estingly there is an association between human saliva and the inhibition of acid secretion. Thus in 1949 it was found that the injection of human saliva into animals would inhibit gastric acid secretion[15] and it was found later that the sublingual gland provided the greatest amount of inhibitory material[16]. The extract called sialogastrone caused the inhibition of secretion in pylorus ligated rats by over 50% at doses of 30 mg/kg. Further purification gave material with antisecretory activity at 50 μg/kg in rats but the product behaved as a compound with a molecular weight of about 150,000[17]. At the same time inhibitory material (MW 50,000) was also obtained from mouse submaxillary glands with an activity of 25 μg/kg but these workers found none of the sexual dimorphism associated with epidermal growth factor[18]. This sex difference is quite striking; the male mouse contains ca. 1000 ng/mg wet tissue and the female only ca. 70 ng/ml[19], but treatment of the female with testosterone causes a dramatic rise in the levels of EGF[14]. Smaller amounts were also observed in rat salivary glands[1], but inhibition of acid secretion can be effected in rats, albeit at higher doses than are necessary in the dog. A recent publication described the identification of immunoreactive EGF in the plasma of pregnant human females but not in non-pregnant females or males[20] and levels up to 6 ng/ml were reported during early pregnancy. However, if the doses administered to dogs ~ 0.5 μg/kg i.v., were calculated as blood levels then the maximum briefly attainable would be about 6 ng/ml and this represents near maximal inhibition of gastric acid secretion. If similar doses applied to the human then a persistent blood level of 6 ng/ml would presumably be associated with greatly reduced acid secretion unless, of course, sensitivity was reduced during pregnancy. This may relate to the known low incidence of peptic ulceration during pregnancy.

Among the known properties of EGF in causing cell proliferation is the stimulation of ornithine decarboxylase[21]. More recently it was reported that histidine decarboxylase was also stimulated by EGF in skin and other tissues although gastric mucosa was not amongst those studied[22]. Nevertheless a number of other inhibitors of gastric secretion are also associated with increased histidine decarboxylase activity in the gastric mucosa even though histamine itself is a stimulant of acid secretion[23]! Possibly EGF exerts a similar effect upon gastric mucosa.

The doses of EGF required to affect gastric secretion in dogs are in the same region as those for the hormone gastrin[24], and are low compared to those required for the other observable effects, so that it is tempting to speculate that it is a truly physiological effect. Indeed it may well be that comparable peptides from different species are involved in the control of gastric acid secretion.

Résumé. Le facteur de croissance épidermique (EGF) a été isolé à partir des glandes sous-maxillaires des souris. On a trouvé que ce facteur est un inhibiteur de la sécrétion gastrique. Chez les chiens des doses aussi faibles que 0,1 μg/kg font baisser la sécrétion gastrique provoquée par divers stimulants.

J. M. BOWER, R. CAMBLE, H. GREGORY, E. L. GERRING and I. R. WILLSHIRE

Imperial Chemical Industries Ltd., Pharmaceutical Division, Mereside Alderley Park, Macclesfield (Cheshire SK10 4TG, England) 4 March 1975.

[15] C. F. CODE, H. V. RATKE, G. R. LIVERMORE and W. LUNDBERG, Fedn. Proc. *8*, 26 (1949).
[16] R. MENGUY and M. BERLINSKI, Amer. J. dig. Dis. *12*, 1 (1967).
[17] M. KOBAYASHI and M. YAMAMOTO, J. pharm. Soc. Japan *89*, 222 (1969).
[18] M. KOBAYASHI and M. YAMAMOTO, J. pharm. Soc. Japan *92*, 226 (1972).
[19] R. L. BYYNY, D. N. ORTH and S. COHEN, Endocrinology *90*, 1261 (1972).
[20] I. G. ANCES, Am. J. Obstet. Gynec. *115*, 357 (1973).
[21] M. STASTRY and S. COHEN, Biochim. biophys. Acta. *204*, 578 (1970).
[22] P. T. BLOSSE, E. L. FENTON, S. HENNINGSSON, G. KAHLSON and E. ROSENGREN, Experientia *30*, 23 (1974).
[23] D. V. MAUDSLEY, Y. KOBAYASHI, E. WILLIAMSON and L. BOVAIRD, Nature New Biol. *245*, 148 (1973).
[24] R. A. GREGORY and H. J. TRACY, Gut *5*, 103 (1964).

