ENZYME-LIKE GLOBULINS FROM SERUM REPRODUCING THE VASCULAR PHENOMENA OF INFLAMMATION.

I. AN ACTIVABLE PERMEABILITY FACTOR AND ITS INHIBITOR IN GUINEA-PIG SERUM.

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THE reactions of capillary blood vessels in early stages of inflammation include vasodilatation, increased stickiness of the endothelial lining, increased permeability to soluble blood substances and changes facilitating the migration of leucocytes into the tissue spaces. The increase in capillary permeability is prominent among these vascular signs. Endogenous substances have been described as natural mediators of this effect; in the wheal and flare skin reactions of man, and in certain allergic reactions, histamine and Lewis's (1927) H-substance (see, *e.g.*, Ungar, 1953); and in acute inflammation, Menkin's (1940) leukotaxine, which is apparently a family of polypeptides (Duthie and Chain, 1939; Spector, 1951) formed by the breakdown of body proteins. Lewis's H substance is often presumed to be histamine.

Vascular reactions to histamine and leukotaxine are readily studied in the skin of "blued" guinea-pigs; *i.e.*, animals with the vital dye Pontamine Sky Blue in their circulation. Both substances increase capillary permeability to the dye, and thereby induce an intense blueing at the site of an intracutaneous injection. The capillaries recover their normal low permeability after a characteristic period of 10–20 min., and become resistant to further induction of blueing, by either histamine or leukotaxine, for several hours. Since blueing by leukotaxine, like blueing by histamine, is antagonised by mepyramine, it appears that leukotaxine acts by liberating endogenous histamine (Miles and Miles, 1952). However, the increased capillary permeability following injury is not always associated with a demonstrable liberation of histamine; and it may differ from histamine-induced permeability in its time course. Thus we have induced blueing in the guinea-pig skin by pinching, scratching, pressure; by the application of a turpentine poultice or mild blistering agents; by the intracutaneous injection of hypotonic saline, or of calcium chloride (0·02 M in 0·6 per cent saline); but in none of these instances did mepyramine maleate (neoantergan) antagonise the blueing effect. We found, moreover, that normal low permeability of the capillaries was not restored until after the lapse of two or more hours.

We obtained our first hint of an endogenous permeability-increasing substance other than histamine or leukotaxine while investigating cross-resistance to histamine-blueing. Nearly all substances which induce blueing, whether they are demonstrably histamine-liberators or not, will for a few hours render the site of intracutaneous injection resistant to further blueing agents, whereas physiological saline or Ringer's solution, which do not blue, do not induce resistance (Miles and Miles, 1952). Fresh guinea-pig serum, however, itself

with very little blueing activity, and without any modifying action on blueing by histamine injected simultaneously with it, induced a substantial histamine resistance after the lapse of an hour. This contradiction was to some extent resolved when we discovered that the serum contained the precursor of a powerful permeability-inducing substance, which could be simply unmasked by a 1/100 dilution of the serum in 0·85 per cent saline (Mackay, Miles, Schachter and Wilhelm, 1953).

Mammalian sera have been shown to contain substances which are toxic or otherwise pharmacologically active in animals of an alien species (*e.g.*, Brodie, 1900). Any estimate of the significance of the toxicity of such zoologically heterologous sera must, however, be dominated by considerations of species incompatibility; but the existence in homologous sera of the precursor of a strong permeability factor (PF), readily activable *in vitro* by simple dilution, and, as we later found, of an inhibitor of this factor (IPF), suggested to us that we might be dealing with endogenous substances of pathological if not physiological importance. In this paper we describe the conditions of the *in vitro* activation of PF and its relation to IPF.

MATERIALS AND METHODS.

Albino guinea-pigs, 450–650 g. body wt. were used. Some had been immunised with B.C.G. for previous tuberculin assay, but all results obtained with such animals were confirmed with normal guinea-pigs.

