ENDOTHELIAL CONTRACTION INDUCED BY HISTAMINE-TYPE MEDIATORS

An Electron Microscopic Study

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ABSTRACT

Previous work has shown that endogenous chemical mediators, of which histamine is the prototype, increase the permeability of blood vessels by causing gaps to appear between endothelial cells. In the present paper, morphologic and statistical evidence is presented, to suggest that endothelial cells contract under the influence of mediators, and that this contraction causes the formation of intercellular gaps. Histamine, serotonin, and bradykinin were injected subcutaneously into the scrotum of the rat, and the vessels of the underlying cremaster muscle were examined by electron microscopy. To eliminate the vascular collapse induced by routine fixation, in one series of animals (including controls) the root of the cremaster was constricted for 2–4 min prior to sacrifice, and the tissues were fixed under conditions of mild venous congestion. Electron micrographs were taken of 599 nuclei from the endothelium of small blood vessels representing the various experimental situations. Nuclear deformations were classified into four types of increasing tightness (notches, foldsl closing folds, and pinches. In the latter the apposed surfaces of the nuclear membrane are in contact). It was found that: (1) venous congestion tends to straighten the nuclei in al groups; (2) mediators cause a highly significant increase in the number of pinches ($P < 0.001$), also if the vessels are distended by venous congestion; (3) fixation without venous congestion causes vascular collapse. The degree of endothelial recoil, as measured by nuclear pinches, is very different from that caused by mediators ($P < 0.001$). (4) Pinched nuclei are more frequent in leaking vessels, and in cells adjacent to gaps ($P < 0.001$); (5) mediators also induce, in the endothelium, cytoplasmic changes suggestive of contraction, and similar to those of contracted smooth muscle; (6) there is no evidence of pericyte contraction under the conditions tested. Occasional pericytes appeared to receive fine nerve endings. Various hypotheses to explain nuclear pinching are discussed; the only satisfactory explanation is that which requires endothelial contraction.

INTRODUCTION

Blood vessels may be induced to leak by several mechanisms (1). Apart from direct injury of the endothelium, such as may be produced by physical trauma, the best known agents of vascular leakage

are the so-called chemical mediators. These endogenous substances, of which histamine is the prototype, are especially important in that they are liberated within the tissues in almost every form of local injury. Their mechanism of action began to be clarified when it was shown that they cause small gaps to appear between endothelial cells (2), particularly in the venules (3). The ultimate step, however, remained to be established: that is, how the molecules of chemical mediators succeed in creating intercellular gaps.

This particular process has received very little attention, even though the basic fact, endothelial detachment, had been recognized or suspected long before the advent of electron microscopy (4). In 1958, Miles reviewed several possible mechanisms (5); of these, the depolymerization of a presumed cement substance (6) was soon ruled out when electron microscopy failed to demonstrate a recognizable layer of endothelial cement. As far as we know, only one theory has been studied experimentally (7): it held that the gaps were formed by an increase in hydrostatic pressure, secondary to a constriction of the larger veins (which contain smooth muscle). This concept appeared especially plausible because it seemed to explain, at long last, an intriguing coincidence: the same substances that cause blood vessels to leak (histamine, serotonin, bradykinin) also induce smooth muscle to contract (8). However, studies of living blood vessels in various laboratories, including our own (9–13), have shown that vascular leakage occurs independently of venous spasm. The hydrostatic theory of vascular leakage, as it was expressed, is no longer tenable.

In the meantime, another concept suggested itself. In reviewing electron micrographs of histamine-injected tissues, embedded and stained with the improved methods that became available after 1960, we noticed that some endothelial nuclei of leaking vessels appeared tightly folded, as if they had been forced into a more globular shape by cellular contraction (14). The same occurred after serotonin and bradykinin. The phenomenon, clear enough per se, stood the test of statistical analysis. We are therefore proposing, as a mechanism of action for the histamine-type mediators, that contraction does occur, but in the endothelial cells themselves.[1]

[1] Presumably this is the mechanism that Sir Ashley Miles had in mind, when he wrote ten years ago: "It is tempting to think of the outraged endothelial cell drawing in its skirts, and thereby exposing, at the intercellular junctions, more of an underlying filtration surface . . ." (5)

MATERIALS AND METHODS

Rat cremaster muscles were used, not only because they are rich in blood vessels, convenient to treat with mediators, and to fix, but also because their particular topography makes it easy to produce venous congestion. Fixation under mild venous congestion was adopted when it became apparent that the fixatives induced a constriction of the blood vessels, thus interfering with the experiment.

Venous congestion was produced at first by placing a small pneumatic cuff around the root of the cremaster. This method proved to be too traumatic, and was eventually replaced by a much simpler one, as follows. Under anesthesia with sodium pentobarbital (Diabutal, Diamond Lab., Inc., Des Moines, Iowa), 6 mg/100 g, the skin over the left groin was shaved and incised over the root of the cremaster, where the latter arises from the abdominal wall as a flattened tubular structure about 1 cm in diameter (hereafter referred to as *peduncle*). By blunt dissection, a No. 31 Eberhard Faber rubber band was carefully drawn behind the peduncle, then looped within itself; the loop was gently closed, but not tightened, around the peduncle. To produce venous congestion the loose end of the loop was given 6 full turns, and thus maintained for 2–4 min. It was found empirically that this method produced a reproducible, mild degree of venous congestion. Carbon black injected i.v. appeared very rapidly beyond the constriction; complete blackening took a few seconds longer than on the control side. On whole mounts of the congested cremaster (without carbon injection) the capillary network was much better visualized than in noncongested controls, because red blood cells were present in most capillaries. By electron microscopy, many blood vessels cut in cross section were circular in outline, and contained blood; a few remained closed. Extravasation of red blood cells was never observed. It was concluded that this form of venous congestion was strong enough to keep the vessels stretched during fixation without creating significant injury.

The rats were of the Sprague-Dawley (Holtzman) strain and weighed 250 to 350 g. Six rats were used as controls without congestion; the cremasters were fixed in situ immediately after clamping of the blood supply (2). Of these cremasters, 3 were fixed in 2.5 or 3% glutaraldehyde in cacodylate buffer (2½ to 4 hr) and post-fixed in 2% osmium tetroxide in veronal buffer (2 hr) (15); one was fixed in osmium tetroxide-veronal only, another in Trump fixative (16) and another in Karnovsky fixative (17). Five additional cremasters were fixed in situ after venous congestion;

fixation was in glutaraldehyde-cacodylate with post-fixation in osmium tetroxide-Veronal. All these control animals received carbon black i.v. 3–6 min before sacrifice (Pelikan "biological ink", 0.1 ml/100 g body wt.; John Henschel and Co., Inc., Farmingdale, L.I., N.Y.).

In 9 rats a chemical mediator was injected subcutaneously in the scrotum, carbon black was given i.v., and the animal was sacrificed 3–6 min later (without venous congestion). The mediators, dissolved in saline, were: histamine phosphate (Eli Lilly and Co., Indianapolis, Ind.), 0.1–0.05 ml of a 0.1 mg/ml solution (4 cremasters fixed in glutaraldehyde-osmium tetroxide, 1 in osmium tetroxide alone, 1 in Karnovsky fixative); bradykinin triacetate (Sigma Chemical Co., St. Louis, Mo.). 0.05–0.1 ml of a solution containing 0.05–0.1 mg/ml (Karnovsky or Trump fixation); and serotonin creatinine sulfate (Nutritional Biochemicals Corp., Cleveland, O.), 0.1 ml of a 0.1 mg/ml solution (Karnovsky fixation).

In 5 rats the chemical mediator was administered in conjunction with venous congestion: the peduncle was surgically prepared as described, carbon was given i.v. and the mediator was injected locally; 2–3 min later venous congestion was started and continued for 2–4 min; then the peduncle was clamped and fixation was accomplished in situ as usual. It would have been possible to reverse the protocol and to start the venous congestion *before* injecting the mediator. However, pilot experiments showed that in 15 min venous congestion alone can produce some degree of vascular leakage (possibly as a result of mast cell degranulation and liberation of endogenous mediators). We therefore chose the sequence that would allow us to obtain the desired effect of the congestion (filling and slight distension of the blood vessels) within a time short enough to avoid significant side effects.

All tissues were dehydrated in graded alcohols, embedded in Epon 812, cut with an LKB Ultrotome, stained with a saturated solution of uranyl acetate and Reynolds lead citrate (18), and examined with a Philips EM 200 electron microscope.

EXPERIMENTAL PROCEDURES AND RESULTS

Since the principal aim of this study was to compare the profiles of two populations of nuclei, the first steps were (a) to collect representative samples of nuclei, and (b) to establish criteria for their comparison.

A total of 599 nuclei were assembled, by scanning whole grids from treated animals as well as from controls, and photographing all the endothelial nuclei in small blood vessels (capillaries, venules and arterioles). We eliminated all the nuclei deformed by artefacts, overlapping the edge of the grid, or represented only by a grazing section. The electron micrographs were taken as often as possible at the same enlargement (7,500 × on the EM screen, enlarged 4 × by printing); the different types of indentations were then marked on the prints and counted. The nuclei were subdivided as follows: noncongested controls, 100; congested controls, 118; histamine alone, 92; bradykinin or serotonin, 105; histamine plus congestion, 149; bradykinin plus congestion, 35.

Classification and Significance of the Nuclear Indentations

A preliminary survey showed that all indentations in the nuclear profile could be reduced to four types, that could also be considered as roughly corresponding to four degrees of nuclear compression. Despite some overlap, these types, shown in the scheme (Fig. 1), were usually easy to identify: (A) *Notches*, i.e. shallow indentations with converging sides; (B) *Folds*, with parallel sides; (C) *Closing folds*, the same but narrower at the mouth; (D) *Pinches*, i.e. folds pressed together so tightly that the opposing faces of the outer nuclear membrane came in contact; this could extend along the entire length of the fold, or occur only at the neck. Occasionally, the lips of the fold seemed to have been forced against each other even more tightly, so that the gutter-shaped area of outer nuclear membrane lining the deep part of the fold had been "pinched off"; then, by fusion of the margins, the pinched-off portion had given rise, in cross section, to a circular profile (Fig. 1, bottom right; see also Figs. 12, 15).

After we had thus classified the various types of nuclear deformations, our next step was to verify their incidence under different experimental conditions. To this purpose, on each electron micrograph, each nuclear indentation was labelled according to its type (in case of doubt, the lower-ranking designation was applied). Thereafter, the electron micrographs were separated into four groups corresponding to the four main experimental situations (animals injected with mediator, with or without congestion; controls, with or without congestion); and the total number of deformations was counted in each group. The results, expressed as number of deformations per 100 nuclei, are shown in Table I. It is clear that *qualitative* differences do not exist between injected animals and controls; quantitative differences are

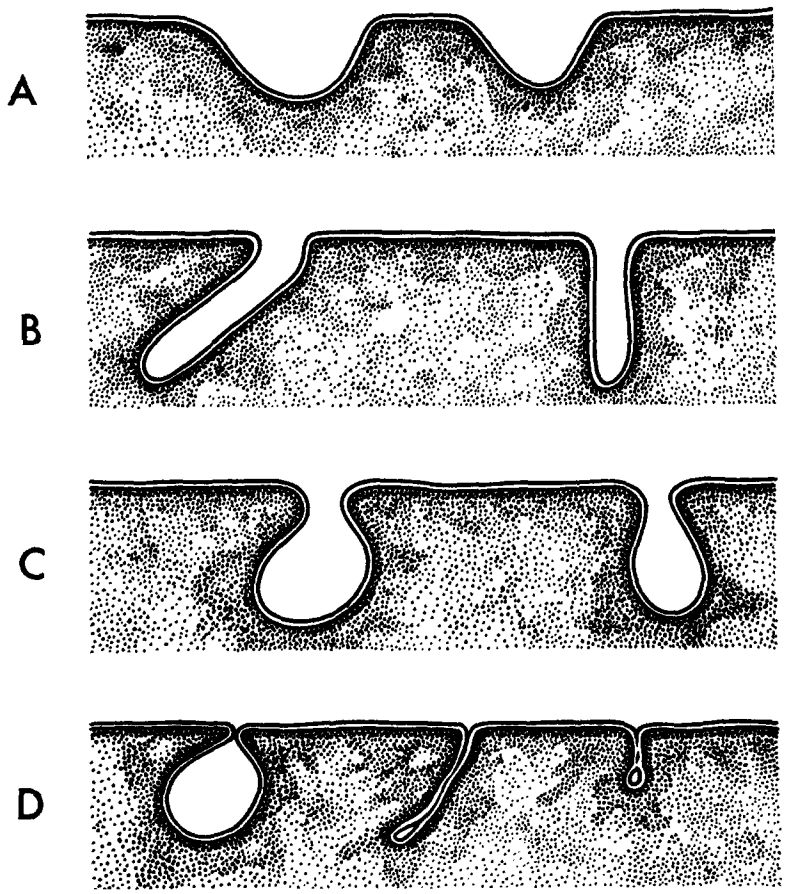


FIGURE 1 Scheme of the indentations observed in endothelial nuclei (normal and after histamine-type mediators): (A) *notches*, i.e. indentations with converging sides; (B) *folds*, with parallel sides; (C) *closing folds*, with a constriction at the mouth, in the manner of a bottleneck; (D) *pinches*, in which the opposing surfaces are pressed together so tightly that the opposing nuclear membranes come in contact along a part or all of the fold. The recess of nuclear membrane in the deepest part of the pocket may become pinched off (right; see also Figs. 12, 15).

obvious, however, especially as regards the "pinches": their occurrence is so low in controls (particularly after congestion) and so drastically increased after injection of mediators, that the pinches come close to representing a specific change, and thus the easiest to detect even before resorting to quantitative methods.