Modification by Calcium Dobesilate of Histamine Effects on Capillary Ultrastructure

As is well known, the existence of close contacts between cells is the most peculiar feature of epithelial structures. In the vascular endothelium, and particularly at the blood capillary level, intercellular bonds through interdigitation create a selective barrier between the vascular compartment and the interstitial fluid[1]. The stability of these intercellular contacts depends on the chemical composition of the cell membrane, and more precisely of the cell coat, as has been shown in several experimental studies[2]. On the other hand, endothelial cells, and bonds between them, constitue the major structural base of capillary permeability and capillary resistence. Several endogenous chemicals, and among them particularly histamine, are capable of disturbing capillary function, leading to an increase in capillary permeability and a fall in capillary resistence. Histamine produced and stored in mast cells is released in response to a local injury[3], following an antigen-antibody reaction, or even through the effects of physiological or pharmacological substances. In fact, a role for histamine has been postulated in connection with the estrogen-induced increase in capillary permeability at the endometrial level[4].

Since the classical studies of MAJNO and PALADE[5], it is generally accepted that histamine effects on capillary permeability mainly result from an action of histamine on postcapillary venules. The increase in capillary permeability would be the result of separation on endothelial cells at their boundaries, due to the contraction and shrinkage of these cells with formation of stomata[6]. It must be stressed, however, that the evidence in favour of a true contraction of the endothelial cell under the effects of histamine is only weak, and consists mainly in the similitude with the effects of histamine on smooth muscle.

[1] W. BLOOM and D. W. FAWCETT, in *Textbook of Histology* (W. B. Saunders, Philadelphia 1968), p. 358.
[2] J. Z. BORYSENKO and J. P. REVEL, Am. J. Anat. *137*, 403 (1973).
[3] W. G. SPECTOR, Pharmac. Rev. *10*, 475 (1958).
[4] C. D. TURNER, in *General Endocrinology* (W. B. Saunders, Philadelphia 1966), p. 439.
[5] G. MAJNO and G. E. PALADE, J. biophys. Biochem. Cyt. *11*, 571 (1961).
[6] G. MAJNO, M. S. SHEA and M. LEVENTHAL, J. Cell Biol. *42*, 648 (1969).