The skin of the trunk was depilated, after clipping away the hair, by a paste consisting of yellow barium sulphide 2 parts, and the detergent "Tide" 1 part, mixed to a smooth paste with 10 per cent glycerol in water 10 min. before use (Pitesky and Last, 1948). The depilated area was thoroughly washed with warm water. For permeability tests each animal received 65–75 mg. Pontamine Sky Blue 6 X (G. T. Gurr) per kg. body wt., given intravenously in a 5 per cent solution in 0·425 per cent saline. Animals so injected are referred to as "blued" and were used for tests without delay. The sites of pre-existing inflammation or of injury during depilation become bright blue within a few minutes of blueing; animals with such lesions were discarded.

Intracutaneous injections were given into the skin of the trunk in the region posterior to the shoulder blade and anterior to the knee joint in the sitting animal, and omitting the thin skin about 3–4 cm. on each side of the ventral midline. All solutions for injection were made up in 0·85 per cent saline, which by itself induces no blueing. The volume injected was 0·1 ml., which initially raises a bleb 9–11 mm. in diameter. With short-bevel no. 26 gauge needles, a small moderately pale area of traumatic blueing 1–4 mm. in diameter develops at the centre of the bleb. Consequently, it is only when the diameter of the area of blueing exceeds 3·5–4 mm. that the increase in capillary permeability induced by an injected substance can be considered significant.

The sites of the various intracutaneous injections in any one test were partly randomised and each injection was made in at least four guinea-pigs. Within the area used for these tests, the skin behaves uniformly to substances which increase capillary permeability. These intracutaneous injections were randomised not so much in the anticipation of heterogeneity, as to disguise the order of serial dilutions and so forth, and thus eliminate in subsequent measurements the unavoidable bias that occurs when the relation of lesions to the experimental design can be recognised from their position on the skin. Besides lesion diameter of the blue areas at the sites of injection, the intensity of colour of these lesions was recorded in a grade of ascending intensity: tr. (= trace), $\pm$, $\pm$, +, +$\pm$, ++, +++ and ++++. In general, the intensity of colour was roughly proportional to the lesion diameter, but the evaluation of the intensity of colour was very useful in demonstrating, *e.g.*, the efficacy of certain inhibitors, where lesion-colour, *i.e.*, intensity of exudation, was affected more than lesion diameter. In all the graphs of lesion diameter, each point is the mean of at least 4 lesions each in 1 animal and sometimes of as many as 16 lesions, 4 from each animal.

The duration of increased capillary permeability, the induction of resistance to permeability factors, and the avidity of tissues for the permeability factors, were measured by the methods of Miles and Miles (1952).

" Fresh " serum was collected by cutting the throats of stunned guinea-pigs, after the hair of the throat had been removed by close clipping. Unless otherwise stated, the blood was allowed to clot at room temperature for 5 min., the clot separated from the walls of the container, and the serum harvested after a further period of not more than 2 hr. at 4°. Throughout the year, " room temperature " ranged from 18–26°, but for most of the time the range was 19–22°.

The soya bean trypsin inhibitor (SBTI) and pancreatic trypsin inhibitor (PTI) were supplied by Worthington Biochemical Sales Co., New Jersey, U.S.A. The SBTI proved to be equal in potency to a sample of the highly purified material made by Dr. M. Kunitz, which we obtained through the courtesy of Dr. R. G. Macfarlane. The concentrations of substances in mixtures are given as final concentrations, unless otherwise stated.

Activation of a Permeability Factor (PF/Dil) by Dilution of Fresh Serum.

When 0·1 ml. volumes of serial dilutions of fresh guinea-pig serum in 0·85 per cent saline are injected intracutaneously into " blued " guinea-pigs, within 3 min. capillary permeability is substantially increased and the circulating dye exudes into the injection site to form a circular blue lesion. Fig. 1 summarises

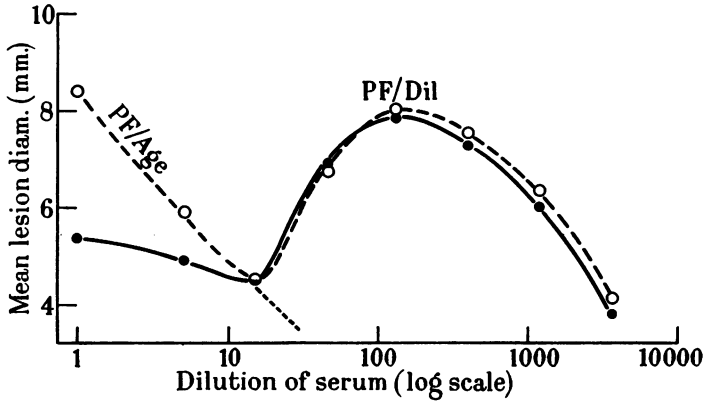

FIG. 1.—The activation of the permeability factor PF/Dil by dilution of fresh guinea-pig serum in 0·85 per cent saline, ●——●. A second permeability factor, PF/Age, which has matured in the same specimen of serum held at 2° for 6 days, superimposed on the PF/Dil activation curve, ○— —○.