STATISTICAL STUDIES: For the reason just mentioned, and for simplicity, we restricted our further statistical inquiry to the significance of the pinches. The nuclei of the various experimental groups were classified in fourfold contingency tables, on the basis of their distribution according to two dichotomies (control and treated; with and without pinches) (Tables II–IV). In Table IV, because of a low value in one "cell", Yates' correction for continuity was applied (20). The significance of all the associations tested was uniformly high ($P < 0.001$).

The findings in each experimental group will now be described in greater detail.

Controls

WITHOUT CONGESTION: In general, the blood vessles were collapsed or contained very little blood (Fig. 2); some were completely closed, their lumen being reduced to a virtual slit (this seemed to occur especially with Trump and Karnovsky

TABLE I

Type and Frequency of Nuclear Deformations in the Four Main Experimental Groups

Data expressed as number of deformations per 100 nuclei

		Type of nuclear deformation			
		Notches	Folds	Closing folds	Pinches
Without venous congestion	controls (100)‖	622.0* (573.1–670.9)	130.0 (107.7–152.3)	108.0 (87.6–128.4)	11.0‡ (4.77–21.49)
	after injection of a mediator§ (197)‖	426.4 (397.6–455.2)	135.5 (119.2–151.8)	66.0 (54.7–77.3)	166.0 (148.0–184.0)
With venous congestion	controls (118)‖	107.6 (88.9–126.3)	8.5‡ (3.50–17.07)	13.6‡ (6.93–23.75)	1.7‡ (0.126–7.13)
	after injection of a mediator§ (184)‖	202.2 (181.7–222.7)	44.0 (34.4–53.6)	37.5 (28.6–46.4)	48.4 (38.4–58.4)

* 95% confidence limits are given in parentheses, and are based on the standard error of a Poisson series (e.g., $622 \pm 1.96 \sqrt{622}$).

‡ Where numbers are small, 98% confidence limits are obtained from the Table of Garwood (*Biometrika* **28:1** 437. 1936.) and given in parentheses.

§ Histamine, bradykinin or serotonin (results pooled).

‖ Figures in brackets represent the total number of nuclei actually counted.

The table shows that *without venous congestion* the mediators cause a 15-fold increase in the number of pinches (from 11 to 166 per 100 nuclei). The other types of indentations remain unchanged or actually decrease, as if the pinches were formed at their expense.

When the vessels are previously distended by *venous congestion*, the mediators cause a significant increase in all types of nuclear indentations; the most striking increase affects the pinches (from 1.7 to 48.4 per 100 nuclei).

fixatives). In this case the nuclei were invariably thrown into folds, usually multiple (Fig. 4). Otherwise, most endothelial nuclei were irregular in outline: only 3 in 100 were free of significant indentations, whereas folds, closing folds, or pinches were present in approximately four of every five nuclei (Table II, top).

WITH CONGESTION: A very different picture emerged from the congested controls: the nuclei were much smoother in outline (Fig. 5), a difference that also was obvious at a cursory look. The number of "folded" nuclei was one in five, whereas it had been four in five without congestion (Table II). The number of notches per 100 nuclei dropped to 108, whereas it had been 622 without congestion; and pinches per 100 nuclei dropped from 11 to barely 2 (Table I).

TABLE II

Effect of Venous Congestion on the Identations of Endothelial Nuclei

For the purposes of this table, the nuclei were subdivided into two groups according to the degree of indentation: *smooth* (i.e. free of indentations, or with nothing more than "notches") and *folded* (i.e. showing "folds", "closing folds" or "pinches").

	Smooth nuclei	Folded nuclei
Normal controls (100 nuclei)	22	78
Congested controls (118 nuclei)	100	18

$\chi^2 = 86.46$ Degrees of freedom = 1 $P < 0.001$

Animals Injected with Mediators

Whether the rats had been injected with histamine, serotonin, or bradykinin, the results concerning the nuclei were qualitatively the same, and will therefore be described together.

WITHOUT CONGESTION: A low-power survey showed many vessels altered in the manner

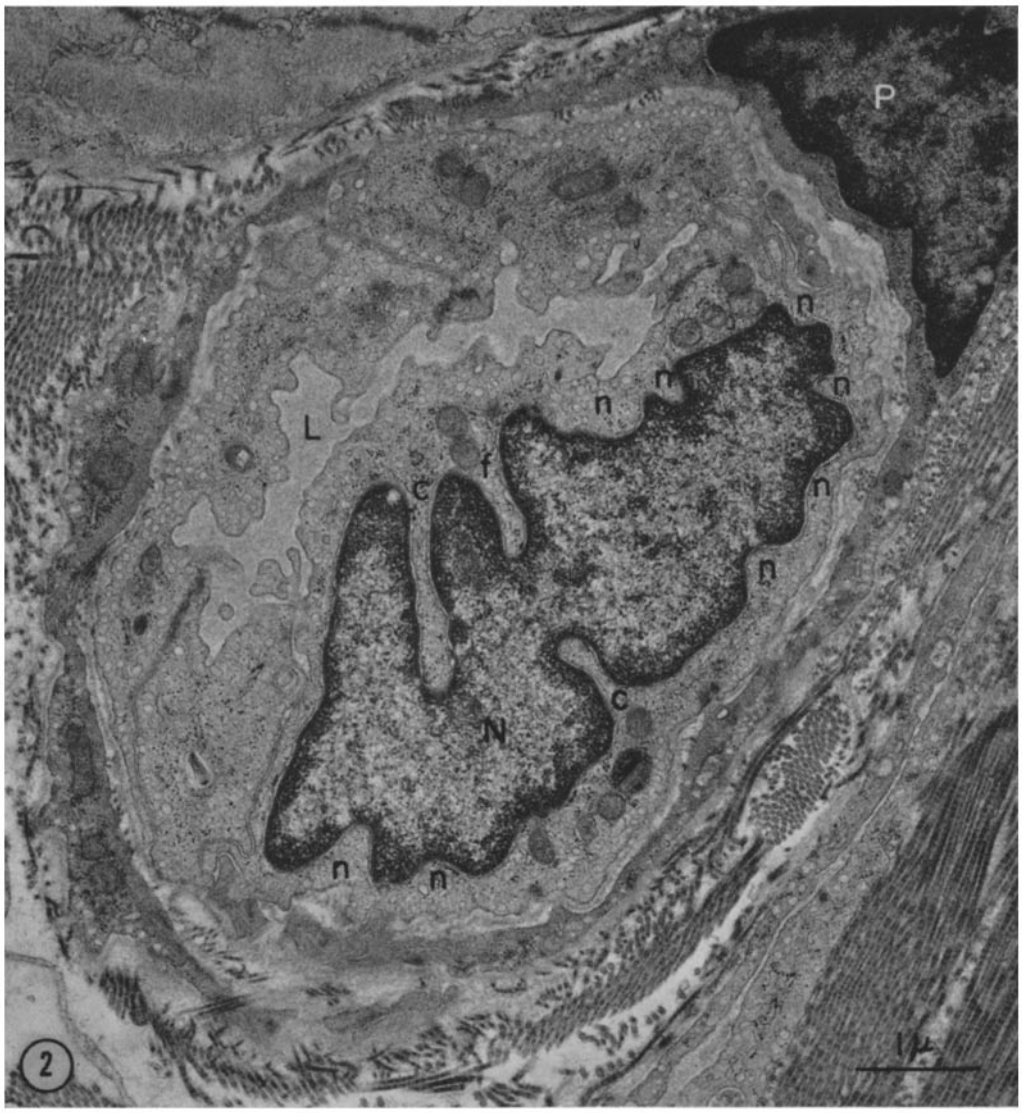


FIGURE 2 Normal blood vessel, collapsed, fixed without venous congestion. The indentations of the endothelial nucleus (*N*) are typical for a vessel in the collapsed state. Each indentation is labeled, to exemplify the nomenclature: *n* = notch, *f* = fold, *c* = closing fold; pinches are absent. (The vessel is probably an arterial capillary: the pericyte (*P*) forms an almost complete sheath, and in parts resembles a smooth muscle cell; the lumen (*L*) is surrounded by 4 cells, a high number for a true capillary.) Fixation: Glutaraldehyde 3%-cacodylate-sucrose, postfixation OsO_4-Veronal.

that is characteristic for histamine-type mediators (2): deposits of carbon black were present between the endothelium and the basement membrane, indicating that carbon-laden plasma had escaped across a gap in the endothelium (e.g. Figs. 8, 9); in some venules the typical gaps between endothelial cells were visible, either open or plugged by a platelet (Fig. 7). Nuclear changes were most striking in these leaking vessels: many endothelial nuclei bulged conspicuously into the lumen, much more so than in the controls (Figs. 6–8) and their overall shape was more rounded than normal. Both changes were quite obvious, even though it would be difficult to translate them into quantita-

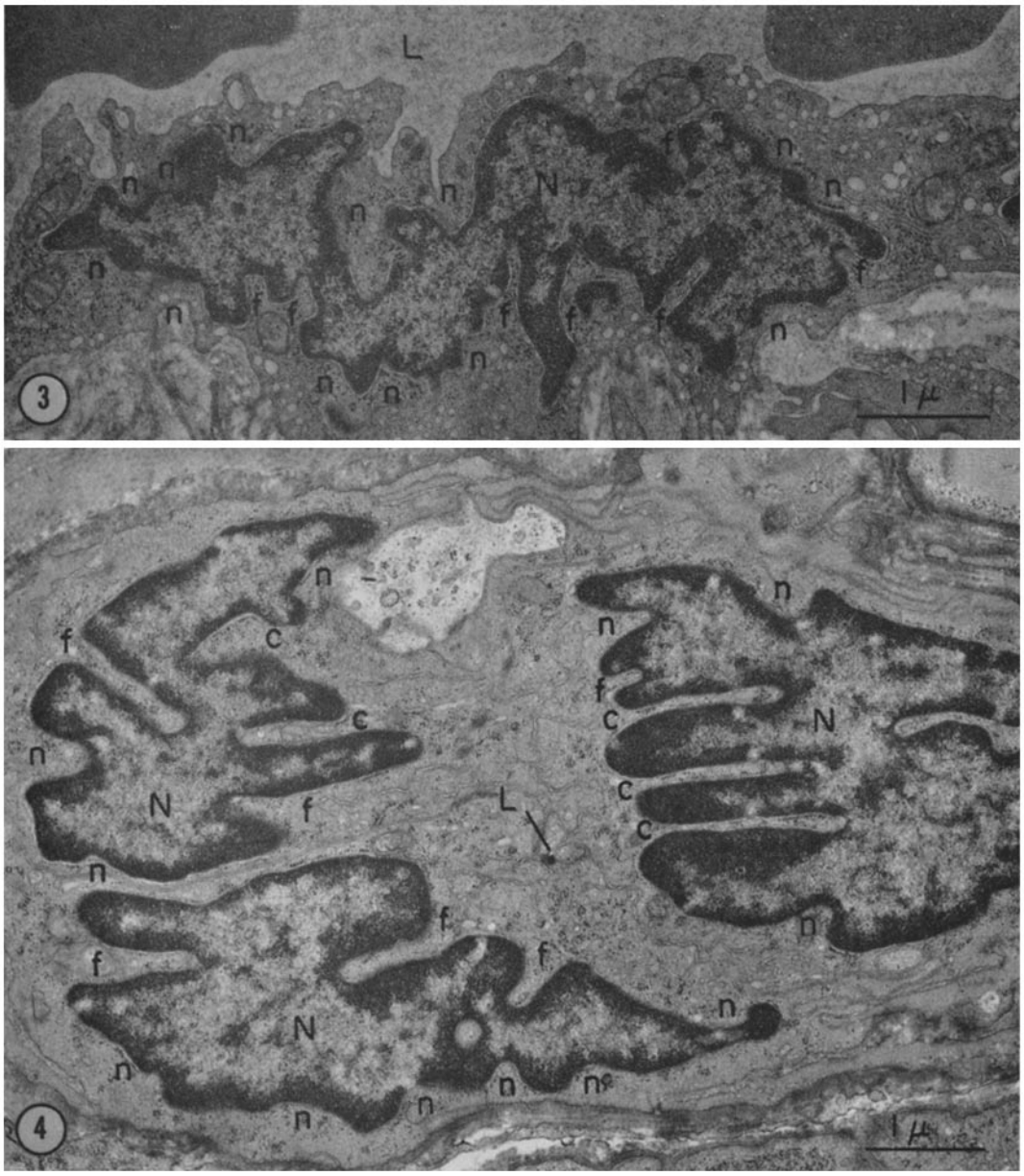


FIGURE 3 Wall of a normal venule, fixed without congestion. The nucleus (*N*) shows the irregular outline typically found in collapsed vessels; it includes 13 notches (*n*) and 7 folds (*f*). No pinches. Fixation: Glutarald. 2.5%-cacodylate, postfixation OsO_4-collidine.

FIGURE 4 Normal vessel, probably a venule, completely collapsed; Karnovsky fixation without congestion. Lumen (*L*) reduced to a virtual slit. Though the nuclei are indented by deep folds, none of these is tight enough to qualify as a pinch. (Same lettering as for Fig. 2.)

