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The Microscope in the History of Pathology

With a Note on the Pathology of Fat Cells

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Summary. Between the birth of the microscope and the birth of microscopic pathology there is a puzzling gap of almost 250 years. Six reasons have been given to explain it: secrecy of the art, high cost of the apparatus, technical difficulty, the notion that the microscope was a toy, lack of new ideas, neglect by Universities. Suspecting that poor optics must have been another major factor in discouraging microscopy, we placed ourselves in the conditions of an experimenter trying to understand acute inflammation in a transparent tissue shortly before 1830 (i.e. before the invention of Lister's achromatic objective and of the condenser). We used a Culpeper-type microscope, and a piece of inflamed omentum, fresh, unfixed and unstained. The resolution proved inadequate to recognize the tissue changes of acute inflammation; and the interpretation of these changes was further complicated by the optical artefact known as "reticular image".

On the other hand, using the same system, we made an observation that is scarcely possible in paraffin-embedded tissues: despite the poor optics of an ancient microscope, we saw cellular blebs arising from adipocytes—possibly a novel finding.

Why did the microscope take so long to become a tool for medical research? This is, indeed, one of the perplexing facts of medical history (Ackerknecht, 1955, p. 127; Belloni, 1961, p. 284; Hughes, 1959, p. 5). Between the time it was invented—around the year 1800 (Clay and Court, 1932) — and the early 1800's, microscopic anatomy made relatively little progress; a body of knowledge worth the name of *microscopic pathology* did not develop at all.

The beginnings had seemed to promise otherwise. Galileo, one of the contenders for the invention of the microscope, claimed in 1614 that he had seen "flies as big as lambs" (Belloni, 1969, p. 179). Malpighi saw blood circulating in capillaries in 1661, Hooke described cells in 1665, and about the same time Leeuwenhoek was catching the first glimpse of bacteria. But after this heroic age the art withered, except in the hands of a few masters. Morgagni, a pupil of Malpighi, could have written his entire masterpiece, the corner-stone of modern pathology (Morgagni, 1761), without using a microscope—he rarely did (Belloni, 1961, p. 283)—and Bichat saw no need for it in describing the differences between the tissues (Bichat, 1799).

On the whole, during the 1700's, the microscope drifted to the status of an interesting toy. One scholar who resented this state of affairs was Henry Baker, F. R. S.; in "an Attempt to excite in Mankind a general Desire of searching into the Wonders of NATURE", he published a delightful manual, *The Microscope Made Easy*. In the preface of the 3rd edition (1744) the current decadence is analyzed (Baker, 1744 p. ii)):

"It is something more than an hundred and twenty Years since the MICROSCOPE was happily invented; and to the valuable Discoveries made thereby, we stand indebted ( ). In such a Length of Time, it was however probable, many more Advantages might have been reaped from it, had not some Difficulties and Discouragements prevented its general Use.

At the Beginning it was confined to very few; who, making a Secret of it, endeavoured all they could to keep it to themselves; and, when it became a little more publick, the Price was fixt so high, that the most Curious and Industrious, who have not always the greatest Share on Money, could not conveniently get at it. Of late Years, indeed, the Expence has been much less; but then new Discouragements have started up from Mistake and Prejudice.

For Many have been frighted from the Use of it, imagining it required great Skill in Optics, and abundance of other Learning ( ) whereas nothing is really needful but good Glasses, good Eyes, a little Practice, and a common Understanding, to distinguish what is seen; and a Love of Truth, to give a faithful Account thereof. Others have considered it a meer Play-thing ( ). Many again, have laid the Microscope aside, ( ) for want of knowing what Objects to examine...".

Freely summarized, Baker's explanations for the slow progress of scientific microscopy are five: (1) *secrecy*, (2) *price*, (3) *technical difficulty*, (4) *misuse as a toy*, and (5) *lack of ideas*.

Baker himself was not a physician; and after him there is no evidence at all that physicians took up his plea. The turning point did not come until the next century, when the father of Lord Lister perfected the achromatic objective (J. J. Lister, 1830). Shortly thereafter the microscope began to creep into medical schools. At Oxford, microscopical demonstrations began in 1845: the students sat "at tables furnished with little railroads on which ran microscopes charged with illustrations of the lecture, alternatively with trays of coffee. A few senior men came from time to time, but could not force their minds into the new groove ( ). Dr. Kidd, after examining some delicate morphological preparation—made answer first, that he did not believe in it, and, secondly, that if it were true he did not think God meant us to know it" (Tuckwell, 1907, quoted by Hughes, 1959 p. 12).

