HF and PF (Table 1). In the guinea-pig the hypotension was

TABLE 1

HYPOTENSIVE EFFECT AND PERMEABILITY FACTOR CONTENT OF FIXED DOSES OF GUINEA-PIG, RAT AND RABBIT SERUM FRACTIONS IN THREE SPECIES OF TEST ANIMAL

With the rabbit as test species, doses of 2 mg of fraction were used; with the rat, 0·2 mg; and with the guinea-pig, 0·2 mg of fractions from guinea-pig and rat, and 0·4 mg of fractions from rabbit; EBD = effective blueing dose (see Methods). Hypotensive effect: tr = trace, + to ++++ = weak to pronounced

Source of fraction	Fraction	Potency in Guinea-pig: Hypotension	Guinea-pig: EBD	Rat: Hypotension	Rat: EBD	Rabbit: Hypotension	Rabbit: EBD
Guinea-pig	G2/1	++++	380	++++	220	++	260
	G2/2	+++	26	++++	8·8	+	208
	G3	+	3	+	1	0	104
	AP	0	0	0	0	0	0
Rat	G2/1	+++	240	+++	76	++	800
	G2/2	++	<2	tr.	0·7	+	102
	G3	+	2	tr.	0·7	tr.	23·3
	AP	0	0	0	0	0	0
Rabbit	G2/1	+	340	+++	24	+	180
	G2/2	0	4·5	+++	4·3	++	51
	G4	+	<4·5	0	<4·3	0	19
	AP	0	0	0	0	0	0

immediate and lasted 3 to 4 min. The hypotensive response of the rat was slower than that of the guinea-pig. Estimation of the EHD was more difficult in the rat owing to fluctuations in the resting pressure and greater inconsistency in responses to a given dose. Though the rabbit was comparatively insusceptible to the HF in all the fractions, the association of high HF and PF potency is also evident in this animal.

Similar results were obtained when hypotensive potency was measured as EHD/mg. Table 2 lists the mean potencies of guinea-pig serum fractions and the corresponding

TABLE 2

HYPOTENSIVE AND PERMEABILITY INCREASING POTENCY AND POTENCY RATIOS OF SEVEN GUINEA-PIG SERUM FRACTIONS IN TERMS OF EFFECTIVE DOSES PER MG, IN THREE SPECIES OF TEST ANIMAL

EHD = Effective hypotensive dose; EBD = effective blueing dose (see Methods)

		Potency in								
		Guinea-pig			Rat			Rabbit		
Type of fraction	Batch	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*
G2/1R	33	83·3	8,912	0·93	20·0	251·2	8·0	0·83	26·6	3·1
	34	54·6	7,496	0·73	12·9	171·2	7·5	0·71	20·6	3·5
	40	41·7	5,624	0·74	8·7	108·0	8·0	<0·50	17·3	<2·9
G2	Pool	39·2	7,645	0·51	14·1	72·2	19·5	0·71	23·1	3·1
G2/2	33	13·4	1,561	0·86	4·3	25·6	16·9	0·21	8·2	2·6
	34	34·7	3,921	0·88	9·2	62·5	14·7	0·35	5·0	7·1
	40	12·0	1,043	1·16	2·9	14·4	20·0	3·21	48·9	6·6
		Mean ratio		0·83			13·5			4·3
		Coefficient of variation (%)		24·00			41·4			45·3

HF : PF potency ratios in each species of test animal. The ratios are similar for the seven fractions in each species but, in terms of PF potency, the rat's blood pressure is least (ratios 7.5 to 20.0) and the guinea-pig's most (0.51 to 1.16) susceptible to HF.

As previously recorded (Miles & Wilhelm, 1960), guinea-pig PF is most potent in the guinea-pig and least so in the rabbit. The same relation holds for the HF. If for each fraction the PF and HF potencies in the guinea-pig are rated as 1,000, the potencies in rat and rabbit, with the exception of those for G2/2(40), lie within the following ranges:

	PF	HF
Guinea-pig	1,000	1,000
Rat	9·4 to 28·2	208 to 360
Rabbit	1·3 to 5·2	<12·0 to 18·1

With G2/2(40), the corresponding figures for the rat were within the ranges cited, but in the rabbit the PF figure was 46.9 and the HF 267, indicating a peculiarly high potency for this fraction. Nevertheless its HF : PF potency ratio in the rabbit—6.6—is of the same order of size as that for the other fractions (2.6 to 7.1).