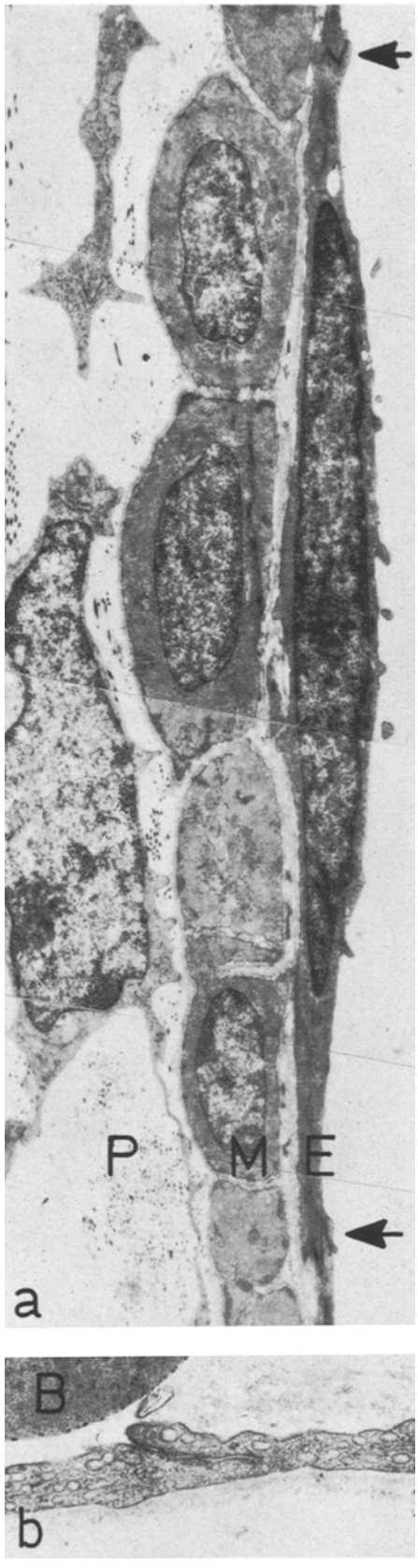

Fig. 1. Blood vessels of the dermis of a control rat. a) Wall of an arteriole. (←) cell contacts between endothelial cells. E, endothel; M, smooth muscle; P, perivascular connective tissue. ×10,000. b) Detail of the junction between cells of a capillary. Some pinocytotic vesicles are visible. B, red blood cell. ×32,000.

Depolimerization of the intercellular cement[7] has been advanced as a posible explanation for histamine-induced capillary permeability. Traditional electron microscopy techniques have failed to demonstrate the existence of such a 'cement'; but recently specific histochemical methods have shown the existence of a glycoprotein layer in the external surface of cells. This cell coat has been proved to exist in the endothelial cells[8], and may, in fact, fulfil the functions of the intercellular cement of classical histology textbooks. Thus, it has been shown that altering the cell surface charges results in a modification of intercellular adhesiveness as well as of cellular junctions and shape[9,10]. This might, perhaps, suggest again the hypothesis postulating a depolimerization of the intercellular cement, now being reformulated as a modification of the endothelial cell coat.

Calcium dobesilate (Calcium 2, 5-dihydroxybenzenesulfonate) is a drug that has been shown to antagonize increases in capillary permeability induced by a variety of substances, and among them histamine[11]. This led us to

[7] A. A. Miles, in *Lectures on the Scientific Basis of Medicine* (The Atholone Press of the Univ. London 1958–59), vol. 8, p. 198.
[8] P. W. Brandt, Circulation *26*, 1075 (1962).
[9] F. Clementi and G. F. Palade, J. Cell Biol. *42*, 706 (1969).
[10] P. Mestres, *in preparation*.
[11] J. Thomas, N. Dorme, M. Sergant, T. Raynaud and P. Bouvet, Annls. Pharm. franc. *30*, 415 (1972).

Fig. 2. Longitudinal section of a venule in the dermis of a histamine-treated rat. Endothelial cells show several finger-like processes. Arrows indicate that contacts are strongly altered when compared with those of control animals. Nuclei of endothelial cells are strongly infolded. Pinocytotic vesicles are present in the cytoplasm. ×21,000.

study the effects of this drug at the capillary ultrastructural level, and to investigate whether it would modify histamine-induced changes in capillary structure. The following is a preliminary report on the effects of histamine on the blood capillaries of the mouse skin, with or without concomitant treatment with calcium dobesilate.

Methods. 60 adult male albino mice were used. Their weight ranged between 30 and 35 g. Calcium dobesilate or saline were given per os daily for 5 days. On the 5th day, 2 h after the administration of the last dose of calcium dobesilate or saline, an i.v. injection of histamine or saline was given, according to the following schedule: Group A: Saline p.o. + saline i.v.; Group B: Calcium dobesilate (50 mg/kg) p.o. + saline i.v.; Group C: Calcium dobesilate (500 mg/kg) p.o. + saline i.v.; Group D: Saline p.o. + histamine (0.09 mg) i.v.; Group E: Calcium dobesilate (50 mg/kg) p.o. + histamine (0.09 mg) i.v.; Group F: Calcium dobesilate (500 mg/kg) p.o. + histamine (0.09 mg) i.v.

Histamine was given as 0.15 ml per mouse of a solution containing 60 mg/100 ml. Mice were killed by a blow on the head 5 min after the injection of histamine, and pieces of dorsal skin were removed and prepared for electron microscopy by fixation in 3% glutaraldehyde (pH 7.3), post-fixation in osmium 1% osmium tetraoxide and embedding in Durcupan ACM. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results and discussion. Capillary vessels in control animals are made up of endothelial cells with several degrees of pinocytotic vesiculation. Nuclei are flat and discoid and the cell membrane shows no folding. Intercellular junctions appear as zones of greater density at the cell membrane, separated by a gap of about 100 Å. Cytoplasmic filaments abound in the neighborhood of intercellular junctions, and this is more marked in endothelial cells of arterioles than in those of capillaries and venules. The cell surface on the vessel lumen, as well as that in contact with the basement membrane is smooth, or shows at most a few finger-like processes (Figure 1).