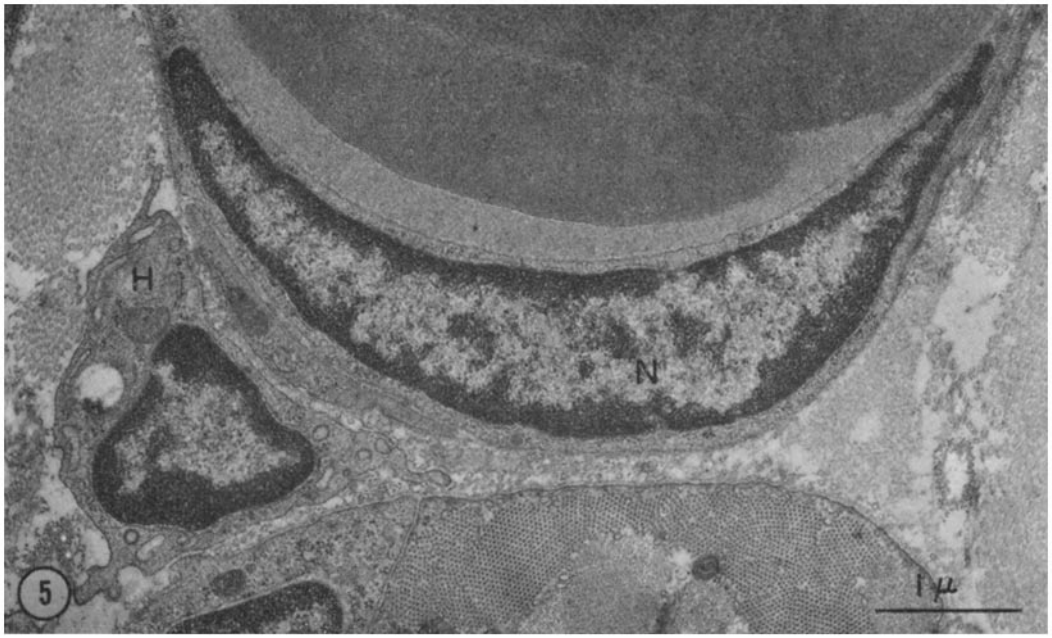


FIGURE 5 Wall of a normal vessel (capillary or venule) fixed with venous congestion. The endothelial nucleus (*N*) is free of indentations and does not bulge into the lumen; the rim of cytoplasm below the nucleus is very thin. *H* = histiocyte. Fixation: Glutarald. 3%-cacodylate. Postfixation OsO_4-collidine.

tive data. Closer inspection showed that some nuclei were indented by as many as 5 to 8 pinches (Figs. 6, 7, 9), *a number never seen in noncongested controls* (where the highest count was 3, observed once only). More than half of the nuclei showed one or more pinches, whereas in noncongested controls only one in ten of the nuclei showed a pinch. Many pinches were so tightly pressed that they appeared as fine slits (Fig. 7) easily overlooked when scanning at low powers. Short bundles of fibrils were sometimes closely applied to the nucleus, either as a single strand of uniform density, or with patches of denser material at irregular intervals (Fig. 6).

Other changes were found outside the nucleus. The base of some cells gave rise to structures resembling pseudopodia, particularly in the region underlying the nucleus (Figs. 6–8); and often the cytoplasm contained circular profiles consisting of two parallel and concentric membranes. These curious profiles were present in all the mediator-injected specimens, whether congested or not. To explain them we see two possible mechanisms: (a) in some cases we may assume the sequence of events reconstructed in Figs. 6 and 7: as the cytoplasm flows during cellular contraction, it deforms some cisternae of ER, causing them to assume a concave shape. A grazing section of this concavity will yield a circular profile (Fig. 14). (b) When the circular profiles appear free of ribosomes, and lie beneath the nucleus (e.g. Fig. 16), they may represent infoldings of the cell membrane, i.e. cross sections of former pseudopodia (such as shown in Figs. 7, 17, 18) surrounded by the bulk of the cytoplasm now flowing into the subnuclear bulge.

WITH CONGESTION: In essence, the findings were the same as in the group just described, though the venous congestion had attenuated certain features and accentuated others. As expected from the study of the controls, the congestion had the effect of "straightening out" many of the nuclei: the fraction of nuclei with pinches dropped from ½ to ⅓, but the difference between nuclei of experimental groups and controls remained highly significant (Table IV). The bulging of endothelial cells, though diminished, was still obvious: Figs. 10 and 11 compare two venules of similar size; both venules were submitted to venous congestion, whereas the second was submitted also to the effect of histamine: in the control (Fig. 10)

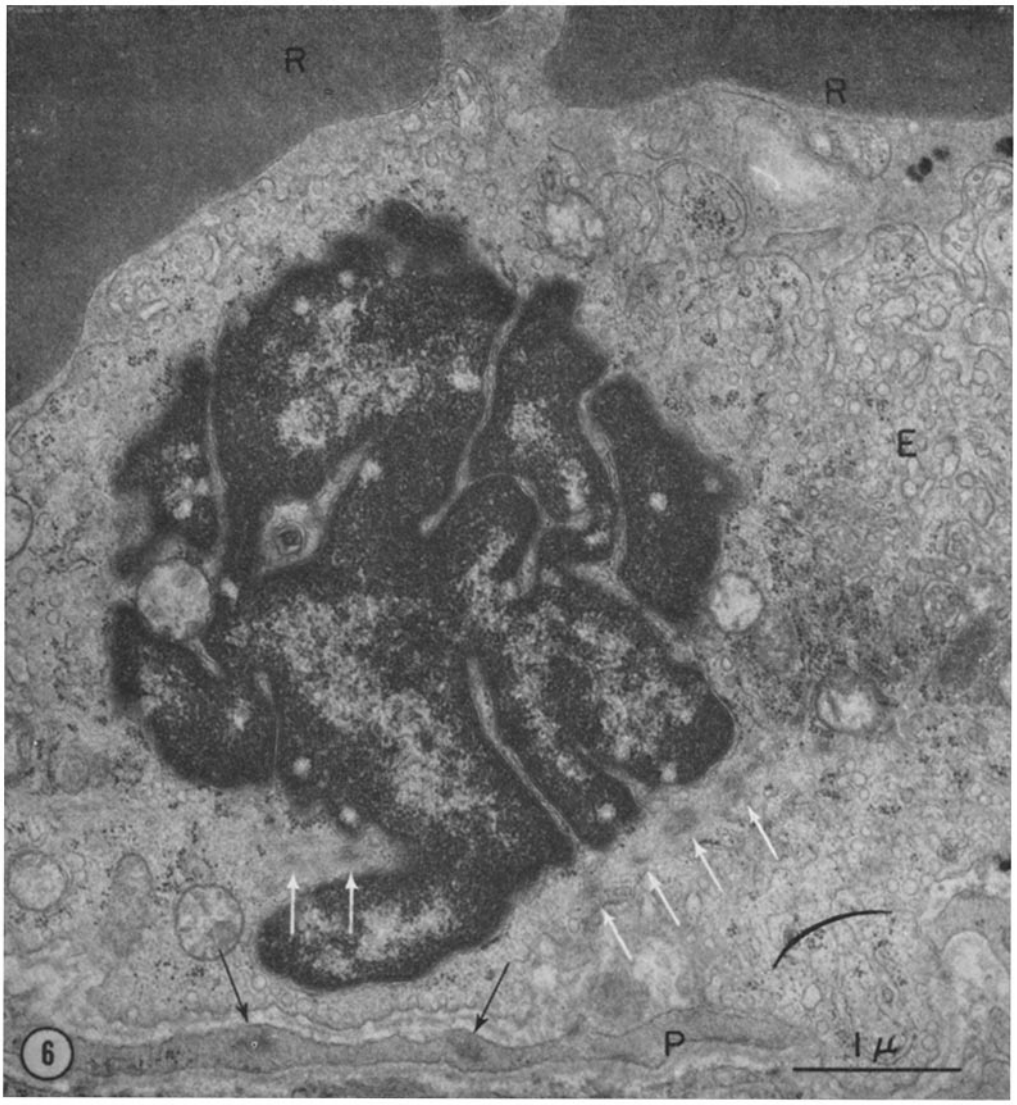


FIGURE 6 Venule, 6 min after histamine; fixation without congestion. The nucleus is rounded and bulges into the lumen (above). Note the narrow folds in the nuclear membrane: of the 8 folds visible, 7 are of the tightest variety (pinches). In this nucleus they are especially conspicuous because the section courses through the denser peripheral part of the nuclear chromatin. *White arrows* point to a juxtanuclear band of fibrillar material, including "dense bodies" at fairly regular intervals. *Black arrows:* "dense bodies" in a pericyte (P). *Bracket:* a portion of the endothelial cell (*E*) is bulging outwards through a discontinuity in the pericyte sheath. The bulge includes a buckled cisterna of endoplasmic reticulum; cross sections of such deformed cisternae give rise to circular profiles (see also Figs. 7, 8, 14, 16). Fixation: Glutarald. 3%—cacodylate, postfixation OsO_4-veronal.

the endothelial cell, though thicker where it contains the nucleus, does not actually bulge into the lumen. In this group the *sub-nuclear cytoplasm* is much thicker, as if the cytoplasm had flowed centripetally to gather around the nucleus (see also Figs. 12 and 14–18); the difference from the controls (Fig. 5) is striking. This subnuclear zone is often rich in ribosomes and poor in vesicles and

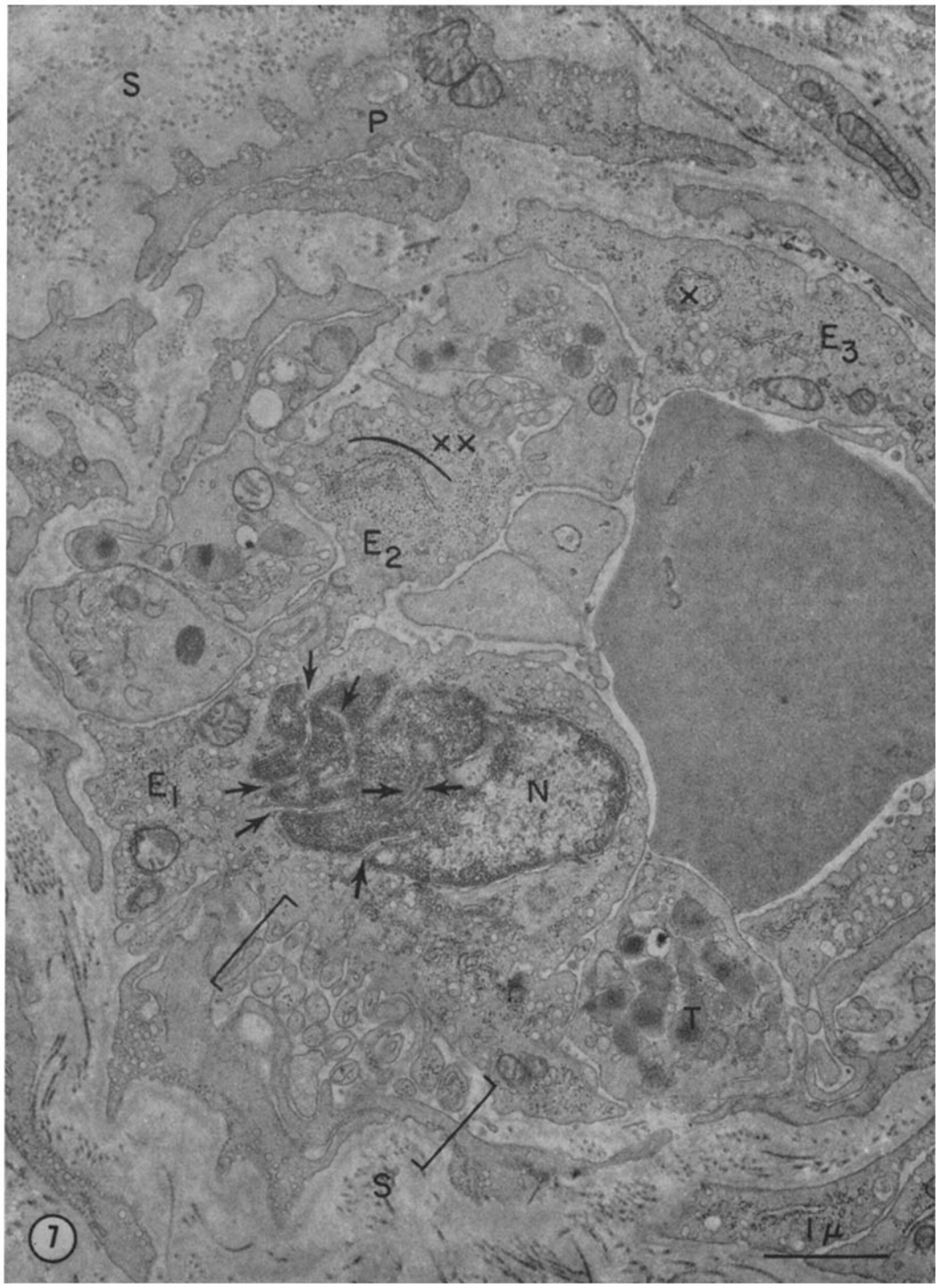


FIGURE 7 Venule, 6 min after histamine; fixation without congestion. Extreme degree of contraction of the endothelial cell E_1 and possibly also of E_2. The nucleus of E_1 (N) bulges toward the lumen and contains 6 or 7 pinches (arrows). Numerous projections emerge from the base of this cell (square brackets). x = circular profile of endoplasmic reticulum. *xx:* below the bracket, a cisterna of ER seems to have been bent into a concave shape (either by cellular contraction or by intracellular flow): this is the likeliest explanation for the image at x (but see also Fig. 16). T = a thrombocyte stopping a gap next to the contracted cell E_1. Note second gap between E_2 and E_3. P = pericyte, S = extracellular space. Fixation: Glutarald. 2.5%—cacodylate, postfixation: OsO_4-collidine.