The individual HF : PF ratios from the guinea-pig tests in Table 2 cluster more closely round the mean than those from the rat tests. It is to be noted also that the G2/1R(33) chosen as the standard preparation was, except for G2/2(40) in the rabbit, the most potent in all three species; the greater coefficients of variation of the rat and rabbit mean ratios are largely due to deviation from the ratio for the standard by the ratios for G2(Pool) and the G2/2 fractions. These last fractions are predominantly γ-globulins, in contrast to G2/1 preparations, which are predominantly α- and β-globulins.

The results are expressed graphically in Fig. 2. For each animal the fraction G2/1R(33) was taken as the standard with an arbitrary PF and HF potency of 100; and the values for the remaining six fractions were adjusted accordingly. The ratio of unity for the standard then, by definition, lies on the diagonal line representing HF : PF ratios of unity and, if HF and PF were in all instances identical, the values for the other fractions should

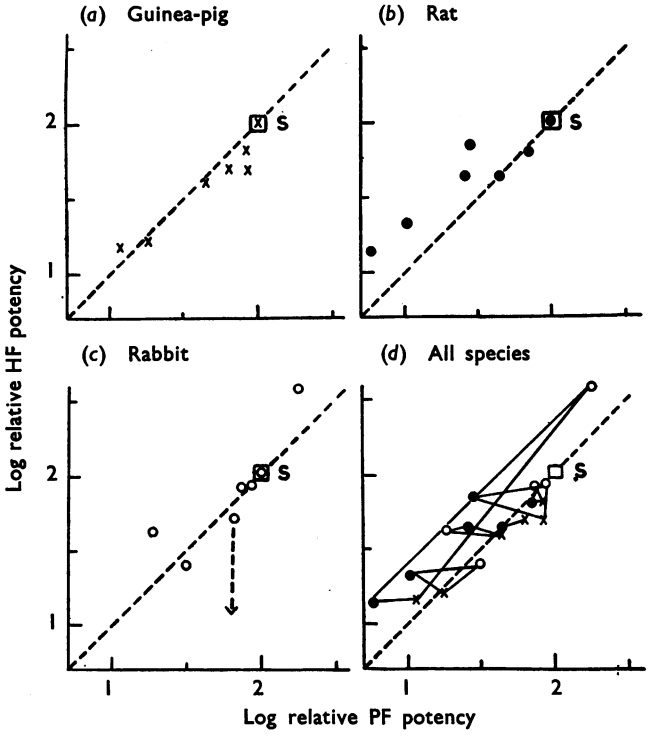

Fig. 2. The relation of hypotensive (HF) and permeability-increasing (PF) potencies of seven fractions of guinea-pig serum. For each of the three test animals an HF and PF potency of 100 is assigned to the "standard" fraction (S), for which the potency ratio is consequently unity, and the values in Table 2 for the remaining six fractions have been adjusted accordingly. (*a*) HF : PF potency ratios in the guinea-pig, ×; (*b*) in the rat, ●; (*c*) in the rabbit, ○; and (*d*) potency ratios in all three species, the points for each of the six fractions being joined by continuous lines.

also lie on it. The assignment to the standard of the same arbitrary potency for measurements in all three species eliminates, of course, the wide differences in species susceptibility to the guinea-pig factors.