In this and succeeding Figures serum dilutions are recorded as reciprocals.

the effect of dilutions that have stood for 1 hr. on the bench before injection. Maximum lesion diameter and maximum intensity of blueing occur in the 1/100–1/400 dilutions. Smaller lesions are produced by 1/1 to 1/15 serum, and the blueing is relatively feeble (±). Beyond 1/400, the blueing is still intense, but the size of the lesion declines with further dilution, and at about 1/5000 it is little more than that caused by needle trauma. This phenomenon was observed consistently with over 100 preparations, of either single sera or pools of sera. It cannot be attributed to any incompatibility between individual animals because it is readily elicited in guinea-pigs tested with their own serum, obtained from blood drawn 4 hr. previously. It was also observed in serum after storage for 17 days at 2° and for 82 days at — 10°. Serum was similarly activated by

dilution in phosphate buffer, pH 8, I = 0·2, and in mammalian Ringer's solution. Preparations of citrated plasma and heparinised plasma, like fresh serum, were activated by dilution with saline. This permeability factor we call PF/Dil, to distinguish it from a second permeability factor, PF/Age, which appears in serum on storage (see below).

The degree of activation in saline also depends on the temperature and age of the serum dilution. A few experiments indicated that maturation of PF/Dil in diluted serum was slow at 2° and about equally rapid at 20° and 37°. Its time course at room temperature is summarised in the three-dimensional graph

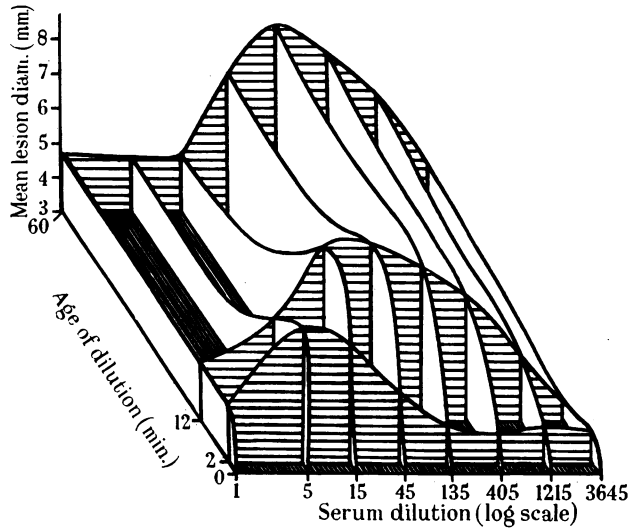

FIG. 2.—The progressive activation at room temperature of PF/Dil in serial saline dilutions of guinea-pig serum after intervals of 2, 12 and 60 min.

(Fig. 2), the curves of which were fitted by eye from the results of a titration of a fresh serum from a single guinea-pig, tested 2, 10–15 and 60 min. after dilution. The maximum activity occurs first in low dilution and shifts to a higher dilution of about 1/135 within the hour (*cf.* Fig. 1). Similar titrations revealed that beyond 1 hr. there was a continued but slower shift of the maximum ; thus, in dilutions standing 1 day at 2°, the peak moves to about 1/1200, and after 4–7 days, to about 1/3600. The end-point also shifts to higher dilutions, though proportionately less than the peak (Fig. 3). We adopted activation after 1–1½ hr. at room temperature as a routine ; and unless otherwise stated PF/Dil is the material so activated. It is evident from the time course of the 1/5, 1/15 and even the 1/45 dilutions, that the striking absence of a permeability factor in less dilute serum at 1 hr. represents the end result of a rapid initial activation and a slower subsequent inactivation, whereas in the more dilute serum, 1/135 and beyond, there is increasing activation during the whole period.