This points out another obstacle to the success of the microscope: its *late acceptance by the Universities*. It was used and developed by outsiders, or by amateurs, until it became impossible for medical schools to ignore it.

At this point, if we add the short-sightedness of Academia, the number of barriers between man and microscope has mounted to six. But there was yet a seventh, which Baker could not yet appreciate: *distrust*,—the fear of studying optical artefacts, of chasing shadows instead of dealing with reality. This attitude is common in the writings of the eighteenth and early nineteenth century, criticizing the microscope (Zanobio, 1960; Belloni, 1962), and recalls the pessimistic comments that we used to hear during the early days of electron microscopy.

Of course there was some substance to these criticisms: we no longer realize what it means to look through uncorrected lenses. However, it is practically impossible to appreciate, from the literature, *to what extent optics were a limiting factor*. There have been excellent studies of the resolution of ancient microscopes

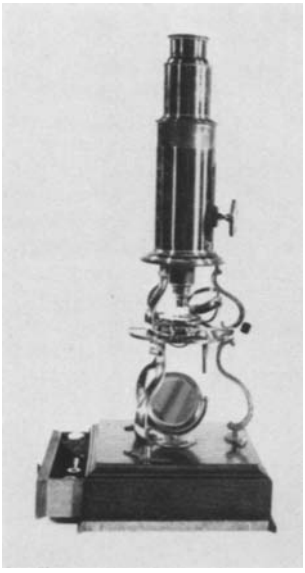

Fig. 1. The ancient compound microscope of the "Culpeper" type, used for the present study

(Otto, 1965, 1966; Bradbury, 1967 p. 151) and even of the optical artefacts that they produced (Zanobio, 1960) but none, as far as we know, from the point of view of the pathologist. The key question, as we see it, is *to know how we could have fared using a microscope with uncorrected lenses and no condenser.*

We therefore performed a series of experiments compatible with the years 1780–1830, using a microscope of the time, and aiming to test the difficulty of "rediscovering" the process of acute inflammation in a piece of transparent tissue. Laboratory studies of this kind are now recognized as a useful adjunct to medical history (Belloni, 1970; Clarke, 1971): we like to refer to them as "experimental history".

Materials and Methods

An ancient compound microscope was obtained from the Museum of Science of Geneva (Fig. 1). It had no condenser, a set of three objectives, rackwork focusing, a rotating plane and concave mirror, and belonged to the general type known as "Culpeper" (Clay and Court, 1932 p. 108). As to its date: we know that it had belonged to Horace-Bénédict de Saussure, who died in 1799; and thanks to Mr. G. L'E. Turner, Senior Assistant Curator, Museum of the History of Science, Oxford, we can add that it was definitely made in London, where that model was produced until about 1830. The lenses were checked by M. Wiegandt, Maître Opticien, who kindly cleaned them and found them to be in good condition; we used only the medium-power objective, which gave the best image. The overall enlargement was about 240 ×.

As a tissue preparation we chose fresh omentum, because (a) it had already been used by Malpighi for his *De Omento* in 1665 (Belloni, 1967), (b) it required no embedding (a much later technique,—about 1860) and (c) it lends itself perhaps better than any other tissue to discovering the major events of inflammation.

To irritate the tissue we used a method compatible with the time, both technically and conceptually: 10 rats were given 1 cc of a 5% emulsion of turpentine intraperitoneally; 6 hours later they were killed. The omentum was usually hyperemic, rolled up to its root and maintained there by fine fibrinous adhesions. It was gently spread out in tap water, mounted between slide and coverslip, checked under a modern microscope to make sure that the typical features of acute inflammation were visible (some preparations were almost normal, others were outright killed by the turpentine). Two or three suitable fields, showing a venule with typical emigration of polymorphs, were selected, brought under the "Culpeper" microscope, and photographed in color and black and white with a 35 mm Nikon reflex camera mounted above the ocular, using artificial light, and the concave reflecting mirror. Thereafter the same fields were photographed at almost identical enlargement with a Zeiss Photomicroscope II. Finally the coverslip was removed, the unfixed preparation was stained with Oil red O and toluidine blue and the same fields were once again photographed with the Zeiss.