Treatment with histamine induces marked changes in the morphology of endothelial cells. Folding and protrusion towards the capillary lumen is seen in all cells and some lose their contacts and separate from the basement

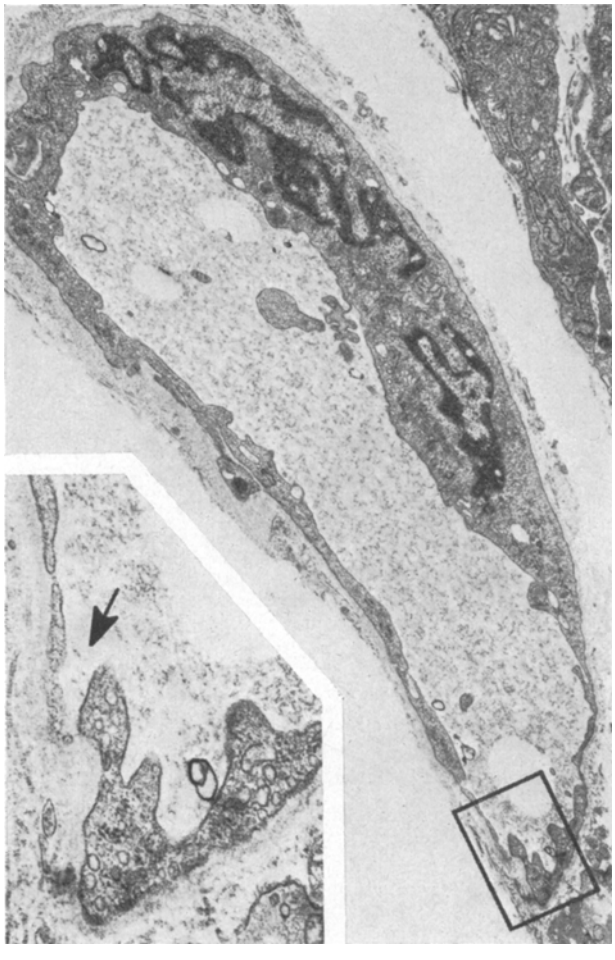

Fig. 3. Cross section of a capillary in the dermis of an histamine-treated rat. Some polymorphous protrusions are present on the surface of the endothelial cell. The nucleus shows an irregular shape as a result of deep infolding. ×8,000. There is a large gap in the capillary wall (→). ×32,000.