IPF, the inhibitor of PF/Dil.

The appearance of a peak in low dilution after 2 min. and its later shift to high dilution rules out any hypothesis of activation only in high dilution. We

may postulate, in addition to PF, a substance inhibiting it, symbolised by IPF, which in the lower dilution antagonises the activated PF. It would be simplest to assume that in undiluted serum the inactive form of PF was a complex of PF and IPF. This entails the additional assumption that after the rapid splitting of the hypothetical (PF + IPF) complex into free PF and IPF on dilution, a further change initiates a slow recombination of the split products. We should then expect to find inhibiting amounts of IPF wherever PF was manifested, including dilutions of 1/135 or greater; to explain the progressive uninhibited maturation of PF at, *e.g.*, 1/135 (Fig. 2), we must further assume a destruction of the newly liberated IPF in the highest dilutions.

The most economical hypothesis avoiding these additional assumptions is that the inactive precursor of PF/Dil in native serum is quite distinct from the

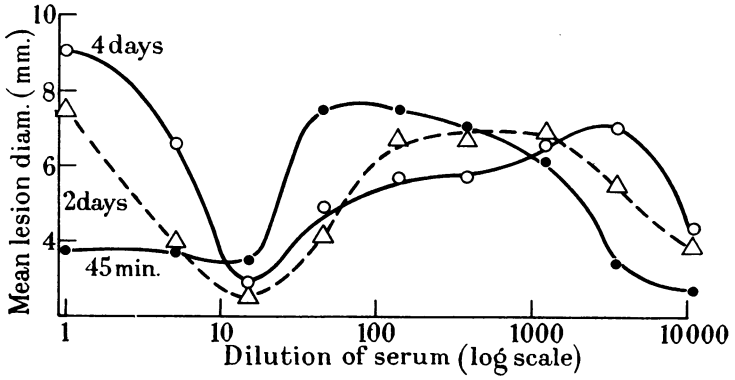

FIG. 3.—The progressive activation of PF/Dil in serial serum dilutions after an interval of 45 min. at room temperature, and subsequently after 2 and 4 days at 2°.

IPF; that there is in native serum a slowly-acting IPF, which is either not very potent or is present in small amounts, so that, at any rate after the lapse of 1 hr., it fails to inhibit all the PF which is activated in dilutions beyond 1/50. The maximum PF activity in dilutions of about 1/135, then, represents the end point of effective IPF inhibition after 1 hr.; after 24 hr. or more, part of the free PF present at 1 hr. has been inhibited by the small amount of slowly-acting IPF present, and the maximum PF activity moves into a more dilute reacting system, where, because of its progressive activation, more PF is present than there was after 1 hr.

The presence of free IPF in lower dilutions is evident from the inhibitory action of 1/2, 1/5 and 1/15 serum on a solution of activated PF/Dil. Four identical series of dilutions of a serum, over a range including the point of maximum activation, were activated by standing for 90 min.; an equal volume of saline was added to each dilution of the first series, and to those of the other three were added respectively equal volumes of the same serum, undiluted and freshly diluted 1/2·5 and 1/7·5. These mixtures were injected after a further 30 min. at room temperature. A detailed analysis of the resulting curves is difficult because of the complexity of the reacting systems in the mixtures, but, in general, it is clear from Fig. 4, which records those for 1/5 and 1/15 serum, that activated PF/Dil

is inhibited by moderately dilute sera, and that 1/15 serum has a greater IPF potency than 1/5 or 1/2 serum. (The curve for 1/2 serum, which is not recorded, was only slightly above that for 1/5 serum.) This suggests that some IPF is activated by dilution. However, the degree of dilution necessary for activation is less than that for PF/Dil. It can further be deduced that in 1/15 serum there is IPF in excess of the amount needed to keep in check any contained PF activated at that dilution. We must therefore conclude that the small activity of recently made low dilutions of serum (*cf.* the 2 min. curve in Fig. 2) is due not only to the action of relatively concentrated IPF, but also to a feebler activation of the PF-precursor in low dilutions. IPF acts relatively slowly; for maximum inhibition of PF/Dil, the mixtures with 1/5 serum must be held for at least 30 min. at room temperature before intracutaneous injection.

