BRADYKININ AND OTHER CAPILLARY ACTIVE FACTORS

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IT IS now established that one of the most potent pharmacological actions of bradykinin, kallidin, and other kinins, is to increase capillary permeability (Schachter, 1960). Since endogeneous enzymes exist which might cause the release of these peptides *in vivo*, we also studied their action. Our experiments demonstrate that these enzymes also increase permeability and that they probably do so by means of the kinin which they release. Our results also indicate that serum kallikrein and not plasmin is the agent in plasma which releases kallidin and increases vascular permeability.

BRADYKININ AND OTHER KININS

The intradermal injection of bradykinin in a guinea-pig with circulating blue dye regularly increases the passage of dye through the blood vessels and produces skin lesions of a more intense blue colour than that produced by histamine (Fig. 1). After injection of bradykinin, visible blueing appears within 2–3 min, whereas the delay after injection of histamine is 4–6 min. The dose-response (response is the mean diameter of blue area) curve for bradykinin also differs from that of

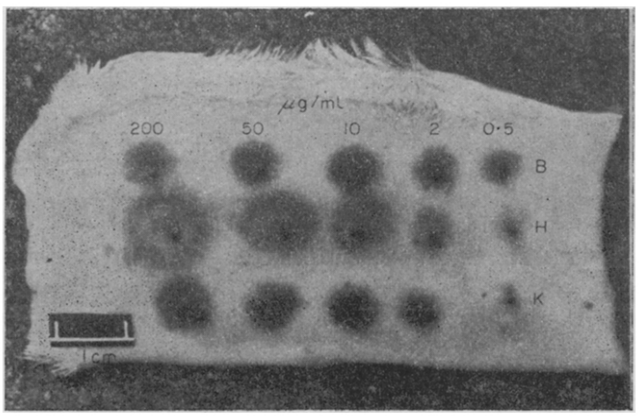

FIG. 1. Skin of guinea-pig with circulating pontamine blue dye showing lesions produced by intradermal injections (0·1 ml.) of B, bradykinin 900 μ/mg); H, histamine; K, serum kallikrein (35 μ/mg protein). (Bhoola *et al.*, 1960.)

histamine in that it shows a pronounced flattening with the higher concentrations (Bhoola *et al.*, 1960). Bradykinin, however, produces larger lesions than histamine in low concentrations. Its effect is not inhibited by prior treatment of the guinea-pig with the antihistamine drug, mepyramine (0·1 mg/kg i.v.).

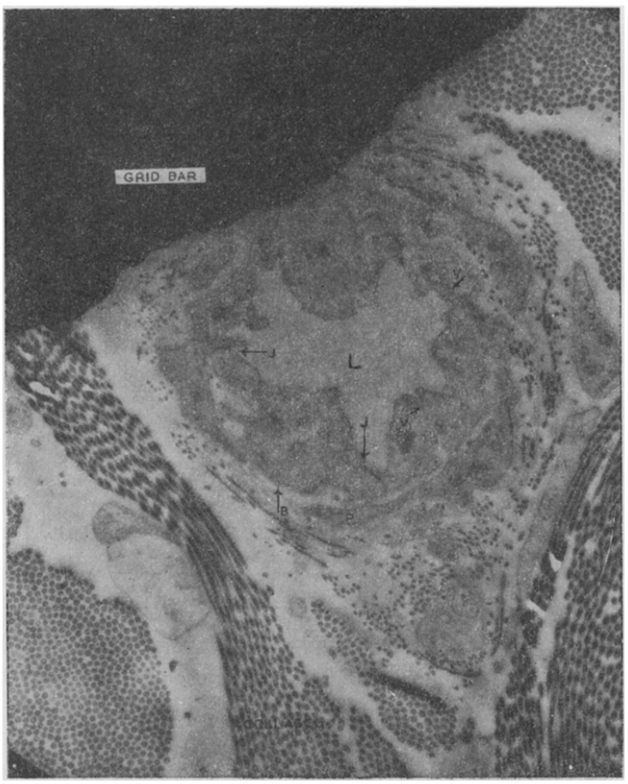

FIG. 2(a). Capillary from control (saline injected) guinea-pig skin. The lumen (L) is surrounded by a continuous layer of endothelial cells with several junctions (J) showing electron dense material. The basement membrane (B) lies outside the endothelial layer and is never in direct contact with the lumen. Note numerous pinocytotic vesicles (V). P, pericapillary cell. × 17,500 (Gray, E. G., Morley, J. and Schachter, M. unpublished).