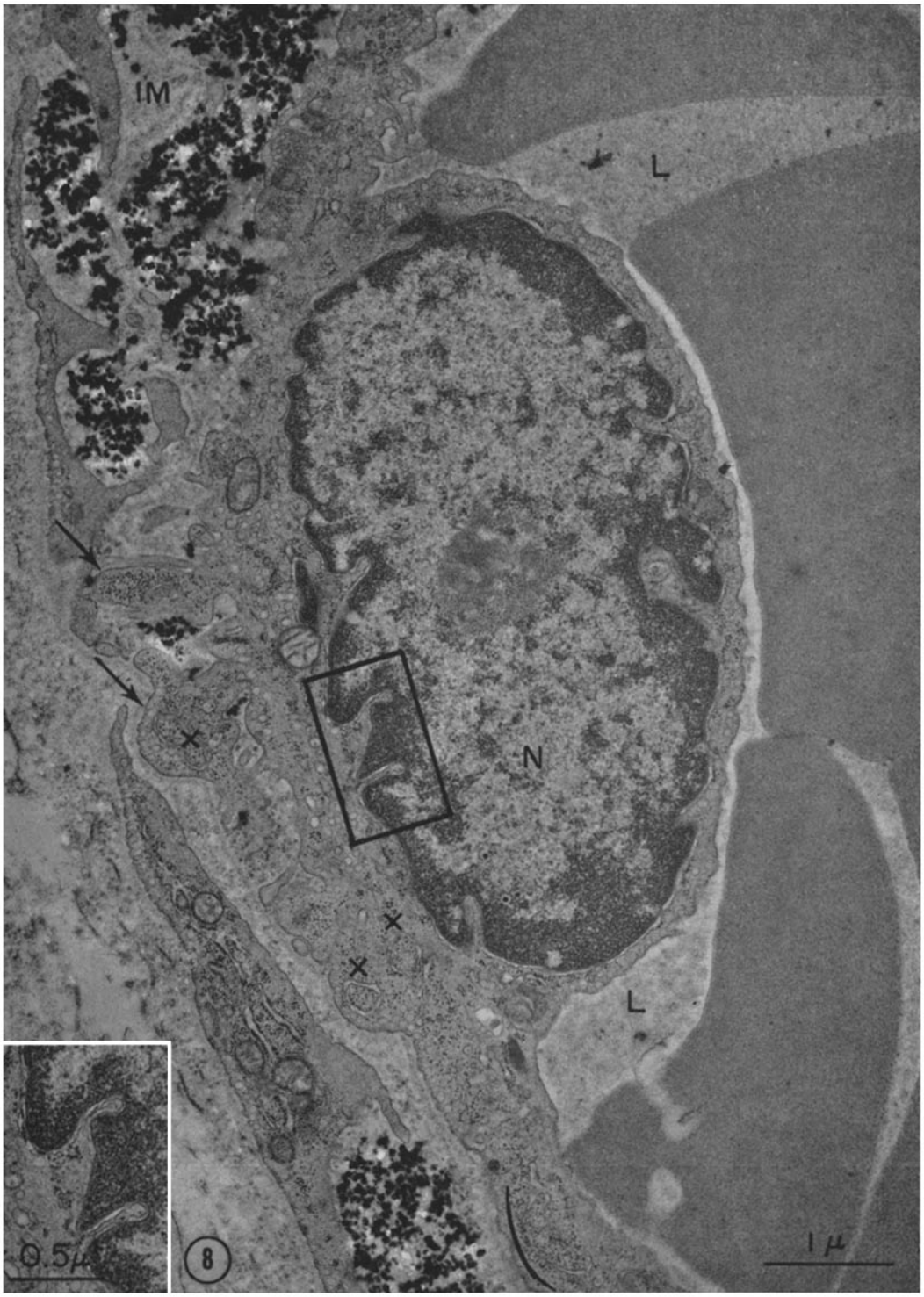


FIGURE 8 Venule, 6 min after histamine; fixation without congestion. Typical bulging of the endothelial nucleus (*N*) into the lumen. Note two tight nuclear pinches (in rectangular area and insert) and cytoplasmic protrusions from the base of the cell (arrows). *Round bracket* = a curved cisterna of ER, and x = similar structures sectioned in a different plane (but see also Fig. 16 for a different origin of circular profiles). *IM* = intramural masses of carbon. Fixation: Glutarald. 2,5%—cacodylate, post-fixation OsO_4-collidine.

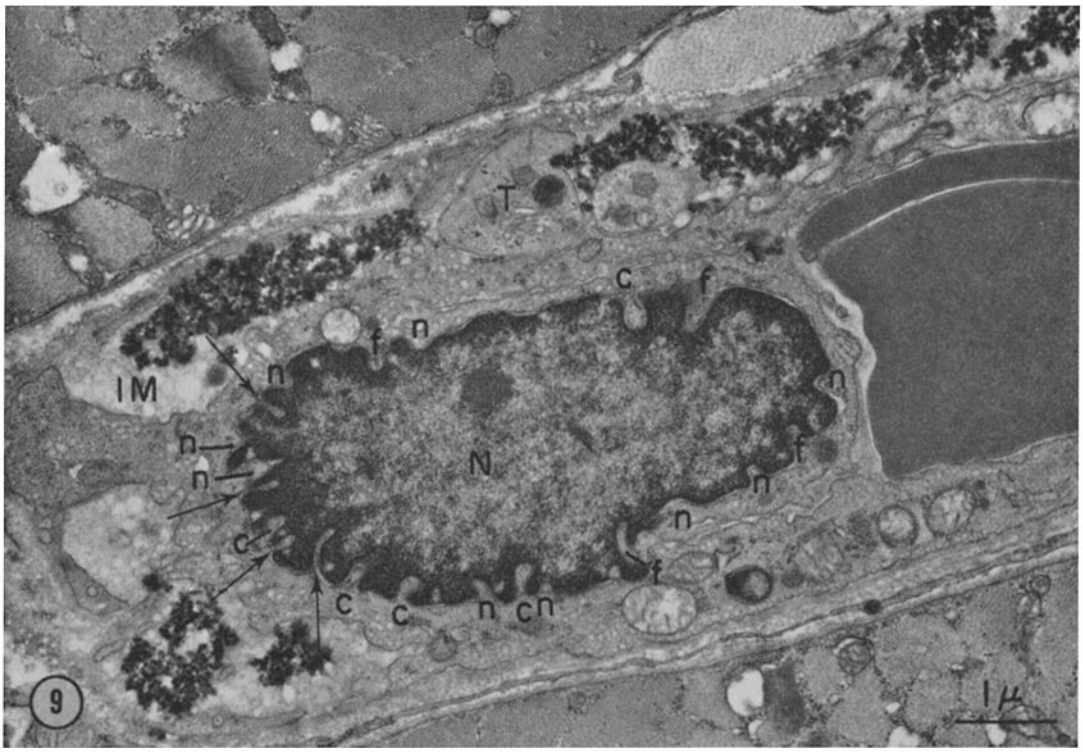


FIGURE 9 Venule or capillary 3 min after bradykinin; fixation without congestion. Note the scalloped outline of the nucleus: at least 4 of its indentations are tight enough to qualify as pinches (arrows). *IM* = intramural masses of carbon particles, chylomicra, and lipoproteins, evidence of leakage. *T* = intramural thrombocyte. Fixation: Karnovsky.

other organelles, as if the cytoplasm, flowing centripetally towards the nucleus, carried with it most easily the smallest particles (Figs. 15–17).

In several instances the subnuclear area also contained a faintly fibrillar material, forming an indistinct juxtanuclear band, either uniform or with the denser patches (Fig. 19) already described (Fig. 6); occasionally, this band seemed connected to a dense patch of cytoplasm against the basal plasmalemma (Fig. 19), a structure resembling the half desmosomes described by Stehbens (19) in the endothelia of blood vessels and lymphatics in the frog. Lastly, the subnuclear zone was often indented by folds of the plasma membrane (Figs. 17, 19) such as were never seen in controls.

DISCUSSION

Our principal finding is a change in shape of certain endothelial nuclei, after injection of a histamine-type mediator; from this (and other) evidence we argue that the corresponding endothelial cells have undergone active contraction. It is therefore necessary to prove, in the first place, that our observation is valid.

Correlation Between Shape of Nuclei, Shape of Endothelial Cells and Shape of Vessels

The shape of the nucleus is undoubtedly related to the mechanical stresses operating within the endothelial cell: witness the effect of venous congestion, which stretches the cells and correspondingly abolishes most of the nuclear indentations (Figs. 2, 3, 4 compared with Fig. 5). The change in shape is confirmed by statistical analysis (Table II). Similar deformations occur in other cell types: it has long been known that the nuclei of smooth muscle become folded and twisted as a result of contraction (21–23). Nuclear folds have been described in contractile cells that surround the seminiferous tubules in man (24) and they are obvious also in contracted skeletal muscle (Fig. 20). The

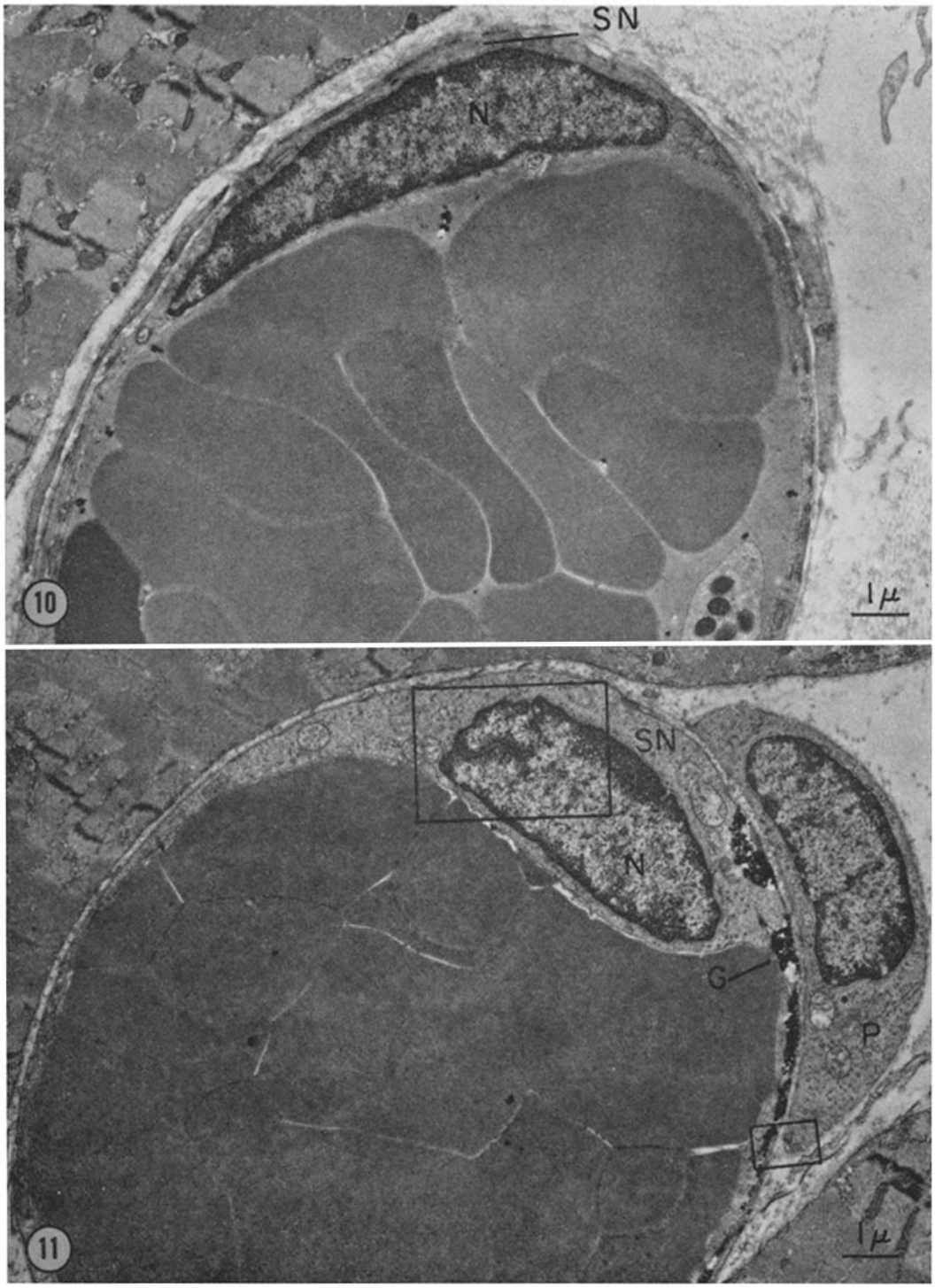


FIGURE 10, 11 Two venules of comparable size, fixed with venous congestion. (Fixation: Glutarald. 3%, postfixation OsO_4-collidine). Fig. 10: *Control.* Note long, flat endothelial nucleus, and thin layer of subnuclear cytoplasm (*SN*). Fig. 11: *6 min after histamine.* The nucleus is more rounded, bulges into the lumen, and shows several pinches. The subnuclear cytoplasm forms a thicker layer. *P* = pericyte. *G* = endothelial gap. Areas in rectangles enlarged in Fig. 12.