Properties of PF/Dil.

Heat-resistance.—The degree of heating which inactivates complement, 30 min. at 56°, does not affect PF/Dil. As Fig. 5 shows, the PF activity of high

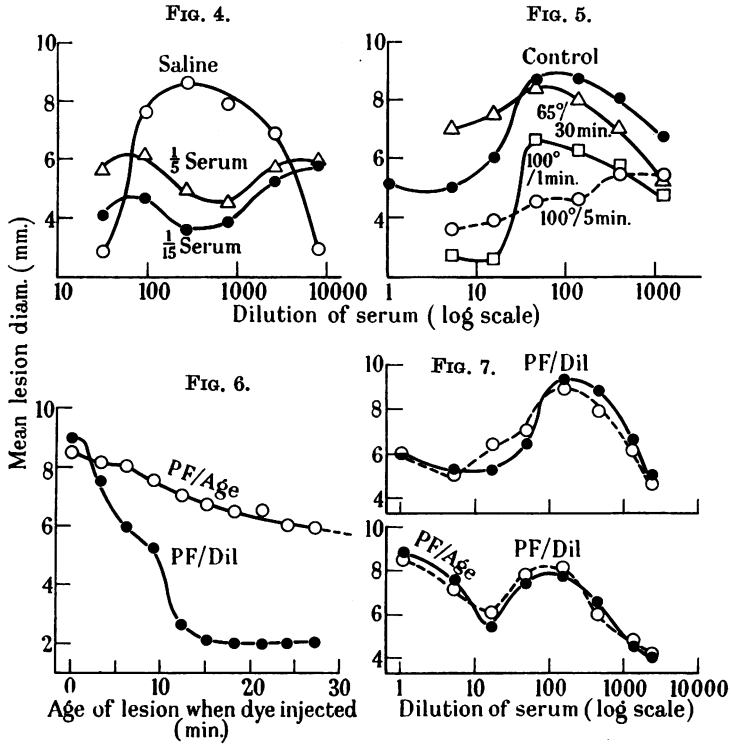

Fig. 4.—The inhibition of PF/Dil by an inhibitor, IPF, present in low dilutions of fresh serum.
Fig. 5.—The heat lability of PF/Dil. The effect of heating serial dilutions of serum in which PF/Dil has been activated.
Fig. 6.—The duration of increased permeability induced by PF/Dil and PF/Age (see text).
Fig. 7.—The inability of the antihistaminic drug mepyramine maleate to modify the permeability effect of PF/Dil and PF/Age. The broken lines connect readings from guinea-pigs given mepyramine, 20 mg./kg., 2 hr. before testing; solid lines connect readings from untreated controls.

dilutions of serum is almost completely destroyed after 5 min. at 100°, but heating does not destroy the activity proportionally in all dilutions. With progressively greater heating, the zone of inactivity in the less dilute serum widens; and the peak activity declines, but remains in about the same dilution. In the highest dilution, 1/1200, after an initial decline, the activity changes little, and indeed is the maximum activity in the dilution series heated for 5 min. at 100°. These results are consistent with the hypothesis that the disappearance of PF is due to its inhibition by IPF, whose slow action is accelerated by heating. In fact the curves in Fig. 5 bear a general resemblance to those in Fig. 3, where the gradual widening of the zone of inactive lower dilutions, with a consequent shift of fresh activity to the higher dilution, is interpreted as due to the slow inhibition of PF by the small quantities of IPF present. Whether this hypothesis is correct or not, in view of the fact that we are dealing, in all dilutions, with a mixture of serum proteins known in other circumstances to form inactive complexes on heating (see, *e.g.*, Bawden and Kleczkowski, 1942; Kleczkowski, 1945) it would be unwise to attribute to the hypothetical factors the heat lability they display as PF/Dil and as serum IPF.

Diffusibility.—PF/Dil is presumably a large-molecular substance, since there was no change in the titration curve of a series of serum dilutions after each had been dialysed in cellophan sacs for 24 hr. at 2° against 0·85 per cent saline.