Cruder preparations of bradykinin and kallidin have been shown also to increase capillary permeability in man (Herxheimer and Schachter, 1959). This has now been confirmed in experiments with Drs. K. D. Bhoola and A. Herxheimer (unpublished) using synthetic bradykinin. Six subjects were injected intradermally (0·05 ml.) on the back with solutions of bradykinin containing 40–200 μg/ml. Weals with a diameter (the mean of the largest diameter and the one at right angles to it) of 25–35 mm were obtained with the highest concentration. The mean diameter of the weal in 4 subjects in response to injection of 0·05 ml. of a bra-

dykinin solution containing 100 μg/ml. was 21·0 mm. The flare reaction, like that produced by histamine, reached a maximum size within 5 min after injection, but disappeared or became very faint, however, after 25–30 min of onset. These findings differ from those of Lewis (1960) who failed to find evidence of weal or flare reactions in man after the intradermal injection of bradykinin.

We have carried out some preliminary electron microscopy studies of the skin capillaries of the guinea-pig after the intradermal injection (0·1 ml.) of synthetic bradykinin (10 μg/ml.). Although the area of skin (on the flank) was one which showed increased capillary permeability to a circulating dye, very few of the capillaries observed showed morphological differences from capillaries in the control (saline injected) area (Fig. 2(a)). A finding, however, which is in accord with the recent observations of Polade (1961) who injected histamine and serotonin into the cremaster muscle of the rat, was a marked discontinuity of the endothelium (with intact subendothelial membrane) in a capillary of skin injec-

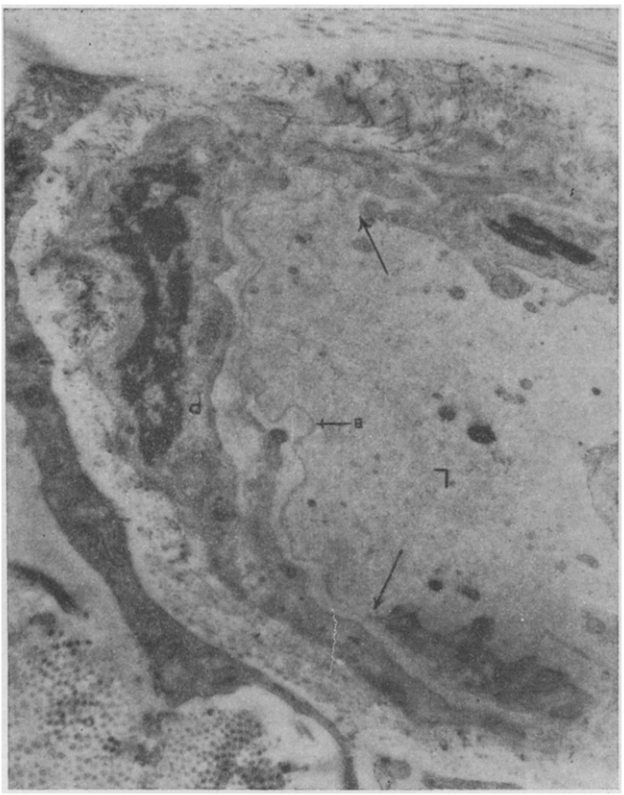

FIG. 2(b). Large capillary from area of bradykinin treated guinea-pig skin. The region between unmarked arrows shows basement membrane (B) in direct contact with lumen (L) due to discontinuity of endothelium lining of lumen. P, pericapillary cell. × 21,000. (Gray E. G., Morley, J. and Schachter, M. unpublished).

ted with bradykinin (Fig. 2(b)). We did not observe any obvious increase in the number of pinocytotic vesicles in the endothelial cells of capillaries of treated skin. In these experiments, skin for microscopic examination was taken 1·0 cm from the intradermal site of injection (i.e., the outer margin of the area showing increased permeability to a circulating dye) in order to avoid changes caused by skin puncture and injection of fluid. Since the area of skin proved to be relatively avascular, we are pursuing these investigations with more vascularized tissues and with techniques which will permit close contact of bradykinin to capillaries without mechanical injury.

Kallidin (produced from ox serum globulin by human salivary kallikrein), which is either identical with, or closely related to bradykinin (Holdstock, *et al.*, 1957; Pierce and Webster, 1961), increased capillary permeability in the skin of the guinea-pig, rabbit and man, in the same way that bradykinin does (Holdstock *et al.*, 1957).

Partially purified wasp kinin is more effective on a weight basis in increasing permeability in the skin of the guinea-pig and rabbit than is histamine. Also, concentrations of wasp kinin which were equivalent in activity to kallidin and bradykinin in contracting smooth muscle were considerably more potent in causing leakage of circulating dye on intradermal injection in these animals (Holdstock *et al.*, 1957). It is possible therefore that wasp kinin and other kinins may prove to be even more active on capillaries than bradykinin.