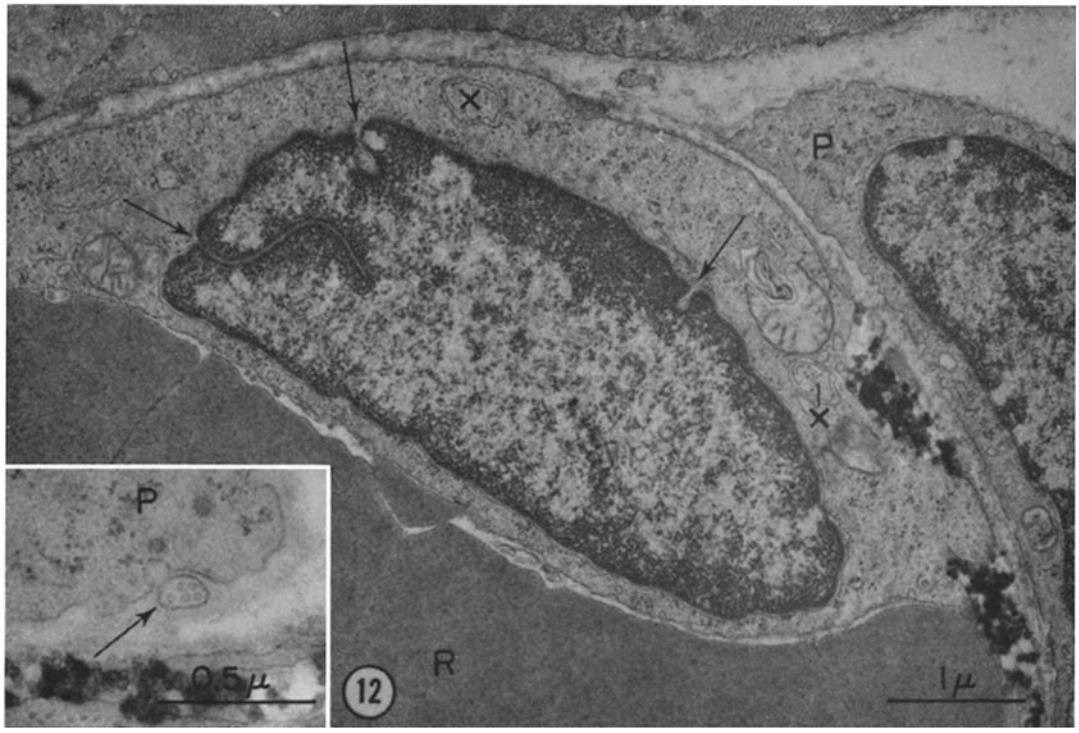


FIGURE 12 Details of Fig. 11. Arrows point to 3 very tight pinches in the nuclear membrane. X = circular profiles as explained in Figs. 7 and 16. *Insert:* other detail from Fig. 11: arrow points to a rounded profile lying between pericyte (P) and endothelium, and closely resembling a very fine axon; compare with Fig. 13. Fixation: Glutarald. 3%—cacodylate, postfixation OsO_4-collidine.

geometric reason for the appearance of folds is simple enough: for a given surface area, the shape that allows it to enclose the largest volume is the sphere. If an oblong nucleus is compressed into a near-spherical shape, this change entails an economy of surface membrane, and the excess membrane is thrown into folds.[2]

When the nuclei of normal control vessels, fixed in routine fashion, are examined in the light of the facts just discussed, one is forced to conclude that almost all endothelial cells are represented in a state of recoil (be it active or passive). This raises the question of a possible fixation artefact, i.e., vascular collapse or spasm induced by fixation.[3] Smooth muscle does contract when placed in fixative (25); this occurs typically with arteries (26, 27) and there are good reasons to believe that

[2] In theory, the nuclear deformations could also represent the effect of intrinsic nuclear contractility, but this is a very unlikely hypothesis. Suffice it to mention that morphologic evidence of overall cellular contraction is found outside the nucleus (see section entitled "Other Morphologic Indications . . .").

[3] The effect of fixation on living capillaries was observed by Dr. Eugene Landis at Harvard Medical School (unpublished experiments). The mesentery of the living, pithed frog was carefully exposed, a capillary field was brought under the objective of a microscope, and after a control period Palade's osmium tetroxide-Veronal fixative (with 2% OsO_4) was poured onto the tissue. The sequence was recorded by a movie camera, then analyzed frame by frame. There was no visible change in the caliber of the capillaries. (We are indebted to Dr. Landis for allowing us to mention these data). This observation suggests that, in the case of the frog mesentery, fixation is rapid enough to preserve the shape and caliber of the *capillaries*. However, the frog mesentery is extremely thin (of the order of 15 μ, or about 20 times less than the cremaster muscle). Fixation of thicker tissues, of course, cannot be as rapid: witness the prolonged twitching that occurs during the fixation of striated muscle.

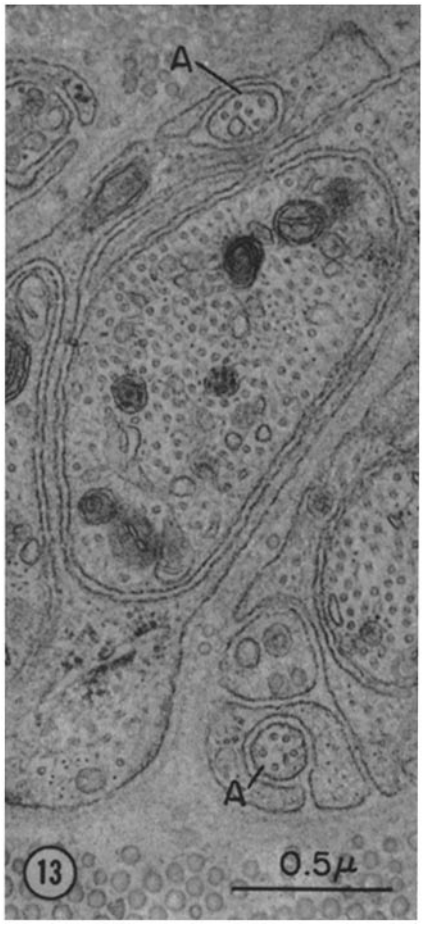


FIGURE 13 Part of a small nerve parallel to an arteriole; same tissue as in Fig. 12. The finest axons (*A*) are very similar to the profile shown in the insert of Fig. 12.

capillaries also tend to contract: unless venous congestion is applied, the lumen almost never appears round in cross section (Figs. 2, 4). It is very unlikely that this preponderance of closed, narrowed or angular lumina should represent the conditions prevailing in vivo. Capillaries observed in the living state give the definite impression that the lumen is cylindrical; when cerebral vessels are fixed by perfusion under physiological pressure, they appear "slightly distended" (28). Though we cannot offer definitive proof, we believe that the shape of our blood vessels fixed with mild venous congestion is more nearly representative of the living condition than the shape obtained by routine fixation. If this is true, and it seems almost inevitably so, then certain details of vascular ultrastructure and function may have to be revised: e.g., we noticed that the so-called endothelial flaps, considered a normal feature of capillary endothelium (29), all but disappeared in distended vessels (in 50 random electron micrographs on noncongested control vessels, at least 20 structures qualified as flaps; in 50 micrographs of congested controls, not a single flap was found).

A contraction of endothelial cells (and vessels) induced by fixation is bound to interfere with the detection of endothelial contraction possibly induced by chemical mediators: hence our decision to fix in two ways, i.e., with and without venous congestion. The series with congestion, admittedly a complicating factor, may be accepted as reliable, for the following reasons: (a) the degree of congestion was relatively mild; extravasation of red blood cells and vascular leakage were never observed; (b) congestion was also applied to the controls; (c) congestion would tend to erase, rather than to exaggerate, our results, by opposing endothelial contraction; (d) this would apply especially to the experimental series, in which some congestion occurs anyway (as an effect of the mediator); and (e) the results were equally significant in the noncongested group (Tables III, IV).

Passive Recoil Versus Active Contraction

We have concluded that the shape of the endothelial nucleus allows certain assumptions as to the state of the cell, whether stretched or "recoiled". The next step is to explore the possibility that the nuclear deformations may differ by degree, or by quality, so as to allow a further distinction between an elastic recoil and an active contraction induced by mediators. This was our main reason for subdividing the main types of nuclear identations into four groups which were thought to represent increasing degrees of nuclear shortening and compression: notches, folds, closing folds, and pinches (Fig. 1). It was our hope that through this classification we would find a pattern characteristic of the histamine-type effect. In this search we were partially rewarded, because the *pinches* are rare enough in the controls to be highly indicative whenever encountered (Table I). The other types of deformations, considered as a group, may be taken as an index of vascular collapse (Tables I, II), but not of the histamine-type effect. This relative specificity of the tightest folds (pinches) suggests that there are *two degrees of cellular "recoil"*:

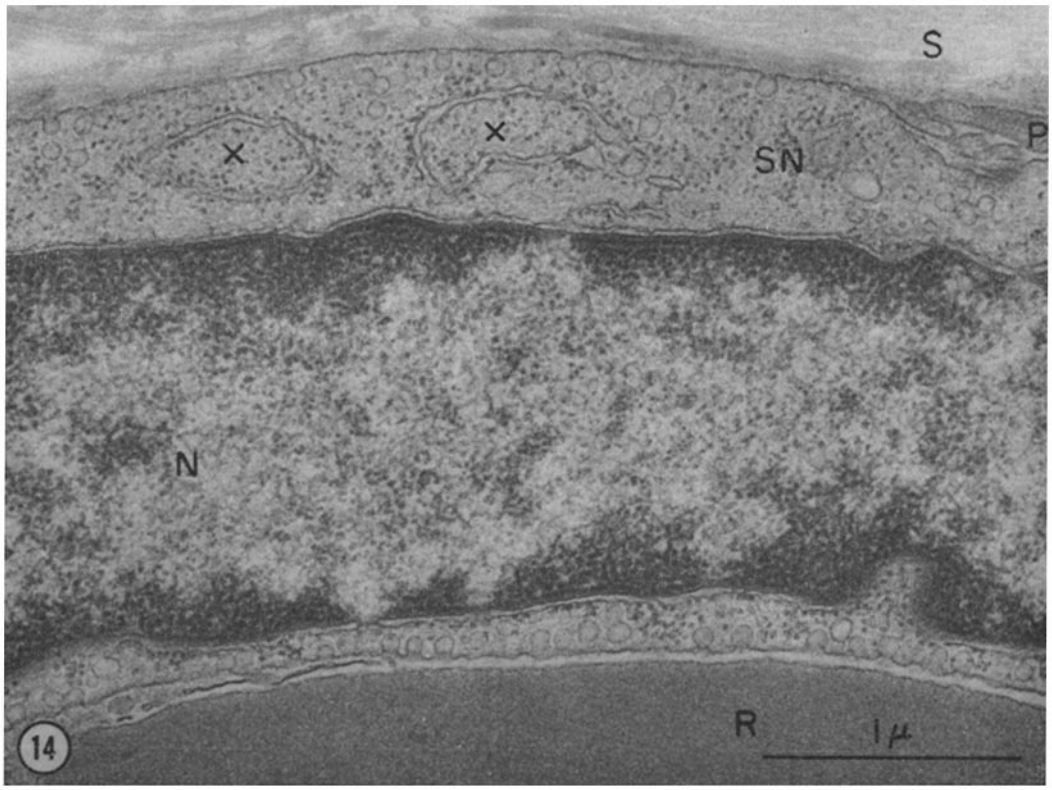


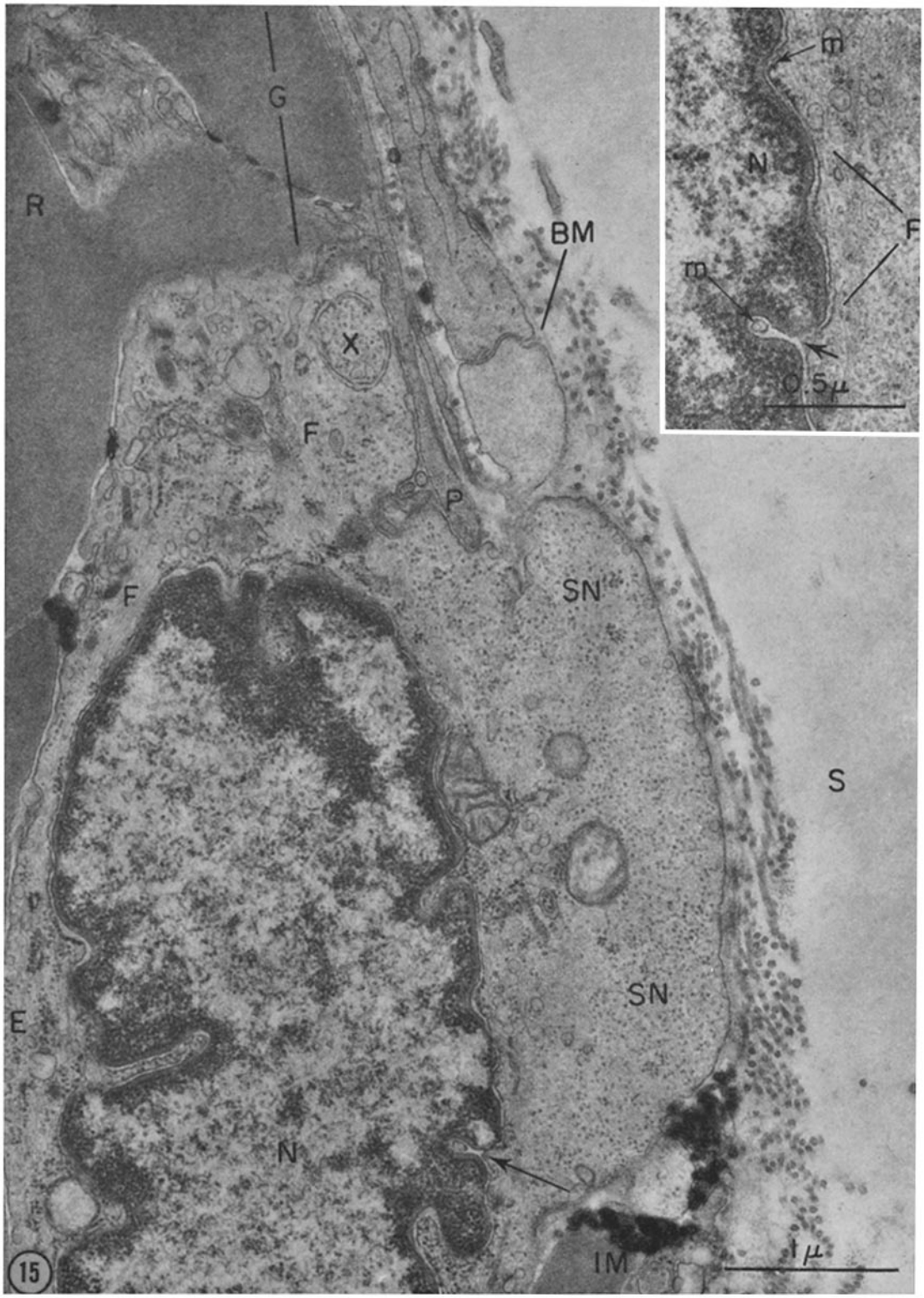
FIGURE 14 Endothelial cell of a venule, 6 min after histamine, fixed with congestion. Characteristic thickness of the subnuclear zone (*SN*) (compare with controls, Figs. 5 and 10), and presence of circular profiles of ER (*x*). Both these structural details suggest that cytoplasm has been displaced towards this part of the cell. *P* = typical electron-dense process of a pericyte. Fixation: Glutarald. 3%, postfixation OsO_4-collidine.

a milder degree, perhaps indicative of a normal cellular tone, and a more powerful degree reflecting active contraction.[4]

An alternative hypothesis could be that the nuclear changes *always* reflect passive recoil: if the

[4] The term "relative specificity" requires further comment. Some pinches occur also in controls; after routine fixation, without venous congestion, approximately one out of 10 nuclei shows a pinch (Tables I, III). The maximum number of pinches ever observed in a single control nucleus was 3. After histamine, or another mediator, the number of pinches in a single nucleus could be as high as 7 or 8. We may therefore speak of "specificity" in terms of pinches per nucleus.

FIGURE 15 Venule, 6 min after histamine; fixed with congestion. Endothelial cell (*E*) at the edge of a gap (*G*). A mass of cytoplasm appears to have accumulated in the subnuclear zone (*SN*); this cytoplasm contains mostly ribosomes, in contrast with that above the nucleus, which is rich in organelles as well as fibrils (*F*). *Arrow:* typical pinch in nucleus (*N*), as shown at higher power in the insert. *R* = red blood cell; *X* = circular profile (ER? pseudopod? see Figs. 7 and 16); *IM* = intramural material (red blood cell and carbon); *BM* = basement membrane. *Insert:* same venule, serial section, higher power. A band of faintly fibrillar material (*F*) lies below the nucleus (*N*). *Arrow* points to a pinch, which is of the tightest variety (Fig. 1): the part of the outer nuclear membrane (*m*) that is in the deepest part of the fold has been pinched off, and appears in cross section as a small round body (*m'*). Fixation: Glutarald. 3%, postfixation OsO_4-collidine.


G
R
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1 μ
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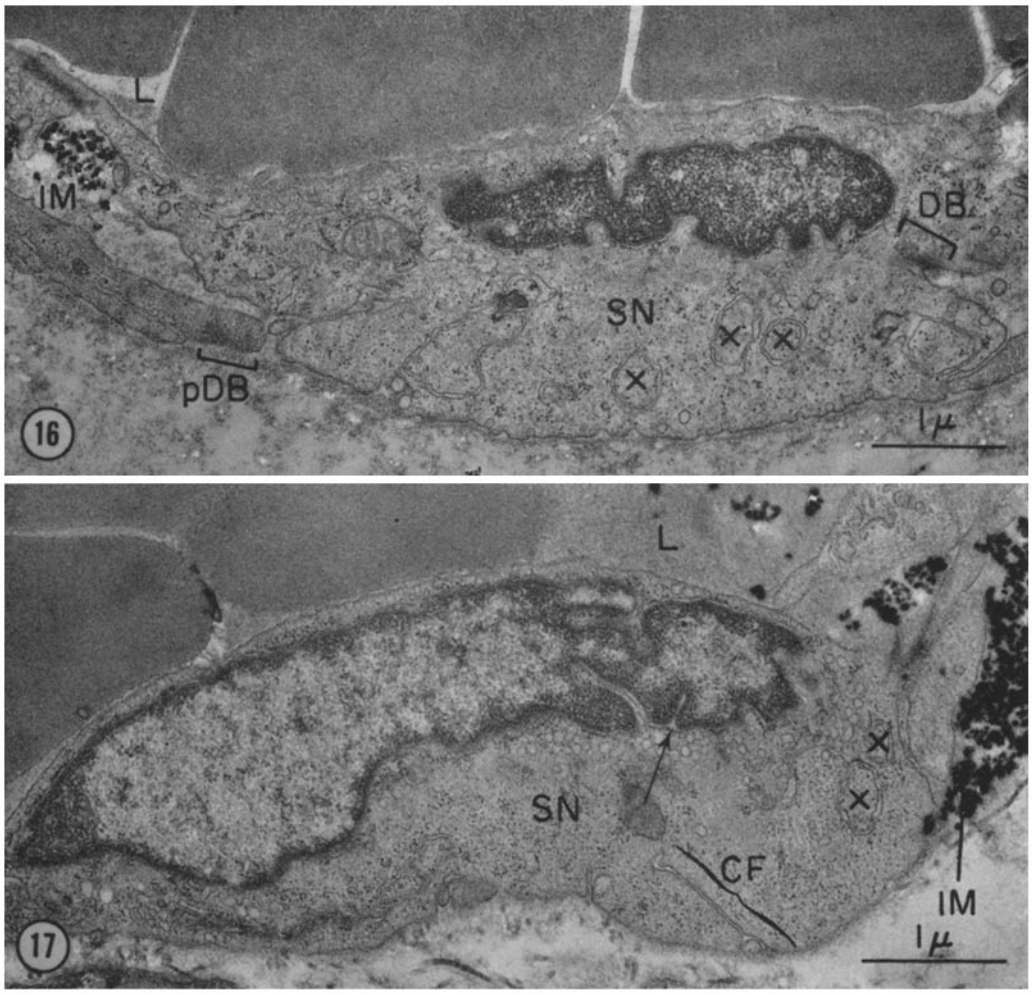

FIGURES 16 and 17 Typical changes in the subnuclear cytoplasm in the endothelial cells of venules, after serotonin or histamine. Both vessels are leaking (note *IM*, intramural deposits of carbon).

FIGURE 16 3 min after serotonin; fixation without congestion. Below the nucleus, the cytoplasm appears drawn in at the site of a "dense body" (*DB*). To the left of the latter, an ill-defined juxtanuclear band of cytoplasm poor in ribosomes and other organelles. Note circular profiles (*x*), that in this instance probably represent infoldings of the cell membrane such as occur at the left ("pseudopodia", see text). A "dense body" is also visible at the tip of a pericyte process (*pDB*). Fixation: Karnovsky.

FIGURE 17 6 min after histamine; fixation with congestion. Despite the congestion, the cell is bulging into the lumen; the nucleus shows several pinches, one of which is extremely tight (arrow). The subnuclear zone is particularly rich in ribosomes, that again tend to spare a juxtanuclear band. *CF* = a deep cytoplasmic fold; *X* = circular profiles, representing either ER or plasma membrane as in Fig. 16. Fixation: Glutarald. 3%, postfixation OsO_4-collidine.

primary effect of the mediators were that of loosening the intercellular junctions, the endothelial cells might be allowed a high degree of elastic recoil. That the mediators may also loosen the intercellular junctions is certainly possible, although no direct evidence is at hand. The only data known to us that might conceivably hint to an effect on junctions are those obtained by Berndt and Gos-

TABLE III

Effect of Mediators Alone (Without Venous Congestion) on the Numbers of Endothelial Nuclei with "Pinches"

	Nuclei with pinches	Nuclei without pinches
(a) Controls (100 nuclei)	11	89
(b) Histamine (92 nuclei)	65	27
(c) All mediators combined (197 nuclei)*	104	93

* The effect of histamine, bradykinin, and serotonin being qualitatively the same, the results may be combined.

χ^2 for (a) (b) = 71.32; degrees of freedom = 1; $P < 0.001$

χ^2 for (a) (c) = 48.82; degrees of freedom = 1; $P < 0.001$

TABLE IV

Effect of a Mediator Plus Venous Congestion on the Numbers of Endothelial Nuclei with "Pinches"

	Nuclei with pinches	Nuclei without pinches
(a) Controls + venous congestion (118 nuclei)	4	114
(b) Histamine + venous congestion (149 nuclei)	46	103
(c) Bradykinin + venous congestion (35 nuclei)	11	24

χ^2 for (a) (b) = 32.69; degrees of freedom = 1; $P < 0.001$

χ^2 for (a) (c) = 20.94 (Yates' correction for continuity applied; degrees of freedom = 1; $P < 0.001$)

selin (30), who showed that histamine and serotonin increase the permeability of the *mesenteric membrane* to ^{86}Rb (though not to ^{32}P phosphate). We have sought a loosening effect in vitro by incubating cremaster muscles in oxygenated Krebs-Ringer medium with glucose, in the presence of histamine; under these conditions the lumen of the vessels collapsed, but not a single gap was found. This experiment, per se, cannot disprove an effect on the interendothelial junctions, but even if these were loosened a significant passive recoil of the cells is out of the question: the intercellular gaps are very small (rarely reaching 1 μ in diameter) and the dimensions of the endothelial

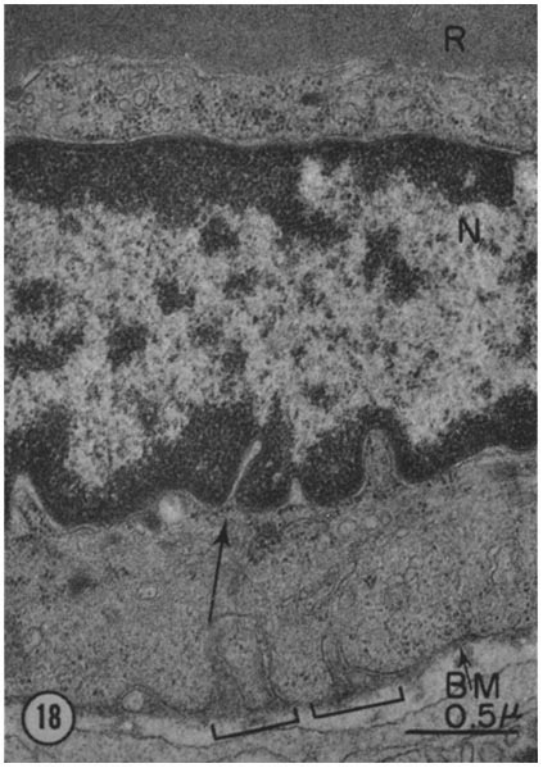

FIGURE 18 Wall of a venule, 6 min after histamine, fixed with congestion. *R* = red blood cell in lumen. Comparison with controls (Figs. 5, 10) shows that the subnuclear zone is wider. The base of the cell is thrown into folds (bracketed area). *Arrow:* pinch in the nuclear membrane. *BM* = basement membrane. Fixation: Glutarald. 3%, postfixation OsO_4-collidine.

cell are of the order of 10 × 30 μ (31). *A passive recoil, if it existed, would be so small that it could not possibly account for the drastic nuclear deformations* that we have seen.

In summary, the most plausible interpretation of the nuclear pinches is that the endothelial cells have contracted.

Relationship Between Nuclear Pinching and Vascular Leakage

While it is readily apparent that a folded nucleus should represent a shortened cell, it remains to be proven that a shortening of the cell may produce vascular leakage. The best we can do, on the basis of electron micrographs, is to infer guilt by association, as follows.