Duration of increased permeability.— In the blued animal, the increased permeability was evident within 3 min. of the intracutaneous injection of a fully activated serum dilution and the lesion reached a maximum size and intensity within 20 min. The duration of permeability was measured by injecting into depilated guinea-pigs 0·1 ml. of 1/135 serum at 3-min. intervals, and giving dye intravenously immediately after the last intracutaneous injection. Thus, when the dye is injected after half an hour, the skin bears lesions varying in age from 0 to 30 min. and injection sites at which the vessels are no longer permeable do not blue. The permeability decreases steadily, and normal low permeability is restored after 15 min. (Fig. 6). The recovered capillaries become resistant to further applications of PF/Dil and of histamine; the resistance is maximal after 1 hr. and is disappearing after 5 hr.

Susceptibility to an antihistamine drug.—Histamine does not appear to be a mediator of the increased permeability. The dilution curves in guinea-pigs receiving 20 mg./kg. mepyramine maleate intraperitoneally 2 hr. before the test, do not differ significantly from those in control animals (Fig. 7). This dose of mepyramine is sufficient to diminish the permeability-increasing potency of powerful histamine-liberators by 3-fold or more (Miles and Miles, 1952).

Susceptibility to protease inhibitors.—The presence in serum of a biologically active, presumably large-molecular substance, activated by dilution, and of an inhibitor for that substance, is strikingly reminiscent of the plasma fibrinolysins and plasma fibrinolysin-inhibitors (Macfarlane and Pilling, 1946). A further general similarity was evident in the susceptibility of PF/Dil to soya bean trypsin inhibitor (*cf.* Kunitz, 1946–7). Equal volumes of solutions of the inhibitor (SBTI) and 1-hr.-old serum dilutions were held for 30 min. at room temperature, with an appropriate saline control series. The effect on the dilution curve is evident in Fig. 8, which shows the full inhibition of PF/Dil by a final concentration of 5 μg./ml. SBTI and partial inhibition by 2·5 μg./ml. Pancreatic trypsin inhibitor (PTI), on the other hand, in final concentrations of 20 μg./ml., only

slightly depressed the dilution curve ; 200 μg./ml. was more obviously inhibitory (Fig. 9), but its effect was only of the same order as that produced by 2·5 μg./ml. of SBTI. In contradistinction to fibrinolysin (Ungar, Damgaard and Hummel, 1952) PF/Dil was unaffected by prior incubation of the serum dilutions for 30 min. at 37° with 0·001 M and 0·01 M sodium salicylate.

Action on tissues.—Intracutaneous PF/Dil induced no obvious histological change, as observed in stained sections of skin excised 30 and 60 min. after the injection of 0·1 ml. of PF/Dil over the range 1/135 to 1/3645. The 60-min.

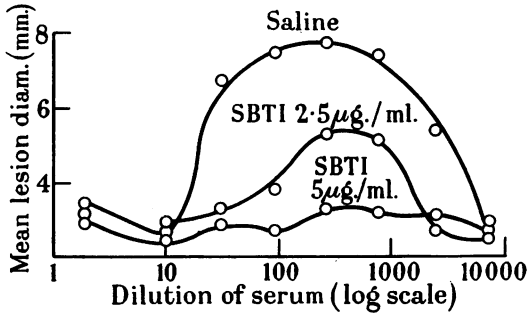

FIG. 8.—The inhibition of PF/Dil in serial dilutions of serum by soya bean trypsin inhibitor.

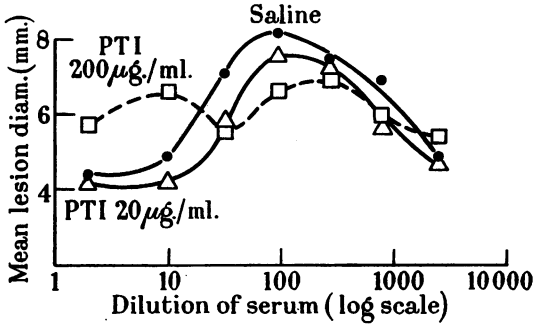

FIG. 9.—The inhibition of PF/Dil in serial dilutions of serum by pancreatic trypsin inhibitor.

lesions with undiluted serum and with 1/15 serum contained a small number, and with 1/5 serum a moderate number, of polymorphonuclear leucocytes in the perivascular tissues.