Other fields, of normal, fresh omentum, were photographed (a) through the ancient microscope, using the concave reflecting mirror, (b) *idem*, using the plane mirror, and (c) through a Zeiss photomicroscope.

Results and Discussion

We made three sets of observations; the first pertained to the quality of the image.

1. A Trap for Early Microscopists: the "Reticular Image"

When the "Culpeper" microscope was used with the concave reflecting mirror, and tested with a diatom, its resolution appeared very poor (Fig. 2). However, modern tissue sections were quite recognizable (Fig. 3); the image was fuzzy but good enough to allow a fair number of histopathologic diagnoses.

It was quite another matter when the plane mirror was used, especially on fresh omentum. For reasons that are not clear to us, the image changed strikingly. It became darker (this was to be anticipated) and at the same time it took on a different quality. It became a murky tangle of wavy, refractile lines, which changed shape and seemed to squirm at the slightest movement of the mirror.

The larger structures, such as fat cells, remained recognizable; but finer details appeared as camouflaged and uninterpretable. Figure 4a gives some idea of the effect, but cannot render the unstable, "wormy" nature of the image, which can only be appreciated by manipulating the microscope itself.

We recognized this as the *reticular image*, a historical artefact that plagued early microscopists. It was described and discussed by Zanobio (1960) and Belloni (1962) and has never been defined in precise physical terms, but consists essentially of the following: using a compound microscope, and transmitted light, at medium and high powers, it is possible to set the illumination in such a way that the image looks as if it were composed of a tangle of wavy, refractile lines— as described above. A peculiarity of this effect is that *it can be obtained with practically any subject*,— and also with modern microscopes; e.g. Zanobio's pictures published by Belloni (1962) were obtained with a modern microscope, at 350–700 ×, using a 60 μ frozen section of liver, or a dried smear of milk; the result is very similar to our own figures. The basic mechanism is presumably a combination of diffraction and interference.

There is no doubt that the reticular image was obtained by early microscopists, because they illustrated it in careful drawings: especially telling are those

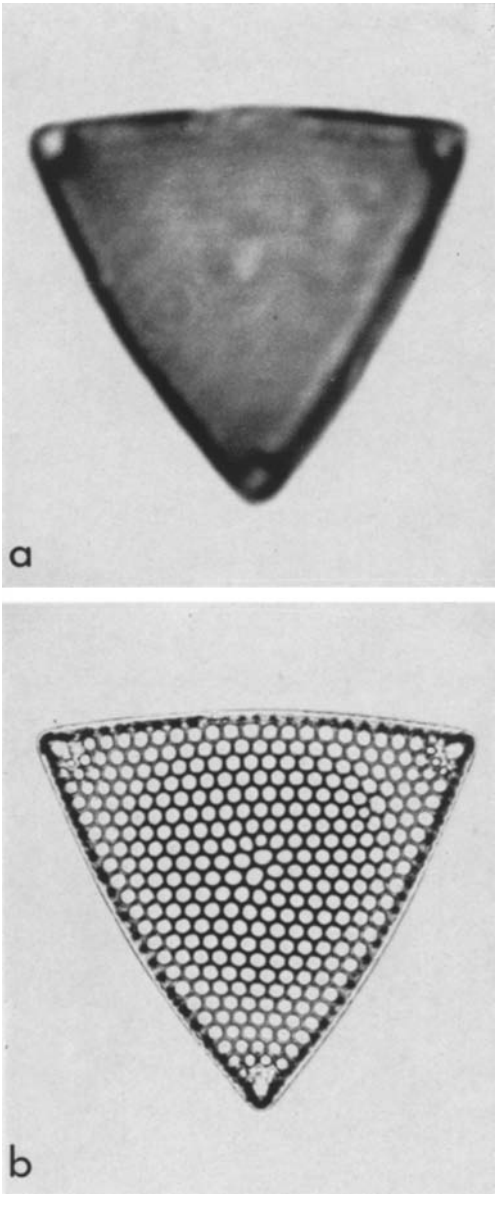

Fig. 2a and b. Demonstrating the resolution of the microscope shown in Fig. 1 (240 ×). A diatom (Triceratium favus) as seen (a) with the ancient microscope, and (b) with a modern microscope

of the Italian biologist Felice Fontana (1781). One of his figures is reproduced here (Fig. 5): note in it the fat cells with *and without* reticular image ("Fig. 19", "Fig. 20").