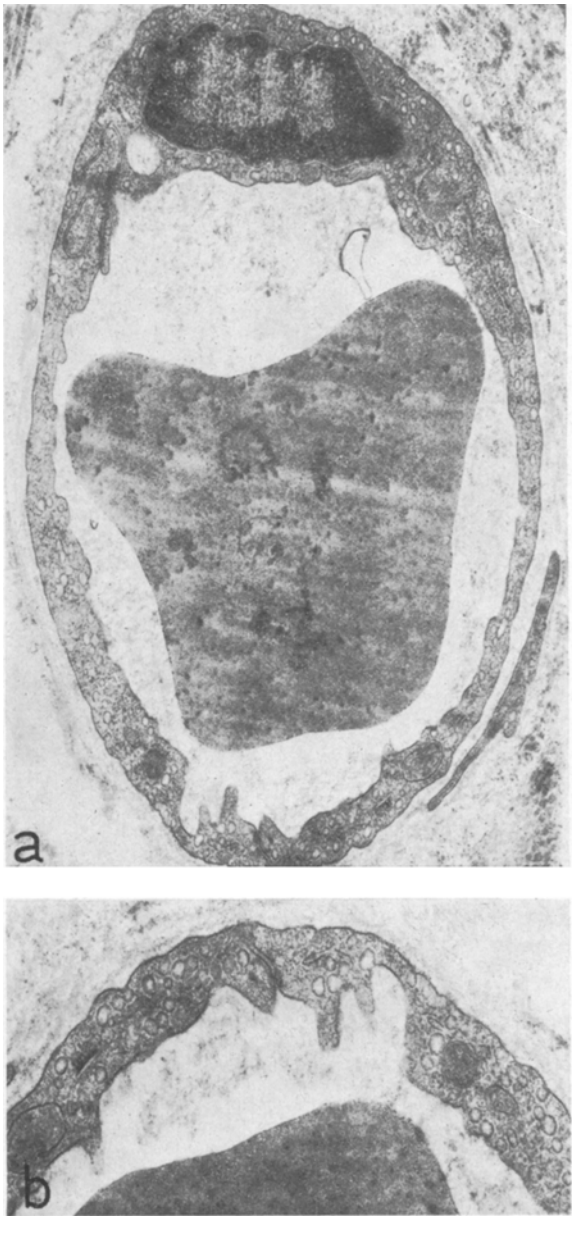

Fig. 4. Blood capillary in the dermis of a rat treated with calcium dobesilate (Group F) before the injection of histamine. The morphology of the endothelial wall is quite similar to that of control animals. a) Transversal section of a capillary. ×18,000; b) detail of the cell contacts ×32,000.

membrane (Figures 2 and 3). On the other hand, nuclei fold in and show a dented appearance (Figure 2). The changes observed are in accordance with those reported by MAJNO et al.[6] and similar to those induced in several kinds of cells by treatment with proteolitic enzymes[12,13] or alkylating agents[2]. Since these substances act by modifying the chemical composition of the cell membrane, it might be suggested that histamine-induced changes could be the result of changes in the cell coat. Histamine could bind, perhaps, to the cell coat and react with negatively charged loci, as happens in plasma proteins[14]. This would result in a diminution of binding forces with a subsequent disruption of intercellular junctions. In order to test this hypothesis, studies aimed at eliciting histamine effects on the cell surface are now in progress at our laboratory. Capillary structures of animals treated with calcium dobesilate alone do not differ significantly from those of control animals (Figure 4). On the other hand, capillary responses to histamine in dobesilate-treated rats markedly differ from those of untreated animals. In fact, endothelial cells appear normal in all senses and no changes are seen in the morphology of capillary venules. Thus, it seems that calcium dobesilate is able to protect the experimental animal from histamine injury at the capillary level. The mechanism of this protection is not clear. Calcium dobesilate is completely devoid of any antihistamine activity in the isolated guinea-pig ileum, as well as in other biological preparations[15], but it has been shown to counteract increases in capillary permeability induced by a variety of causes[11]. We believe that any possible interpretation should take into account the possibility that histamine reaction with the cell coat could be the initial cause of changes in cell morphology. In other words, it would be reasonable to explore whether the endothelial cell contraction, as suggested by MAJNO et al.[6], could be the result of changes in cellular adhesiveness. Capillary protection by calcium dobesilate might well be the result of a direct effect on the cell coat with secondary changes on cellular adhesiveness[16,17].

Summary. The ultrastructure of blood capillaries and venules are studied in rat skin. After i.v. injection of histamine the luminal surface of the endothelial cells show protrusions of variable size. Numerous gaps have be found in the capillary wall, specially in the venules. These alterations are not observed in the animals that were treated with calcium dobesilate before the administration of histamine. In these cases the capillary structure are indistinguishable of the controls. The possible effects of the histamine and calcium dobesilate on the cell coat and cell junctions of the endothelial capillary cells are discussed.

P. MESTRES, L. RODRIGUEZ, S. ERILL and J. LAPORTE

Ruhr-Universität Bochum,
Lehrstuhl für Anatomie I, Postfach 2148,
D-463 Bochum-Querenburg,
(German Federal Republic, BRD), 17 February 1975.