SERUM KALLIKREIN, SALIVARY KALLIKREIN, AND PLASMIN

Our next problem was to see whether kallikrein and plasmin increased capillary permeability, and if so, whether it was due to the release of kallidin (or bradykinin).

Serum kallikrein (prepared from hog serum) increased capillary permeability on intradermal injection in the guinea-pig (Fig. 1) and its effect resembled bradykinin in every respect. This effect of the enzyme, like its ability to release kinin was greatly reduced by incubating it with soya bean trypsin inhibitor (Bhoola *et al.*, 1960). In view of these facts, and since serum kallikrein released detectable amounts of kallidin (assayed on the isolated guinea-pig ileum) when as little as 0·1 unit was incubated with 0·5 ml plasma for 1 min (a kallikrein concentration of 0·01 μg/ml. in terms of the purest kallikrein preparation), we conclude that the effect of serum kallikrein on capillaries is due to the kallidin it releases.

Human salivary kallikrein has also been shown to increase capillary permeability in the skin of man and rabbit (Holdstock *et al.*, 1957; Herxheimer and Schachter, 1959). Like bradykinin, it produced more intense and more rapid blueing in the rabbit than histamine did, and the dose-

response curve also showed considerable flattening with higher concentrations (Fig. 3). In the guinea-pig, however, the intradermal injection of human (or guinea-pig) salivary kallikrein was without effect even in concentrations up to 1000 μg/ml. This is very different from the reaction in the rabbit which is positive with solutions containing as little as 1·0 μg/ml. This lack of sensitivity of guinea-pig capillaries to salivary kallikrein is correlated with its failure to release kinin from guinea-pig plasma (Schachter, 1960). On the other hand, it does release kinin from the plasma of rabbit and man and also increases their capillary permeability. These results support the view that salivary, as well as serum kallikrein, increases permeability by means of the kinin it releases.

It has been suggested that plasmin, the fibrinolytic enzyme in plasma, is a potent releaser of bradykinin (or kallidin), and that various conditions associated with vasodilatation (reactive hyperaemia, shock, etc.,) are due to activation of plasminogen and the subsequent release of bradykinin (Beraldo, 1950; Lewis, 1958; Hilton, 1960). Our results do not support this view. We found that a preparation of human plasmin did

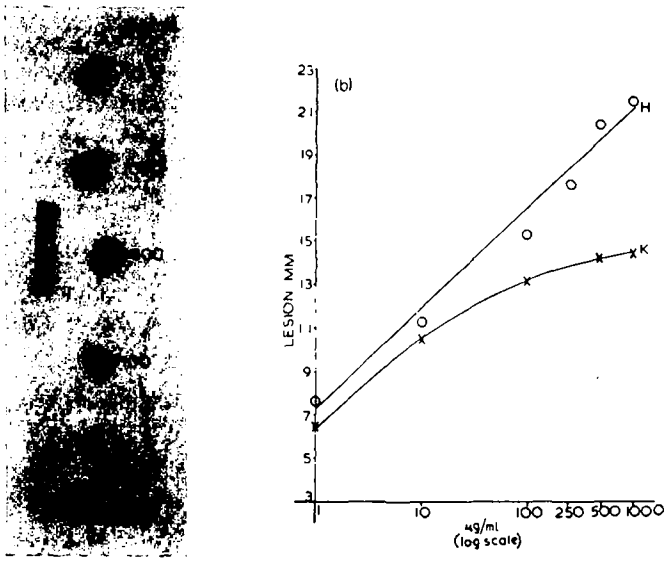

FIG. 3(a). Skin of rabbit with circulating blue dye showing lesions produced by intradermal injection (0·1 ml.) of human salivary kallikrein (K) and histamine (H).

FIG. 3(b). Dose-response relationship for histamine (H) and for human salivary kallikrein (K) in increasing capillary permeability in rabbits in experiments as shown in Fig. 3 (a).

not release kinin from plasma under conditions in which serum kallikrein was very effective (Fig. 4). Plasmin was also practically ineffective in increasing capillary permeability in the guinea-pig (Fig. 5). On the other

hand it was a more active fibrinolytic agent than trypsin or surum kallikrein (Fig. 6). Human salivary kallikrein which releases large amounts of kallidin from human plasma failed to lyse human fibrin. There is no

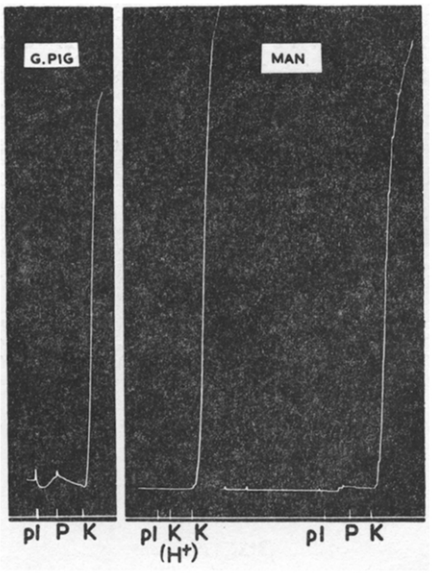

FIG. 4. Serum kallikrein (hog, 35 u/mg protein) is a potent releaser of kallidin from plasma of guinea-pig (left) and of man (right), but human plasmin is without effect.