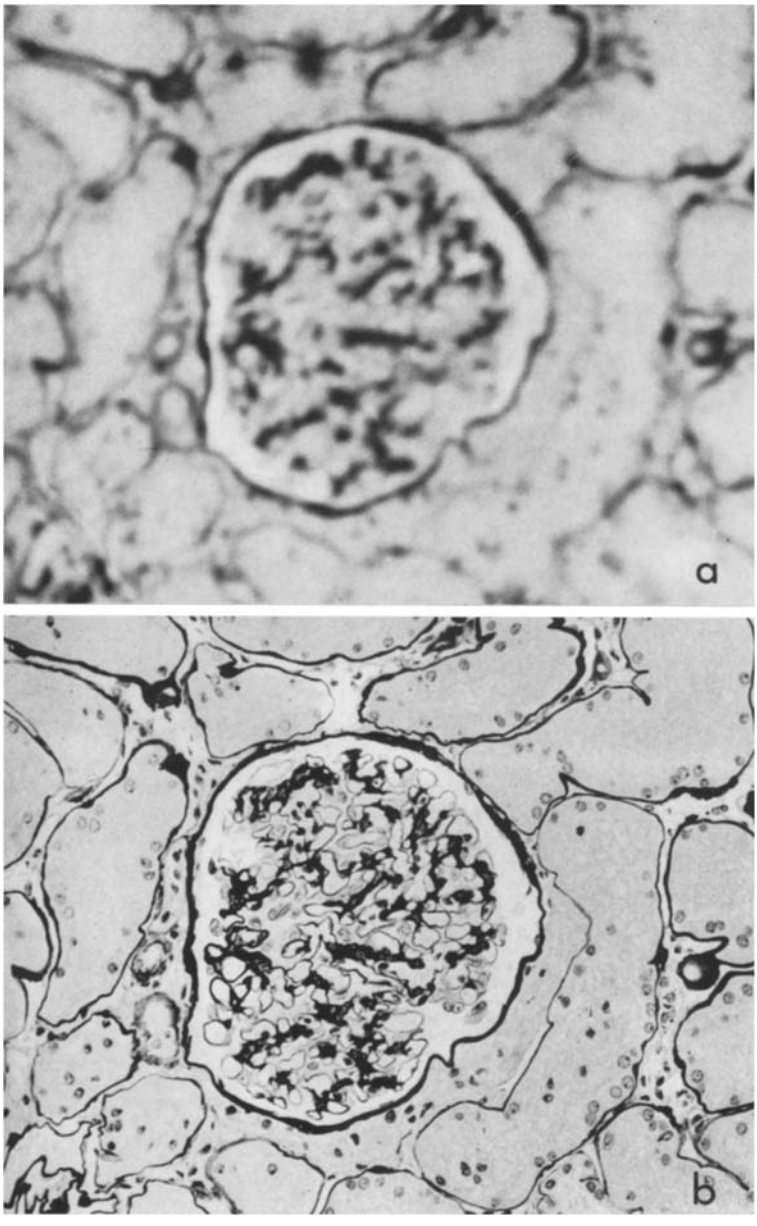

Fig. 3a and b. Demonstrating the resolution of the ancient microscope, with a modern tissue section (kidney, stained with Jones' methenamine silver method). 240 ×. (a) With the ancient microscope. (b) With a modern microscope

Fig. 4a—c. Normal, fresh omentum mounted in tap water. Three views of the same field. 240 ×. (a) With the *plane mirror* one obtains the effect called *reticular image*; though fat cells are still recognizable, the rest of the tissue becomes uninterpretable. (b) With the *concave mirror*, definition is better. (c) Through a modern microscope