[12] A. MOSCONA, Expl. Cell Res. *3*, 535 (1962).
[13] H. DALEN and P. W. TODD, Expl. Cell Res. *66*, 353 (1971).
[14] J. LABAT, B. LEBEL, G. PARROT, J. L. PARROT and J. E. CURTOIS, C. r. Acad. Sci., Paris *263*, 2050 (1966).
[15] E. MARMO, personal communication.
[16] Supported with a grant of the 'Laboratorios Dr. ESTEVE'. Barcelona, Spain.
[17] The authors are indebted to Mrs. T. MESTRES for her help with the preparation of the manuscript.

Cytotoxicity and Carcinogenicity of Pterosins and Pterosides, 1-Indanone Derivatives from Bracken (*Pteridium aquilinum*)

Since bracken fern (*Pteridium aquilinum*) was noticed to produce tumors in experimental animals[1], extensive surveys have been carried out to attain the isolation of the carcinogenic principle(s)[2,3]. We have also conducted the fractionation of the extracts of dried young leaves and rhizomes of bracken along with cytotoxicity and carcinogenicity tests. We have so far isolated and identified more than 30 compounds, in which sesquiterpenes having 1-indanone nucleus, named pterosins, and the glucosides, pterosides, have been shown to be characteristic constituents of the plant[4].

Although there is no simple efficient method for detecting carcinogenicity using small amount of samples such as separated fractions by chromatographies, we have tentatively employed cytotoxicity tests using HeLa cells for the first screening[5]. Nearly 500 fractions separated from the methanol extract of young leaves of bracken were checked by this method. Although several fractions with high cytotoxicity have not been isolated as pure forms, the toxicity of pterosins and pterosides, isolated as the characteristic constituents, will be collectively presented here.

HeLa cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum. For testing toxic concentration and morphological changes, modified panel method was employed[5]. Each cup of panels contained round cover-glasses, and the cells grown on them, were treated with test compounds for 3 days. These cover-glasses were then fixed with Carnoy's fixative and stained with hematoxylin and eosin. Cell injuries were recorded as 0 through 4, where 0 mean no appreciable effect, 4 lethal effect and 1, 2, 3, gradual injuries between 0 and 4.

In Table I, chemical structures of the compounds and the degree of cell injuries are presented. The morphology

[1] I. A. EVANS and J. MASON, Nature, Lond. *208*, 913 (1965). – I.A. EVANS, Cancer Res. *28*, 2252 (1968). – J. M. PRICE and A. M. PAMUKCU, Cancer Res. *28*, 2247 (1968). – I. HIRONO, C. SHIBUYA, K. FUKUSHI and M. HAGA, J. natn. Cancer Inst. *45*, 179 (1970).
[2] H. LEACH, G. D. BARBER, I. A. EVANS and W. C. EVANS, Biochem. J. *124*, 13 (1971).
[3] C.-Y. WANG, A. M. PAMUKCU and G. T. BRYAN, Phytochemistry *12*, 2298 (1973). – K. TAKATORI, S. NAKANO, S. NAGATA, K. OKUMURA, I. HIRONO and M. SHIMIZU, Chem. pharm. Bull., Tokyo *20*, 1087 (1972). – K. AOKI, K. YAMADA and Y. HIRATA, paper presented at Ann. Meeting of chem. Soc. Japan, April 1973 Tokyo, Abstracts of papers, p. 1780. – H. HIKINO, T. TAKAHASHI and T. TAKEMOTO, Chem. pharm. Bull., Tokyo *20*, 210 (1972).
[4] K. YOSHIHIRA, M. FUKUOKA, M. KUROYANAGI and S. NATORI, Chem. pharm. Bull., Tokyo *19*, 1491 (1971); chem. pharm. Bull., Tokyo *20*, 426 (1972). – M. FUKUOKA, M. KUROYANAGI, M. TŌYAMA, K. YOSHIHIRA and S. NATORI, Chem. pharm. Bull., Tokyo *20*, 2282 (1972). – M. KUROYANAGI, M. FUKUOKA, K. YOSHIHIRA and S. NATORI, Chem. pharm. Bull., Tokyo *22*, 723, 2762 (1974).
[5] M. UMEDA, T. YAMASHITA, M. SAITO, S. SEKITA, C. TAKAHASHI, K. YOSHIHIRA, S. NATORI, H. KURATA and S. UDAGAWA, Jap. J. exp. Med. *44*, 83 (1974).