Maturation of a Permeability Factor (PF/Age) during the Ageing of Serum.

We have already noted that undiluted fresh serum (Fig. 1) sometimes induces a feeble blueing in the skin. This appears to be due to a second permeability factor which increases in amount and in blueing potency when the serum is allowed to age. This factor, which we designate PF/Age, is distinct from PF/Dil. In about 80 per cent of all our guinea-pigs, 0·1 ml. of sera less than 2 hr. old induced lesions 3–6 mm. in diameter, which were faintly or very faintly blue, with indistinct edges. In the remaining 20 per cent the lesions were faintly coloured, but 7–9 mm.

in diameter. Pooled serum behaved like single sera, but when animals were tested with their own serum the lesions were much smaller than those induced by the serum of other guinea-pigs. The frequency of the larger lesions increased when the serum was more than 4 hr. old, or when serum was removed from the clot within 30 min. of bleeding, instead of the routine 1–2 hr. The rate of maturation of this factor with age is evident in the Table. PF/Age also matures in

TABLE.—*The Maturation of PF/Age in Undiluted Guinea-pig Serum at 2°.*

Age of serum.	Approximate percentage of animals tested.	Lesion induced by 0·1 ml.	
		Size (mm.).	Intensity of colour.
2 hr.	80	3–6	tr to ±
	20	7–9	± to ±
4–24 hr.	50	3–6	± to +
	50	7–9	
2–3 days	100	8–10	+ to +±
6 days	100	8–11	+++

sera frozen at −10°, but takes many weeks to do so. Like PF/Dil, it does not dialyse through cellophan, is partly destroyed after 30 min. at 65°, and (Fig. 7) retains its potency in mepyramine-treated animals

The distinction of PF/Age from PF/Dil.

The relation of PF/Age to PF/Dil is displayed in Fig. 10, which records the titration of successive samples of a serum held at 2°, the dilutions of each sample

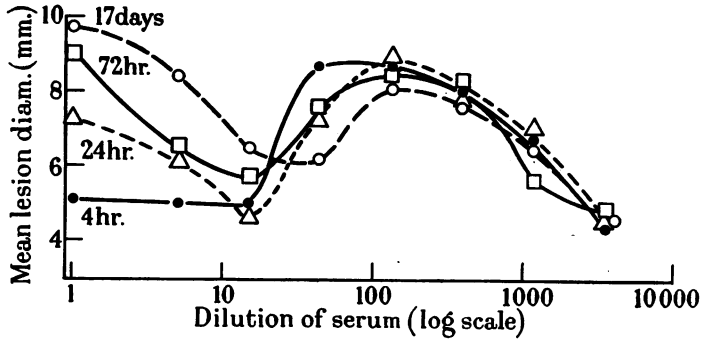

FIG. 10.—The maturation of PF/Age in serum held for varying periods at 2°. The serum samples were injected 90 min. after dilution, so that PF/Dil is also activated.

being held for 1 hr. at room temperature before injection. The PF/Dil maximum remains stationary. (This stationary maximum is not to be confused with the shifting PF/Dil maximum noted previously, which occurs when *serum dilutions*, not whole sera, are allowed to age.) PF/Age, on the other hand, not only increases in potency on ageing as indicated by an increase in the intensity of blueing, but in

amount ; because the end titre determined by extrapolations of the PF/Age curve moves from 50 to 200.

The end titres of PF/Age found in 48-hr.-old serum, and of PF/Dil in 1-hr.-old serum dilutions, provide a rough measure of the relative potency of the two factors. End-points, however, being extrapolations to the 4 mm. line of maximum traumatic blueing, are unsatisfactory responses for comparison. It is reasonable to assume that for both factors the rightward decline of the curves from peak activity represents the progressive dilution of active material. In all titrations, these curves were roughly straight and roughly parallel, with a slope of about $-3{\cdot}5$ to $-4{\cdot}0$ on the logarithmic scale of serum dilutions. It is therefore biometrically valid to compare doses at any desired response level. The 6 mm. lesion is a suitable response for this purpose, being near the mid-point of the decline between maximum diameter and 4 mm. ; we may conveniently define an effective blueing dose (EBD) as the amount in 0·1 ml. producing a 6 mm. lesion. On this basis, one EBD of PF/Age usually occurred at 1/8. In 1-hr.-old dilutions, one EBD of PF/Dil occurred at 1/2,000, and in one- or two-day-old dilutions at values up to 1/12,000. One ml. of serum therefore contains 80 EBD of activable PF/Age and from 20,000 to 120,000 EBD of activable PF/Dil ; so that PF/Age represents 0·4 per cent or less of the total permeability-increasing potency which can be elicited in guinea-pig serum.