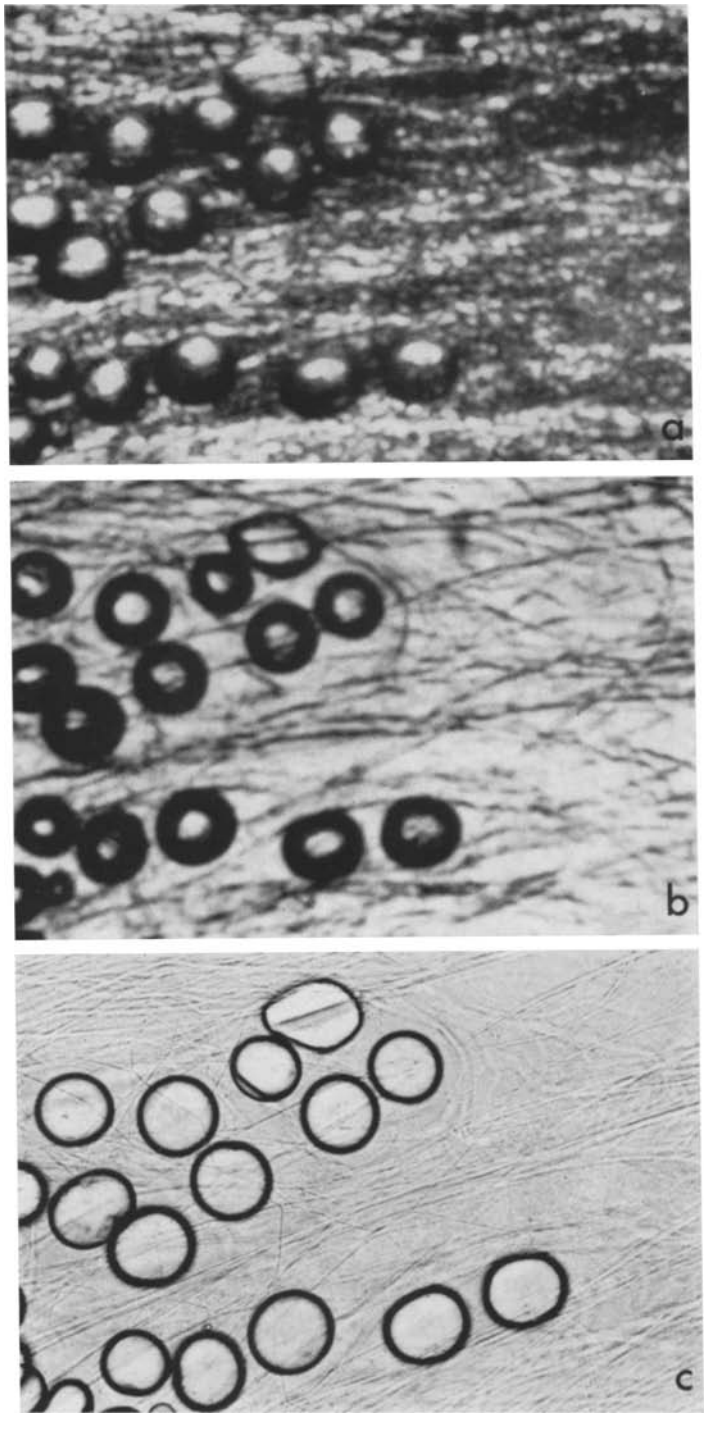

Fig. 4 a—c

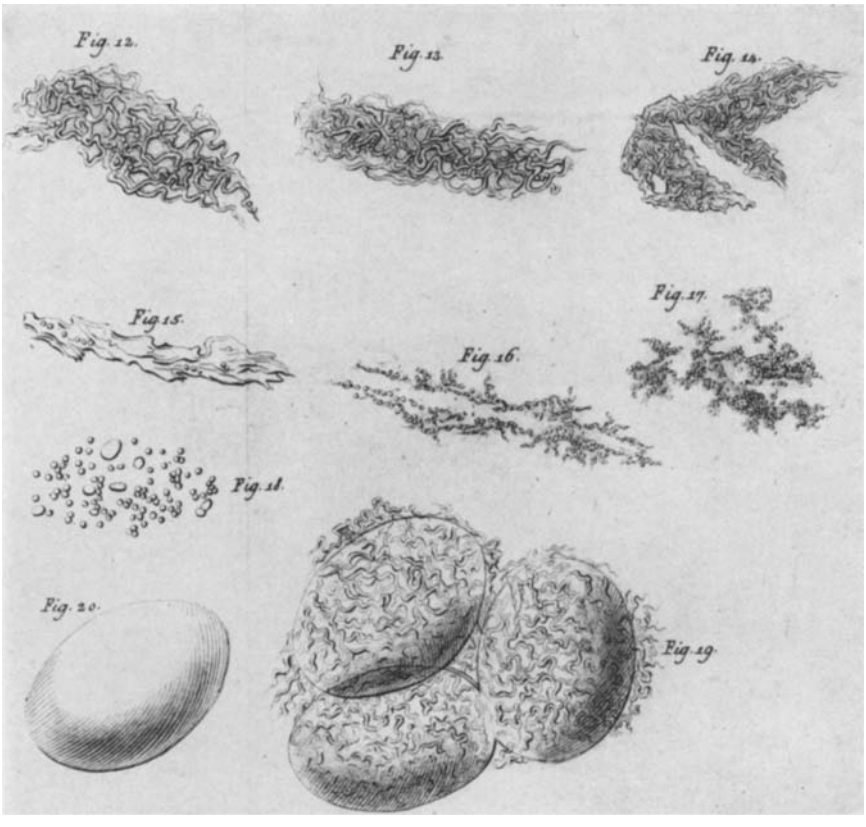

Fig. 5. Fontana's drawings illustrating the phenomenon of the *reticular image* as observed by him on fat cells (Fig. 19), on little scales scraped from the skin (Figs. 12, 13) or from a fingernail (Fig. 14) (Fontana 1781, II, Tav. VIII)

The historical significance of the reticular image is obvious; some microscopists thought that it corresponded to a real structure, i.e. that the basic component of all tissues was some sort of contorted "vermicular" unit; others thought that it was an illusion (Belloni, 1962); and the doubt had to remain for everybody: was the microscope, really, a reliable instrument?

Whoever used ancient microscopes such as ours must have obtained, like us, two kinds of images, and wondered which was the real one. Our photographs illustrate this historic dilemma.

Fig. 6a—c. Mildly inflamed omentum, mounted in tap water (240 ×). (a) Through the ancient microscope, using the concave mirror (i.e. under the best optical conditions): vessels and fat cells are recognizable, but the white blood cells scattered around the tissue cannot be identified as such. The arrow points to a fat cell that seems to be lobulated. (b) The same field, under the same conditions, through a modern microscope. (c) The same field after fixation and staining, and seen through a modern microscope. A few inflammatory cells are well recognizable; but note that the "lobules" of the fat cells have almost disappeared. They were actually cellular blebs