Contractions of isolated guinea-pig ileum in Tyrode solution (17 ml. bath, 35°C, atropine and mepyramine — 10^{-5} g/l.). Continuous line indicates bath not washed out.

pl, dialysed heated (56°C) plasma (0·5 ml. g. pig; 1·0 ml. man); P, human plasmin (1·0 mg); K, serum kallikrein (0·25 u); K(H^+), serum kallikrein (0·25 u + HCl) at pH 2 for 20 min before neutralization and addition to bath. (Bhoola *et al.*, 1960.)

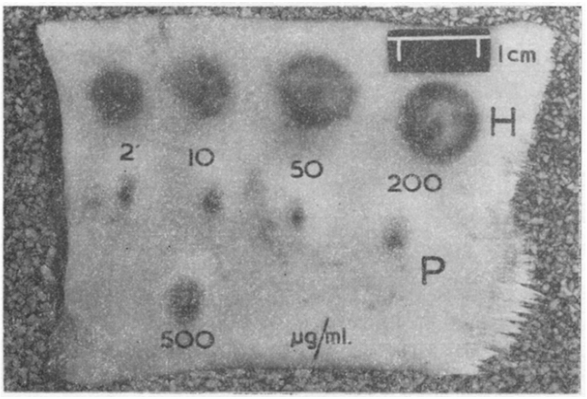

FIG. 5. Skin of guinea-pig with circulating pontamine blue dye. Lesions produced by intradermal injections (0·1 ml.) of H, histamine; P, human plasmin. (Bhoola *et al.*, 1960.)

correlation, therefore, between the fibrinolytic activity of these enzymes and their ability to release kinin.

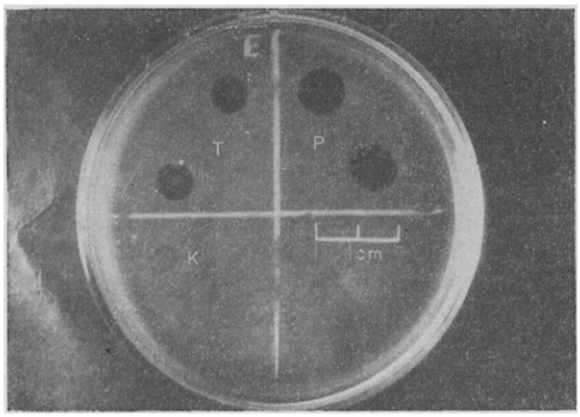

FIG. 6. Lysis of human fibrin (16 hr, 37°C); 0·03 ml. of each solution in concentration of 10 μg/ml. P, plasmin; T, crystalline trypsin; K, serum kallikrein. (Bhoola *et al.*, 1960.)

DISCUSSION

Our results demonstrate that bradykinin (and hence kallidin which appears to be identical with it) is one of the most effective substances in increasing capillary permeability. The same is true of the kallikreins which exert their action by means of the kallidin which they release.

The results also suggest that it is serum kallikreinogen which is activated when kinin rapidly develops in plasma on dilution (Schachter, 1956), on contact with glass (Armstrong *et al.*, 1957), or when diluted plasma acquires permeability enhancing activity (Mackay *et al.*, 1953; Miles and Wilhelm, 1955). Also, that the "permeability globulins" (Miles and Wilhelm, 1955) and "Contact factor" (Margolis, 1958) (both of which have never been distinguished from serum kallikrein) are probably identical with serum kallikrein, since they also increase permeability and release kinin (Armstrong *et al.*, 1957; Lewis, 1958; Margolis, 1958). On the basis of many of the experiments described here we have previously suggested (Bhoola *et al.*, 1960) that those individuals whose blood shows a prolonged clotting time *in vitro* and who are deficient in the so-called Hageman factor in their plasma (Ratnoff and Colopy, 1955) might, in fact, be deficient in the inactive precursor of serum kallikrein, since their sera do not release kinin on contact with glass (Margolis, 1958). This suggestion has recently been confirmed by Webster and Ratnoff (1961) who have found that patients with Hageman trait are deficient in kallikreinogenase, the endogenous activator of kallikreinogen.

The possibility that some clinical disorders with abnormal capillary permeability are associated with defects in the biochemical inactivation of serum kallikrein merits investigation.

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