In the guinea-pig (Fig. 2,*a*), the homologous test animal, the ratios for the six fractions lie close to the line and, within the limits of experimental error, may be considered to conform to the notion of a constant ratio. In the rat (Fig. 2,*b*), two ratios lie on the diagonal, and four on a line roughly parallel to it, but at a distance from it equivalent to a threefold excess of HF over PF potency. In the rabbit (Fig. 2,*c*), three of the five ratios are on the diagonal; the position of the other two indicates a three- to fourfold excess of HF potency. No ratio was obtainable for the sixth fraction G2/1R(40), because for technical reasons the EHD could not be estimated. It lies somewhere on the vertical dotted line in the centre of Fig. 2,*c*. Even allowing for the imprecision of the two assays, in particular of the HF assay, the discrepancies are large enough to suggest heterogeneity of the active principles in the fractions. The plots of Fig. 2,*a*, *b* and *c*, are combined in Fig. 2,*d*—omitting the indeterminate ratio for G2/1R(40) in the rabbit—and lines are drawn joining the points for each of the fractions as determined in the three test animals. Here again there is a strong indication of heterogeneity in the elongated triangle made on the upper part of the

figure by the three ratios for fraction G2/2(40). As already noted, in comparison with its effect in rat and guinea-pig both the PF and HF of this fraction were highly potent in rabbit. By contrast, the triangles for the potency ratios of the remaining five fractions are compact, indicating a similar relative interspecies susceptibility to HF and PF.

Wilhelm *et al.* (1955) noted that the rabbit's blood pressure was peculiarly susceptible to a preparation of crude guinea-pig G2. This preparation was separated electrophoretically into α- and β-globulins; the α-globulins proved to be the more hypotensive in the guinea-pig and the β-globulins in the rabbit. Moreover, the β-globulins hypotensive for the rabbit were more heat-labile than the α-globulins active in the guinea-pig. That the fraction G2/2(40) contained a substantial amount of an active protein resembling the β-globulin is evident from the effect of heat on the fraction, described in the next section.

Effect of heat on guinea-pig serum fractions, tested in guinea-pig and rabbit. Ten mg/ml. samples of G2(Pool), G2/2(34) and the anomalous G2/2(40) were heated at 98 to 100° C for 1 and 5 min. In both guinea-pigs and rabbits HF and PF potencies declined in parallel (Table 3). Fraction G2/2(40) was outstandingly and disproportionately heat-labile in the

TABLE 3

EFFECT OF HEATING TO 98 TO 100° C ON HF AND PF POTENCY OF THREE GUINEA-PIG SERUM FRACTIONS TESTED IN GUINEA-PIG AND RABBIT

Potency is recorded in terms of the unheated sample, to which a value of 1·0 is assigned for both effects in each animal

		Potency in					
		Guinea-pig			Rabbit		
Fraction	Heating (min)	HF (*a*)	PF (*b*)	*a*/*b*	HF (*a*)	PF (*b*)	*a*/*b*
G2(Pool)	0	1·00	1·00	1·00	1·00	1·00	1·00
	1	0·45	0·33	1·34	0·33	0·37	0·89
	5	0·06	0·06	1·00	0·33	0·33	1·00
G2/2(34)	0	1·00	[1·00	1·00	1·00	1·00	1·00
	1	0·33	0·53	0·62	0·25	0·35	0·71
	5	0·25	0·25	1·00	<0·25	0·30	<0·83
G2/2(40)	0	1·00	1·00	1·00	1·00	1·00	1·00
	1	0·86	0·89	0·97	<0·03	0·15	<0·20
	5	0·80	0·84	0·95	<0·03	0·18	<0·16

rabbit. The HF : PF ratios for 1 and 5 min at 98 to 100° C are <0.20 and <0.16, compared with all other values, which lie between 0.71 and 1.34; these results also are consistent with the presence of a relatively large amount of the heat-labile β-globulin HF described by Wilhelm *et al.* (1955).

Rat serum fractions tested in rat and guinea-pig. Table 4 records the potencies and potency ratios of a number of rat serum fractions. As with the guinea-pig serum fractions, both PF and HF are strongest in the G2/1 fraction and weak in G2/2, G3 and AP fractions. The skins of the rat and guinea-pig are about equally susceptible to the PF; systemic susceptibility, on the other hand, is about five-times greater in the guinea-pig than in the rat. The HF : PF ratios are moderately constant, more so in the tests on the heterologous than in the homologous animal, the means having a coefficient of variation of 32% in the one and 64% in the other. The most discrepant values are those for γ-globulin preparations weak in PF.