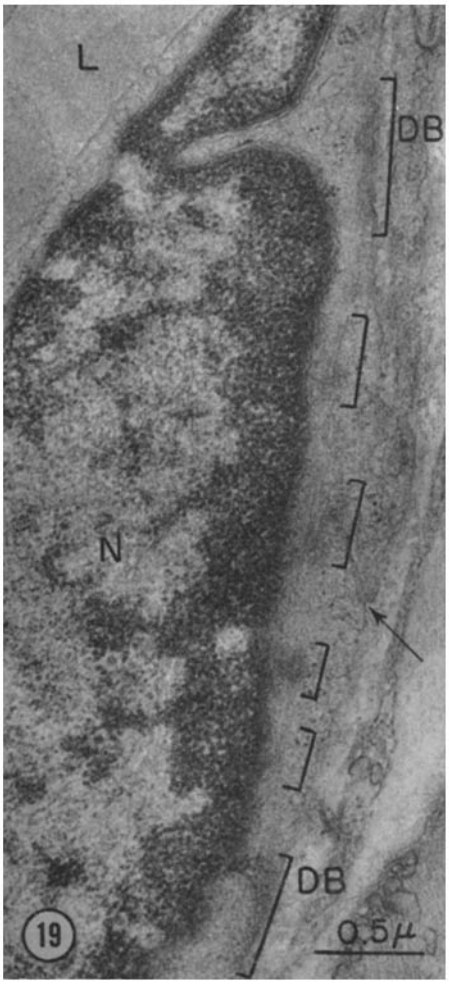

FIGURE 19 "Attachment bodies" and related "dense bodies" in the subnuclear zone; endothelial cell of a small vessel (capillary or venule) 6 min after histamine, fixed with congestion. *L* = lumen. Two "dense bodies" (*DB*) lie against the cell membrane; others (brackets) lie between them in the cytoplasm, at irregular intervals. *Arrow* points to a fold in the plasma membrane at the base of the cell. Fixation: Glutarald. 3%, post-fixation OsO_4-collidine.

First, there is a *significant association between nuclear pinching and vascular leakage* (Table V). To demonstrate it we chose one experimental series in which there would be vascular leakage (histamine or bradykinin, plus congestion) and subdivided all the nuclei into two series: with and without pinches. Then the corresponding vessels were examined for evidence of leakage, i.e., intramural deposits of carbon. Table V shows that

TABLE V

Association Between Nuclear Pinching and Vascular Leakage

The nuclei of one entire series (histamine or bradykinin, with congestion) were subdivided into two groups: with and without pinches. Thereafter the corresponding vessels were examined for evidence of leakage, i.e., intramural deposits of carbon black. The table shows that vessels could leak whether the nucleus (in that particular section) was pinched or not; but leaking vessels contained more pinched nuclei.

	With signs of vascular leakage*	No signs of vascular leakage
Nuclei without pinches (128 nuclei)	35	93
Nuclei with pinches (56 nuclei)	38	18

* determined by the presence of intramural carbon
$\chi^2 = 26.004$ Degrees of freedom = 1 $P < 0.001$

TABLE VI

Association Between Nuclear Pinching and Detachment of the Same Endothelial Cell

The nuclei of one entire series (histamine or bradykinin, with congestion) were subdivided into two groups: with and without pinches. Thereafter the intercellular junctions of the corresponding endothelial cells were examined (*presence or absence of a gap*). This table shows that an intercellular gap could exist, whether the nucleus (in that particular section) was pinched or not; but if the nucleus was pinched, the chance of its cell's being detached was greater.

	Adjacent to gaps	Not adjacent to gaps
Nuclei without pinches (128 nuclei)	15	113
Nuclei with pinches (56 nuclei)	20	36

$\chi^2 = 14.56$ Degrees of freedom = 1 $P < 0.001$

vessels could leak whether the nucleus (in that particular section) was pinched or not; but *leaking vessels contained more pinched nuclei.*

Second, it is possible to sharpen the association even further, and to show that *pinched nuclei tend to occur near endothelial gaps.* This could be shown in the same material as above. Again the nuclei were

subdivided into two series, with and without pinches; then the corresponding endothelial cells were examined for the presence or absence of a gap at the intercellular junction. Table VI shows that an intercellular gap may exist, whether the adjacent nucleus (in that particular section) is pinched or not; but *if a nucleus is pinched, it is likelier to occur in a "detached" cell.*

We may conclude that the phenomenon of nuclear pinching must be *closely related* to the formation of endothelial gaps and to the ensuing vascular leakage.

Other Morphologic Indications of Endothelial Contraction

In addition to the nuclear pinches, other evidence points to a shortening of the endothelial cells: (a) *The nucleus tends to bulge* (i.e. to form a convex surface) towards the lumen of the vessel, even if venous congestion is applied (Figs. 11, 17). (b) *The cytoplasm tends to accumulate in the center of the cell,* below the nucleus (e.g., Figs. 11, 14, 15, 18 compared with Figs. 5, 10). There is evidence that the cytoplasm "flows" into this zone, e.g. the preferential accumulation of ribosomes, the smallest organelles (Figs. 15–17), and the deformation of cisternae of ER, giving rise to unusual circular profiles as in Figs. 7, 8, 12, 14 (only three of these were seen in controls). (c) *The subnuclear cytoplasm is sometimes thrown into folds,* recalling the nuclear pinches (Figs. 16–18). Cellular protrusions also arise from this area (e.g., Figs. 6–8, 15, 19). Neither folds nor protrusions were ever seen in a control; on the other hand, both are characteristic of *contracted smooth muscle cells* (22, 23) which acquire such a jagged outline, even by light microscopy, that they were given the name of "spiny cells" (Stachelzellen (22, 23)).

Problems Raised by the Endothelial Contraction Hypothesis

While the facts discussed above, taken as a whole, weigh heavily in favor of endothelial contraction, several questions remain to be answered: (1) *Why is it that not all endothelial nuclei show signs of contraction?* Of course it is conceivable that a nucleus, though pinched, could be cut at such an angle as not to show any indentation; however, the number of pinches per nucleus (as seen in cross section) does not follow a random Poisson distribu-

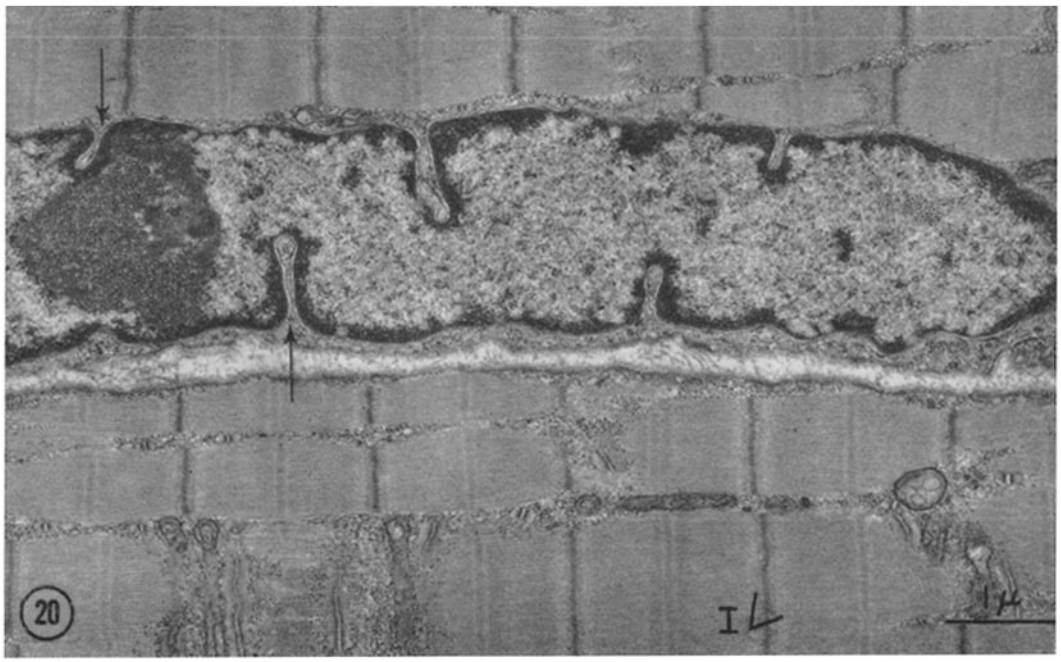

FIGURE 20 Striated muscle fiber, fixed in a state of contraction, as shown by the narrowness of the I bands (*I*). The nucleus shows indentations and pinches (arrows) quite similar to those of the endothelium as illustrated in the preceding figures. Fixation: Glutarald. 2.5%-cacodylate, postfixation OsO_4-collidine.

TABLE VII

Distribution of "Pinches" Among Endothelial Nuclei After Histamine Alone

In a series of 92 nuclei (after histamine) the total number of pinches was 225. Mean number of pinches per nucleus = 2.44. If the numbers of pinches per nucleus were distributed in random fashion, the expected numbers of nuclei with 0, 1, 2, . . . pinches could be calculated from the equation $P[\{\psi\}] = e^{-\lambda}\frac{\lambda^x}{x!}, x = 0, 1, 2, \ldots$, in which λ is taken as approximately equal to 2.4.* The table shows that the numbers of nuclei with 0, 1, 2, . . . pinches *observed* are significantly different from the numbers *expected*, which suggests the existence of more than one population of nuclei.

Number of pinches (x) per nucleus	0	1	2	3	4	5	6	7	8	9 or more
Number of nuclei observed	27	16	13	9	6	7	5	5	4	0
Number of nuclei expected	8.34	20.03	24.04	19.23	11.54	5.54	2.22	0.76	0.23	0.07

* To facilitate the use of a table of Poisson probabilities given in "Modern Probability Theory and Its Applications," E. Parzen, John Wiley & Sons Inc., New York. 1964.

$\chi^2 = 145.898$ Degrees of freedom = 9 $P < 0.001$

tion (Table VII). There are *too many nuclei without pinches*, and there are *too many nuclei with more than four pinches*, which suggests that *there may be more than one population of endothelial cells*. A spotty distribution of contractile cells would actually help to understand the focal, rather than diffuse, distribution of the vascular leaks (3).

(2) *If the venular endothelium contracts, why is there no constriction of the lumen?* Actually, where a cell does contract and *bulge*, a localized restriction of the lumen necessarily occurs (though not accompanied by a reduction of the outer caliber). As to the outer caliber, if cellular contraction occurred along the axis of the vessel, there would be no constriction. There would be either no change, or even a dilatation. This has been specifically observed in vivo: Sanders, Ebert, and Florey (32) described a longitudinal "swelling of the endothelial nucleus" along the axis of a capillary, with restriction of the lumen but no change in the outer caliber. Since the endothelial cell is elongated along the axis of the vessel, we may speculate that its contractile system is predominantly oriented in the same direction.

(3) *Why is the leakage localized preferentially in the venules?* This question, once again, must remain unanswered. Nuclear pinches and some leakage occurred also in capillaries; however, the proportion of "pinches without leak" was higher in the capillaries. The only conclusion offered by our data is that *endothelial contraction in the capillaries does not have the same disrupting effect as in the venules*; the reason may lie in a different degree, or different manner, of contraction; or in a physical mechanism, related to the smaller diameter of the vessel (an example of such mechanism is offered by the Law of Laplace (7): for equal internal pressures, the tangential force tending to disrupt the vascular wall is greater in the larger vessels); or perhaps, more simply, the intercellular junctions of the venules are more easily torn apart than those of the capillaries. Ultrastructural differences have not yet been described, but it is well established that venules are physiologically more permeable than the capillaries (see 29), which may mean that their interendothelial junctions are looser.

(4) *What is the contractile mechanism?* All cells, to some extent, have the capacity to change shape and thus to contract; however, endothelium may be endowed with more than this baseline capacity. Using fluorescein-labeled antibodies, Becker and Murphy found that endothelial cells contain actomyosin similar to that of uterine smooth muscle (33). A structural equivalent of actomyosin in the endothelium is not yet known; it might be represented by the fibrils (34) (Figs. 6, 19) or by the microtubules that are often observed (35). Periodically banded fibrillar structures have also been described recently (36, 37) but we found only 2 in about 1000 electron micrographs. In our material, an ill-defined zone of fibrillar structure was often visible beneath the nucleus; in

some instances this zone also showed "dense bodies" (Fig. 19) similar to those of smooth muscle, which have been considered analogous to the Z band of sarcomeres (25). Smooth muscle and endothelium are in effect closely related cell types, by histogenesis as well as by morphology (29). It should also be kept in mind that a contractile mechanism may be present with little or no morphologic equivalent recognizable by present methods: e.g., platelets contain thrombosthenin, a contractile system of the actomyosin type; this amounts to 15–20 per cent of the platelet protein (38) yet none of the platelet structures can be described as possibly contractile, with the exception of the few microtubules.

THE PERICYTES: We searched for evidence of pericyte contraction, but found none. Nuclear pinches were exceptional, in the experimental as well as in the control series. Yet it was obvious that the body of the pericytes resembled smooth muscle more closely than did endothelium; fibrillar masses devoid of vesicles or ribosomes were more frequent, and so were denser masses that could be interpreted as attachment bodies (Fig. 16). Often the pericyte processes were darker, i.e. more electron-opaque, than the endothelium (Fig. 14); one is reminded that, in smooth muscle, contracted cells (the Stachelzellen) are consistently darker than the relaxed cells (22, 23). This apparent discrepancy may mean only that the pericytes do not respond to the agents tested. However, one should also bear in mind that the peculiar shape of these cells might not allow them to deform their nucleus even if they did indeed contract. Pericytes are much longer than endothelial cells (39); their slender processes, structurally similar to smooth muscle, are embedded in the basement membrane (29). Contraction, if it occurs, probably takes place in these processes, most of which are relatively remote from the nucleus. The ultimate effect would be a local constriction of the vessel (or an increased pressure within the same) rather than an overall shortening of the pericyte body, and of its nucleus.