Besides its characteristic dilution curve, its relatively low potency, and its slow activation in serum, PF/Age has other properties distinguishing it from PF/Dil. It induces exudation more slowly ; blueing is maximum in 60–90 min., compared with 20 min. for PF/Dil, and (Fig. 8) the increased permeability lasts more than 30 min. It is not inhibited on admixture with 1/2 to 1/15 dilutions of fresh serum (IPF) ; it is only slightly affected by high concentrations of SBTI (100 μg./ml.) and not at all by PTI (200 μg./ml.).

These facts, in our opinion, establish PF/Age as distinct from PF/Dil, and our subsequent investigations were confined to the factors activable in *fresh* serum and to those found in fractions of fresh serum.

The significance of our findings is discussed at the conclusion of our paper on the isolation and characterisation of the main permeability factor in fresh serum, and its inhibitor (Wilhelm, Miles and Mackay, 1954).

SUMMARY.

There is in fresh guinea-pig serum and plasma the precursor of a large-molecular substance which increases the permeability of guinea-pig blood capillaries. On the dilution of serum in 0·85 per cent saline, this permeability factor is rapidly and increasingly activated, substantial amounts being formed in dilutions of about 1/200 within 1 hr. at room temperature. It increases permeability as rapidly as histamine, leukotaxine and synthetic histamine-liberators, and, as with these substances, the capillaries recover their normal low permeability within 20 min. In low dilutions (up to 1/50) there is much less activation of the permeability factor and the material activated is antagonised within half an hour by a low-potency inhibitor, which appears to be present as such in serum, though more of it is activated on dilution to about 1/10.

The permeability factor is not antagonised by the anti-histamine drug mepyramine, or by sodium salicylate. It is antagonised moderately by pancreatic trypsin inhibitor and strongly by soya bean trypsin inhibitor.

When guinea-pig serum is allowed to age *in vitro* at 2°, a second permeability factor, distinct from the first, matures within a few days. Less than 0·5 per cent of the permeability-increasing potency activable in the serum can be attributed to this factor.

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REFERENCES.

BAWDEN, F. C. AND KLECZKOWSKI, A.—(1942) *Brit. J. exp. Path.*, **23**, 169, 178.
BRODIE, T. G.—(1900) *J. Physiol.*, **26**, 48.
DUTHIE, E. S. AND CHAIN, E.—(1939) *Brit. J. exp. Path.*, **20**, 417.
KLECZKOWSKI, A.—(1945) *Ibid.*, **26**, 33.
KUNITZ, M.—(1946–7) *J. gen. Physiol.*, **30**, 291.
LEWIS, T.—(1927) ' The Blood Vessels of the Human Skin and their Responses.' London (Shaw & Sons, Ltd.).
MACFARLANE, R. G. AND PILLING, J.—(1946) *Lancet*, ii, 562.
MACKAY, MARGARET E., MILES, A. A., SCHACHTER, M. AND WILHELM, D. L.—(1953) *Nature, Lond.*, **172**, 714.
MENKIN, V.—(1940) ' Dynamics of Inflammation.' New York (Macmillan).
MILES, A. A. AND MILES, E. M.—(1952) *J. Physiol.*, **118**, 228.
PITESKY, I. AND LAST, J. H.—(1948) *Science*, **108**, 657.
SPECTOR, W. G.—(1951) *J. Path. Bact.*, **63**, 93.
UNGAR, G.—(1953) *Int. Arch. Allergy*, **4**, 258.
Idem, DAMGAARD, E. AND HUMMEL, F. P.—(1952) *Amer. J. Physiol.*, **171**, 545.
WILHELM, D. L., MILES, A. A. AND MACKAY, MARGARET E.—(1955) *Brit. J. exp. Path.*, **36**, 82.