TABLE 4

HYPOTENSIVE AND PERMEABILITY INCREASING POTENCY AND POTENCY RATIOS OF FIVE RAT SERUM FRACTIONS IN TERMS OF EFFECTIVE DOSES PER MG IN TWO SPECIES OF TEST ANIMAL

EHD = Effective hypotensive dose; EBD = effective blueing dose (see Methods)

		Potency in					
		Guinea-pig			Rat		
Type of fraction	Batch	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*
G2/1R	10	5·33	595·2	0·90	5·55	50·12	11·07
G2/2	7	0·15	10·3	1·46	0·22	5·61	3·96
	10	0·09	14·9	0·60	0·12	2·82	4·11
G3	10	<0·11	<4·1	—	<0·16	2·58	<6·05
	A	0·05	3·2	1·58	0·03	1·57	1·78
AP1	7	0·12	10·3	1·17	0·33	3·76	8·86
AP	10	<0·04	<0·5	—	<0·03	<0·75	—
		Mean ratio		1·14			5·96
		Coefficient of variation (%)		35·2			64·6

Human serum fractions tested in the guinea-pig. Human PF, fraction B2/1R, and human plasma kallikrein were compared in the guinea-pig (Table 5). Dr Marion Webster kindly gave us four preparations of kallikrein, and assayed the vasodilator potency, both of these and of two of our PF preparations by Frey's test in the hind-limb of the dog (Frey, Kraut & Werle, 1950). Human kallikrein, like human PF/Dil, is a relatively poor permeability factor in the guinea-pig, but for both types of preparation the HF : PF ratios are similar. There is, however, no constant association between either HF or PF potency on the one hand and potency in terms of Frey units on the other. In the light of the rough interspecies parallelism of the HF potencies of rat and guinea-pig plasma fractions, the absence of any parallelism with human plasma fractions, between HF potency in the guinea-pig and Frey unitage in the dog, is noteworthy. It suggests that the hypotensive substances in the human fractions are widely heterogeneous. Alternatively, the vasodilatation upon which the Frey test is based may not, for quantitative or qualitative reasons, be a local expression of the general vasodilatation assumed to be the cause of the fall in blood pressure when these proteases are given intravenously.

TABLE 5

HYPOTENSIVE AND PERMEABILITY INCREASING POTENCY AND POTENCY RATIO OF SIX HUMAN SERUM OR PLASMA FRACTIONS IN TERMS OF EFFECTIVE DOSES PER MG IN THE GUINEA-PIG, AND OF FREY UNITS IN THE DOG

Nt = Not tested. EHD = Effective hypotensive dose; EBD = effective blueing dose (see Methods)

		Potency in			
		Guinea-pig			Dog
Type of fraction	Batch	EHD/mg (*a*)	EBD/mg (*b*)	100*a*/*b*	(Frey units/mg)
B1/R	1	0·64	205	0·31	0·25
	186	1·04	223	0·47	0·14
Kallikrein	V.70.1	0·45	62	0·72	0·05
	V.66.2	Nt	80	Nt	0·11
	V.87.2d2	Nt	258	Nt	0·33
	VII.75.2	6·94	838	0·83	2·00

The effect of inhibitors on guinea-pig serum fractions. The esterase inhibitor dyflos, the trypsin inhibitor from the soya bean and the serum fraction G1S/P that inhibits guinea-pig PF/Dil (Becker, Wilhelm & Miles, 1959) were used.

Soya bean trypsin inhibitor. The relative inhibition by soya bean trypsin inhibitor of the PF and HF in three guinea-pig G2/1R preparations was measured in the guinea-pig and the rat. For the HF tests, doses representing 20 μg/kg in the guinea-pig and 40 μg/kg in the rat—equivalent to about 1 EHD—were held for 10 min at room temperature and immediately assayed. For the PF tests, 0.3 and 25 μg of preparation for guinea-pigs and rats, respectively, representing in the one about 2 EBD and in the other 4 EBD, were used. With HF, inhibition was considered total when the HF effect was eliminated; with PF, when the mean lesion diameter was decreased to 4 mm, a permeability response produced also by the trauma of intracutaneous injection, corresponding to 0.25 EBD. Table 6 records, for each kind of test, estimates of the minimal totally inhibiting dose (MID) of the soya bean trypsin inhibitor.