An unexpected finding, which requires confirmation, was the close relationship between pericytes and profiles similar to very fine axons (Figs. 12, 13). Though they cannot be common (only three were found, incidental to this study), they offer new hope for a physiology of the pericytes, a chapter which is almost completely blank (29).

CORRELATION WITH LIGHT MICROSCOPIC FINDINGS: The debate on endothelial contractility is about a century old (40). In Krogh's monograph, *capillary* contractility was considered an established fact (40). The contractile elements were thought to be the "Rouget cells" rather than the endothelium.[5] Later investigations, particularly those of the Clarks (41, 42) and of Sandison (43), brought about the notion that contractility depends on the endothelium, but occurs in amphibian capillaries only; no significant contraction was seen in capillaries of the rabbit ear chamber. The Clarks conclude that "there may have been an evolutionary loss of contractile power of vascular endothelium from invertebrate to mammal associated with the development of a highly differentiated muscular layer in the arterioles (42)." Zweifach confirmed the contractility of the endothelium in a micromanipulative study of frog capillaries (44): "The endothelial cells could be readily stimulated by prodding their nuclear thickenings whereupon the cell would contract. Each endothelial cell was capable of contracting independently of its neighboring cells. The stimulated cell drew its ends closer to the nuclear thickening, which bulged into the lumen." The same author also mentions that histamine causes endothelial swelling (45, 46), but the experimental details are not given.

Sanders, Ebert, and Florey took issue with the statement that mammalian capillary endothelium does not contract (32). Using the rabbit ear chamber, they noticed spontaneous, active changes in the caliber of the capillaries due to a "swelling of the nucleus" and this effect could be brought about at will by stimulation of the sympathetic chain.

These observations on "nuclear swelling" in vivo presumably refer to the same phenomenon that we described on electron micrographs as "bulging of nuclei" in contracted cells. Pinches, of course, could not be seen in vivo: they are altogether beyond the range of light microscopy. Further studies on the mammalian microcirculation in vivo are needed, to establish which stimuli are able to induce endothelial contraction and "bulging," in which types of vessels, and in how many endothelial cells.

CONCLUSION: At this time, we visualize the

[5] The Rouget cells are probably to be identified with our pericytes; however, Krogh was not inclined to make this identification, feeling that Zimmermann's morphologic study of pericytes, now recognized as a classic (29), "did not inspire complete confidence" (p. 82).

effect of histamine-type mediators as follows: the arterioles dilate (47, 48) (the mechanism of this dilatation is still speculative), thereby raising the pressure in capillaries and venules; some endothelial cells in the venules, and some in the capillaries, contract and tug at each other; in the venules they actually pull apart: this effect is facilitated by the increased intraluminal pressure. Leaks develop, while the bulging nuclei may interfere with normal flow. A direct effect of the mediators on the intercellular junctions is not necessary, but it cannot be excluded as an accessory mechanism.

As far as we know, this pathogenesis of histamine-type vascular leakage fits all the known facts; it reconciles the two different and seemingly unrelated actions of these drugs, (a) stimulation of smooth muscle and (b) increase in vascular permeability: in either case the mechanism would be a cellular contraction. It also explains why substances that induce or prevent the contraction of muscular veins also induce or prevent vascular leakage (49, 50).

It should be emphasized that the present study deals with only *three mediators*. The group of substances that increase vascular permeability has greatly expanded in recent years (51–56) and other cellular mechanisms may be at play in addition to that here proposed.

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REFERENCES

1. COTRAN, R. S., and G. MAJNO. 1964. A light and electron microscopic analysis of vascular injury. *Ann. N. Y. Acad. Sci.* **116**:750.
2. MAJNO, G., and G. E. PALADE. 1961. Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. Biochem. Cytol.* **11**:571.
3. MAJNO, G., G. E. PALADE, and G. I. SCHOEFL. 1961. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. *J. Biophys. Biochem. Cytol.* **11**:607.
4. ARNOLD, J. 1875. Über das Verhalten der Wandungen der Blutgefässe bei der Emigration weisser Blutkörper. *Virchows Arch. Pathol. Anat.* **62**:487.
5. MILES, A. A. 1958–59. Mediators of the vascular phenomena of inflammation. *In* Lectures on the Scient. Basis of Medicine. The Athlone Press of the University of London, England. **8**:198.
6. CHAMBERS, R., and B. W. ZWEIFACH. 1947. Intercellular cement and capillary permeability. *Physiol. Rev.* **27**:436.
7. ROWLEY, D. A. 1964. Venous constriction as the cause of increased permeablity produced by 5-hydroxytryptamine, histamine, bradykinin and 48/80 in the rat. *Brit. J. Exp. Pathol.* **45**:56.
8. SPECTOR, W. G. 1958. Substances which affect capillary permeability. *Pharmacol. Rev.* **10**:475.
9. GRANT, R. T. 1964. Direct observation of skeletal muscle blood vessels (rat cremaster). *J. Physiol. (London).* **172**:123.
10. TAICHMAN, N. S., and P. GOLDHABER. 1964. Microcirculatory stasis and the production of tissue necrosis in the hamster cheek pouch induced by histamine or a histamine liberator. *Angiology.* **15**:515.
11. EBERT, R. H., and R. C. GRAHAM. 1966. Observations on the effect of histamine and serotonin in the rabbit ear chamber. *Angiology.* **17**:402.
12. MAJNO, G., V. GILMORE, and M. LEVENTHAL. 1967. On the mechanism of vascular leakage caused by histamine-type mediators. A microscopic study in vivo. *Cir. Res.* **21**:833.
13. BUCKLEY, I. K., and G. B. RYAN. 1969. Increased vascular permeability: the effect of histamine and serotonin on rat mesenteric blood vessels in vivo. *Amer. J. Pathol.* In press.
14. MAJNO, G., and M. LEVENTHAL. 1967. Pathogenesis of "histamine-type" vascular leakage. *Lancet.* **2**:99.
15. PALADE, G. E. 1952. A study of fixation for electron microscopy. *J. Exp. Med.* **95**:285.
16. TRUMP, B. F., and R. E. BULGER. 1966. New ultrastructural characteristics of cells fixed in a glutaraldehyde-osmium tetroxide mixture. *Lab. Invest.* **15**:368.
17. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**:49A.
18. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.

19. STEHBENS, W. E. 1966. The basal attachment of endothelial cells. *J. Ultrastruct. Res.* **15**:389.
20. FISHER, R. A. 1950. Statistical methods for research workers. Oliver & Boyd Ltd., London. 11th edition.
21. WALLS, E. W. 1960. The microanatomy of muscle. *In* The Structure and Function of Muscle. G. H. Bourne, editor. Academic Press Inc., New York. 1:21.
22. GANSLER, H. 1960. Phasenkontrast und elektronenmikroskopische Untersuchungen zur Morphologie und Funktion der glatten Muskulatur. *Z. Zellforsch. Mikroskop. Anat.* **52**: 60.
23. GANSLER, H. 1961. Struktur und Funktion der glatten Muskulatur. II. Licht- und elektronenmikroskopische Befunde an Hohlorganen von Ratte, Meerschweinchen und Mensch. *Z. Zellforsch. Mikroskop. Anat.* **55**:724.
24. ROSS, M. H., and I. R. LONG. 1966. Contractile cells in human seminiferous tubules. *Science.* **153**:1271.
25. PANNER, B. J., and C. R. HONIG. 1967. Filament ultrastructure and organization in vertebrate smooth muscle. *J. Cell Biol.* **35**:303.
26. PEASE, D. C., and S. MOLINARI. 1960. Electron microscopy of muscular arteries; pial vessels of the cat and monkey. *J. Ultrastruct. Res.* **3**:447.
27. MATTHEWS, M. A., and D. L. GARDNER. 1966. The fine structure of the mesenteric arteries of the rat. *Angiology.* **17**:902.
28. PALAY, S. L., S. M. MCGEE-RUSSELL, S. GORDON, JR., and M. A. GRILLO. 1962. Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium tetroxide. *J. Cell Biol.* **12**:385.
29. MAJNO, G. 1965. Ultrastructure of the vascular membrane. *In* Handbook of Physiology. Circulation. W. F. Hamilton and Philip Dow, editors. American Physiological Society, Washington, D.C. Section 2, **3**:2293.
30. BERNDT, W. O., and R. E. GOSSELIN. 1962. Differential changes in permeability of mesentery to rubidium and phosphate. *Amer. J. Physiol.* **202**:761.
31. BRUNS, R. R., and G. E. PALADE. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. *J. Cell Biol.* **37**:244.
32. SANDERS, A. G., R. H. EBERT, and H. W. FLOREY. 1940. The mechanism of capillary contraction. *Quart. J. Exp. Physiol.* **30**:281.
33. BECKER, C. G., and G. E. MURPHY. 1969. Demonstration of contractile protein in endothelium and cells of the heart valves, endocardium, intima, arteriosclerotic plaques, and Aschoff bodies of rheumatic heart disease. *Amer. J. Pathol.* **55**:1.
34. CECIO, A. 1967. Ultrastrucutral features of cytofilaments in mammalian endothelial cells. *Z. Zellforsch. Mikroskop. Anat.* **83**:40.
35. RHODIN, J. A. G. 1967. The ultrastructure of mammalian arterioles and precapillary sphincters. *J. Ultrastruct Res.* **18**:181.
36. COTRAN, R. S. 1967. The fine structure of the microvasculature in relation to normal and altered permeability. *In* Physical Bases of Circulatory Transport. E. B. Reeve and A. C. Guyton, editors. W. B. Saunders Company, Philadelphia. 249.
37. RÖHLICH, P., and I. OLÀH. 1967. Cross-striated fibrils in the endothelium of the rat myometral arterioles. *J. Ultrastruct. Res.* **18**:667.
38. BETTEX-GALLAND, M., and E. F. LUSCHER. 1965. Thrombosthenin, the contractile protein from blood platelets and its relation to other contractile proteins. *Advan. Protein Chem.* **20**:1.
39. ZIMMERMANN, K. W. 1923. Der feinere Bau der Blutcapillaren. *Z. Anat. Entwicklungsgesch.* **69**: 29.
40. KROGH, A. 1959. The Anatomy and Physiology of Capillaries. Hafner, New York.
41. CLARK, E. R., and E. L. CLARK. 1931. Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. *Amer. J. Anat.* **49**:441.
42. CLARK, E. R., and E. L. CLARK. 1935. Observations on changes in blood vascular endothelium in the living animal. *Amer. J. Anat.* **57**:385.
43. SANDISON, J. C. 1932. Contraction of blood vessels and observations on the circulation in the transparent chamber in the rabbit's ear. *Amer. J. Anat.* **54**:105.
44. ZWEIFACH, B. W. 1934. A micro-manipulative study of blood capillaries. *Anat. Rec.* **59**:83.
45. ZWEIFACH, B. W. 1957. General principles governing the behavior of the microcirculation. *Amer. J. Med.* **23**:684.
46. ZWEIFACH, B. W. 1961. Biologic properties of vascular endothelium. *Angiology.* **12**:507.
47. PARROT, J. -L., and J. THOUVENOT. 1966. Action de l'histamine sur les muscles lisses. *In* Histamine and Anti-histaminics. M. Rochae Silva, editor. Part 1. Handbook of experimental pharmacology. O. Eichler and A. Farah, editors. Springer Verlag, Berlin, Heidelberg, and New York. **18**:202.
48. MARSHALL, J. M. 1967. Comparative aspects of the pharmacology of smooth muscle. *Fed. Proc.* **26**:1104.
49. NORTHOVER, B. J. 1967. The effect of anti-inflammatory drugs on the deposition of colloidal carbon in the walls of venules. *J. Pathol. Bacteriol.* **94**:204.
50. NORTHOVER, B. J. 1967. The antagonism between

anti-inflammatory drugs and substances that constrict veins. *J. Pathol. Bacteriol.* **94**:206.

51. WILHELM, D. L. 1962. The mediation of increased vascular permeability in inflammation. *Pharmacol. Rev.* **14**:251.
52. WILLOUGHBY, D. A., B. BOUGHTON, and H. O. SCHILD. 1963. A factor capable of increasing vascular permeability present in lymph node cells: a possible mediator of the delayed reaction. *Immunology.* **6**:484.
53. FRIMMER, M., and B. ZEIDLER. 1965. Isolierung eines zweiten Polypeptids mit leukotaktischer und permeationsfördernder Wirkung aus Kalbsthymus. *Arch. Pharmakol. Exp. Pathol.* **251**:315.
54. YOSHINAGA, M., I. TASAKI, and H. HAYASHI. 1966. Purification of permeability factors mediating increased vascular response in cutaneous Arthus-type inflammation. *Biochim. Biophys. Acta.* **127**:172.
55. MELMON, K. L., and M. J. CLINE. 1967. Kinins. *Amer. J. Med.* **43**:153.
56. DA SILVA, W., J. W. EISELE, and I. H. LEPOW. 1967. Complement as a mediator of inflammation. III. Purification of the activity with anaphylatoxin properties generated by interaction of the first four components of compleiment and its identification as a cleavage product of C'3. *J. Exp. Med.* **126**:1027.