TABLE 6

THE MINIMUM DOSE (MID) OF SOYA BEAN TRYPSIN INHIBITOR (SBTI) TOTALLY INHIBITING THE HYPOTENSIVE AND PERMEABILITY INCREASING EFFECT OF THREE GUINEA-PIG G2/1R FRACTIONS, TESTED IN GUINEA-PIG AND RAT

EHD = Effective hypotensive dose; EBD = effective blueing dose (see Methods)

		Hypotensive effect			Permeability increasing effect		
			MID			MID	
Test species	Batch of G2/1R	EHD *a*	SBTI (μg) *b*	100*a*/*b*	EBD *a*	SBTI (μg) *b*	*a*/*b*
Guinea-pig	33	1·66	180	0·92	2·67	2·12	1·26
	34	1·09	100	1·09	2·25	1·06	2·12
	40	0·83	80	1·04	1·69	0·79	2·13
Rat	33	0·80	160	0·50	6·28	0·84	7·48
	34	0·52	100	0·52	4·28	0·71	6·04
	40	0·35	60	0·58	2·70	0·31	8·65

There is a close proportionality between the hypotensive potency and the amount of soya bean trypsin inhibitor needed to inhibit it, and a good, though less close, proportionality between PF potency and the inhibitory dose. Precise assays were not made in the rabbit, but it is noteworthy that in this species equivalent hypotensive doses of G2(Pool), G2/2(34) and G2/2(40) were all inactivated by 200 μg of soya bean trypsin inhibitor.

Dyflos. The HF for the guinea-pig in G2(Pool), G2/2(34) and G2/2(40) was destroyed by incubation of 400-μg samples with equal volumes of 5×10^{-4} M-dyflos for 4 hr at 37° C, followed by dialysis against Locke solution for 15 hr at 3° C. Similar treatment of 0.5- to 10-mg samples totally inhibited the hypotensive response in the rabbit.

G1S/P. Fractions of G2/1R(40), G2(Pool) and G2/2(40) in amounts of 30 to 120 μg/kg were incubated for 24 hr at room temperature with the fraction of serum containing the PF inhibitor (Wilhelm *et al.*, 1955), G1S/P(40), at a final concentration of 0.5 and 0.166%. These amounts of G1S/P alone had no hypotensive effect. The HF for guinea-pigs in all three fractions was totally inhibited.

The effect of inhibitors on human plasma fractions tested in the guinea-pig. The relative inhibition of the permeability effect in the guinea-pig was measured on a single preparation each of human B1/R and human kallikrein. Equipotent samples of these fractions were

incubated at room temperature for 30 min with the antihistamine drug triprolidine, with guinea-pig G1S/P, and with the trypsin inhibitors from potato, lima bean, soya bean and eggs (ovomucoid). These drugs had a similar effect (Table 7) on both types of human preparation.

TABLE 7

THE EFFECT OF INHIBITORS ON THE PERMEABILITY INCREASING POTENCY IN THE GUINEA-PIG OF PREPARATIONS OF HUMAN PF/DIL AND HUMAN PLASMA KALLIKREIN

Potency is recorded in terms of the untreated sample to which a value of 1·0 is assigned for each fraction. Nt = Not tested

Inhibitor	Concentration (μg/ml.)	PF potency	
		B1/R1	V-66-2
Nil		1·00	1·00
Triprolidine	100·0	1·00	1·00
Guinea-pig G1S/P	180·0	0·50	0·63
	550·0	0·50	0·50
	1,650·0	0·20	0·13
Potato trypsin inhibitor	0·1	0·33	1·00
	1·0	0·16	0·50
	10·0	0·02	0·13
Lima bean trypsin inhibitor	1·0	0·55	0·63
	10·0	0·55	0·71
Soya bean trypsin inhibitor	0·1	0·33	Nt
	1·0	0·09	0·11
	10·0	0·04	0·05
Ovomucoid	100·0	1·00	1·00

However, the minimum doses (MID) of soya bean trypsin inhibitor totally inhibiting the hypotensive effect in the guinea-pig of equipotent doses of human B1/R and human kallikrein V.70.1 were widely different; they were 200 μg of soya bean trypsin inhibitor for B1/R and 5 μg for human kallikrein.

DISCUSSION

There are a number of inferences about the homogeneity or heterogeneity of the hypotensive and permeability increasing factors in the serum fractions that can be drawn from the numerical relations established by the different tests.

If HF and PF are one substance, which for ease of reference may be designated HPF, the relations would be as follows:

(1) In any one species of test animal, the HF : PF potency ratio would be constant for all preparations.

(2*a*) In any one species of test animal, the HF and the PF effects of all preparations would be decreased to the same proportional extent with progressive inactivation by chemical substances and heat; and

(2*b*) The HF : PF potency ratio would be constant for all degrees of partial inactivation.

(3) On the assumption that there is a constant relative interspecies susceptibility to an active substance—whether HF, PF or HPF—in each species of test animal the preparations would be ranged in the same order of HF and of PF potency.

If the effects are due to more than one HPF, the relationship in (1) is unlikely to hold, unless the HF : PF potency ratio is about the same for each HPF; (2*a*) and (2*b*) will

not hold, because the HPFs are unlikely to have similar relative susceptibilities to a variety of inactivating agents; and (3) will not hold because the HPFs are unlikely to have proportionally the same effect in all species tested.

If HF and PF are distinct single substances, present in varying proportions in the preparations tested, (1) is unlikely to hold; (2*a*) will hold, in so far as the HF and PF potencies are considered separately, but not (2*b*) as regards the constancy of the HF : PF potency ratio after partial inactivation; and (3) will hold.

If HF and PF are distinct and multiple, the relations are likely to be as for multiple HPFs.

The significance of numerical evidence of this kind, obtained with a number of impure preparations of the active substances, depends on the variety of conditions in which the different plasma fractions were prepared. The greater the variety, the less the probability that two distinct factors will be present in proportions constant enough to ensure a constant ratio of potencies. It is evident that the fractions tested were of various degrees of purity. Moreover, the various types of fraction—G2/1, G2/2 and B1/R—were each precipitated from the plasma by substantially different concentrations of ether in buffer solutions of different ionic strength (Kekwick & Mackay, 1954; Mackay, 1955; Mill *et al.*, 1958); these are conditions designed to separate broad categories of the plasma proteins. Each set should therefore result in substantially different mixtures of proteins, including mixtures of HF and PF globulins, if these are indeed distinct substances. The human kallikrein was prepared by yet another technique, involving the removal of unwanted plasma proteins by precipitation with dilute acetone and by absorption with diethylaminoethyl cellulose (see Webster & Pierce, 1961).

The roughly constant proportionality of the HF and PF potencies of most of the serum fractions tested, in both homologous and heterologous species of animal, is some evidence for the identity of HF and PF. The proportionality is the more striking in that for the most part it holds with different HF : PF ratios for tests in three species of animal, among which relative susceptibilities range from 1 to >300. The *prima facie* case for the identity of PF and HF in most of the fractions is strengthened by the similar effect of heat and inhibitors on the PF and HF. However, the inhibitors used are active against a wide variety of proteases and esterases, and might well stand in the same stoichiometric relation to a number of plasma proteases, including more than one kind of protease PF, HF, or HPF. There were, moreover, many discrepancies in the survey that are incompatible with the notion of identity of PF and HF. Thus some guinea-pig serum fractions tended to be more hypotensive in the rat and rabbit than was to be expected on this basis; and the vasoactive potencies of human serum fractions determined in the dog had no constant relation to the HF content measured in the guinea-pig, though in this animal the HF : PF ratios were fairly constant. That one guinea-pig fraction contained a large amount of an anomalously heat-labile HF and PF for the rabbit, and that the HF in human kallikrein preparations differed substantially from the HF in fractions of the B2/1R type in susceptibility to soya bean trypsin inhibitor, are both incompatible with the notion of singularity of a hypothetical HPF.

We have therefore to account for a rough parallelism of effects and some striking discrepancies, discrepancies which are more frequent with serum fractions other than those

containing mainly α- and β-globulins. In general, the discrepancies are incompatible with the notion of a single HPF, or distinct HF and PF, in the plasma of man, guinea-pig and rat.

They are, on the other hand, compatible with the presence in the serum fractions of various proportions of globulin PF/Dil and kininogenase each with HF and PF activity. The HF : PF ratios for either substance alone would presumably be constant, if the components of the systems through which they act *in vivo* were all plentiful and freely available.

In both human and guinea-pig plasma, the globulin PFs are distinct from the kininogenases. In human plasma, the activation of Hageman Factor leads to the activation of kininogenases (kallikreins), which in turn liberate the kinins that are presumably responsible for the permeability and hypotensive effects in injected preparations of the kallikreins (see Margolis, 1960). Activated Hageman Factor also activates human PF/Dil; the two substances, moreover, are distinct (Ratnoff & Miles, 1964). It is postulated elsewhere (Mason & Miles, 1962) that PF activates the kininogenases, thereby introducing another stage into the Hageman Factor—kininogenases—kinin sequence of Margolis, and that an analogous sequence occurs in guinea-pig plasma. On the basis of this amended sequence, namely Hageman Factor (and its analogue in the guinea-pig)—PF/Dil—kininogenases—kinins, injected preparations of PF/Dil also owe their activity to the ultimate liberation of kinins; from the circulating plasma on intravenous injection, and from the plasma proteins of the intercellular fluid on intracutaneous injection.

The interspecies differences in susceptibility to a given fraction may therefore be determined by a variety of factors, such as: differential susceptibility of the various components of the animal's kinin system to the Hageman-like factor, the PF/Dil and the kininogenase isolated from the serum of homologous and heterologous species; and the potency and availability of the endogenous inhibitors of PF and kininogenases and of the kininases that destroy kinins. Judging from the abundance of all components of the kinin system in the plasma of man and guinea-pig, it is unlikely that limiting amounts of any component restrict the response to any great degree; and the interspecies differences in susceptibility are most probably referable to differences in responsiveness of the relevant kinin system to activation by heterologous factors.

The degree to which the HF : PF potency ratios of the fractions vary in a given species of animal could well be accounted for in terms of various proportions of globulin PF and kininogenase, and differing dynamics in the release of kinins, initiated in the one case by the activation of kininogenases, and in the other by direct enzymic attack on the kininogens. If globulin PF/Dil and kininogenase prove to be activated independently by Hageman Factor, and preparations of PF/Dil activate kininogenases only because they are contaminated with Hageman Factor (Margolis, 1963), then PF/Dil itself, which is distinct from Hageman Factor, presumably acts on blood vessels through a system other than that which produces kinins. It is arguable that, in these circumstances, the HF : PF ratios for fractions that appear to be mixtures of PF/Dil and kininogenases would have been more widely scattered than is the observed case. Within a given set of serum fractions, the moderately consistent ranking of HF and PF potencies in homologous and heterologous species of animal, even though species susceptibilities differ widely, is consistent with the notion that injected PF/Dil and kininogenase both act on the same system *in vivo*, namely the kinin-producing system.

SUMMARY

1. There was a roughly constant association of hypotensive and permeability increasing potency among fractions of guinea-pig serum, tested in guinea-pig, rat and rabbit; among rat serum fractions tested in the guinea-pig and rat; and among human serum fractions tested in the guinea-pig.

2. In fractions separated by ether precipitation, α- and β-globulins had the highest potencies, γ-globulins low potencies, and albumins none. In preparations of human kallikrein the two potencies were also roughly associated, but there was no association between them and vasoactivity as measured by the Frey test.

3. The two activities were in general inhibited to the same degree by heat, soya bean trypsin inhibitor and dyflos.

4. With some serum or plasma fractions from each of the three species there were significant deviations from the constant ratios of hypotensive and permeability increasing potencies to be expected if the two activities were due to a single substance, and significant differences in susceptibility to inactivating agents.

5. The results are compatible with the assumption that plasma fractions contain two substances, each with hypotensive and permeability increasing action, one the permeability globulin PF/Dil, the other kininogenase (kallikrein); and that both substances owe their pharmacological activity to the ultimate release of kinins.

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