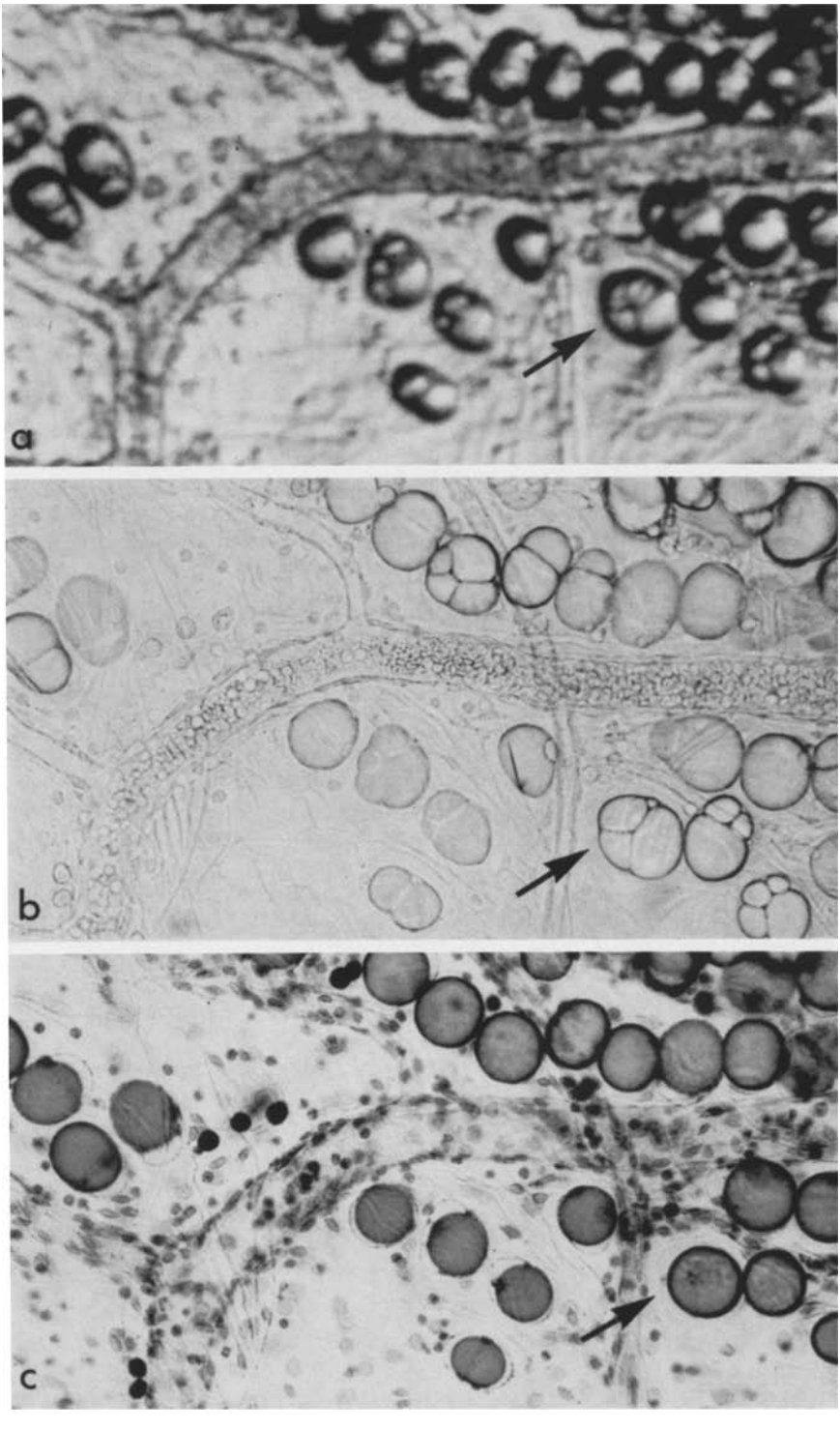

Fig. 6 a—c

2. *Histopathology of Inflammation*

Before the discovery of paraffin embedding, one of the best tissue preparations available was the omentum, because its thickness (in parts as low as 15 micra) approached that of a tissue section. Hence our experiments with the omentum represent near-optimal conditions for the period corresponding to our ancient microscope.

The result is obvious: any pathologist can test his own diagnostic ability on Figs. 6a, 7a, 8a. Vessels and fat cells were well recognizable; blood cells could be distinguished; however, it was not possible to tell whether capillaries were bare channels or whether they had a wall of their own. Though the emigrated white blood cells were visible, to identify them with similar objects in the blood would have been a flight of fancy—closer to prophecy than to microscopy. In other words, an inquisitive physician who would have tried to study "inflamed omentum" under these supposedly optimal conditions, around the year 1800, would have had very little chance of guessing that a basic event of inflammation was the emigration of white blood cells.

When the same microscopic fields were viewed with a good modern microscope, cellular details were beautifully visible though the tissue was mounted in plain water (Figs. 6b, 7b, 8b). And when the same fields were fixed and stained (Figs. 6c, 7c, 8c) the suggestion of diapedesis was even stronger; it might have caused second thoughts even to Virchow, who in 1858 still believed that "pus globules" derived from the connective tissue (Virchow, 1858, Fig. 137).

However, the discovery of diapedesis was not only a matter of good optics: being a dynamic event, it could only be *proven* on a living preparation. Augustus Waller described it in 1846, in the tongue of the living frog (Rather, 1972 p. 91 ff.); Cohnheim, who restudied the process in the 1860's and gave the final description of diapedesis, also used living tissues (Cohnheim, 1867).

In concluding, Henry Baker had left out a major cause of "Difficulty and Discouragement" that slowed down the pace of microscopy: blurry images.

3. *Cellular Blebbing in Adipocytes*

While trying in vain to make our microscope explain diapedesis *on fresh, unstained tissue*, our attention was drawn by fat cells with a very peculiar look: they had a lobulated aspect, quite visible with the ancient microscope (Fig. 6a, 7a), even better on the Zeiss (Fig. 6b, 7b). At first we thought that they represented the less common but well-known fat cells endowed with several fat globules; but to our surprise, the fat stain showed only one red vacuole, usually the largest, *whereas the other vacuoles had greatly shrunk or disappeared* (Fig. 6c,

Fig. 7a—c. Moderately inflamed omentum, mounted in tap water (240 ×). (a) With the ancient microscope, concave mirror. Vessels and fat cells clearly recognizable. Arrow points to a fat cell that appears to be lobulated. (b) Same field, same conditions, with a modern microscope. (c) Same field after fixation and staining. The inflammatory response is obvious; note that the "lobules" of the fat cell (arrow) have disappeared—they were blebs, which fixation has somehow "deflated"

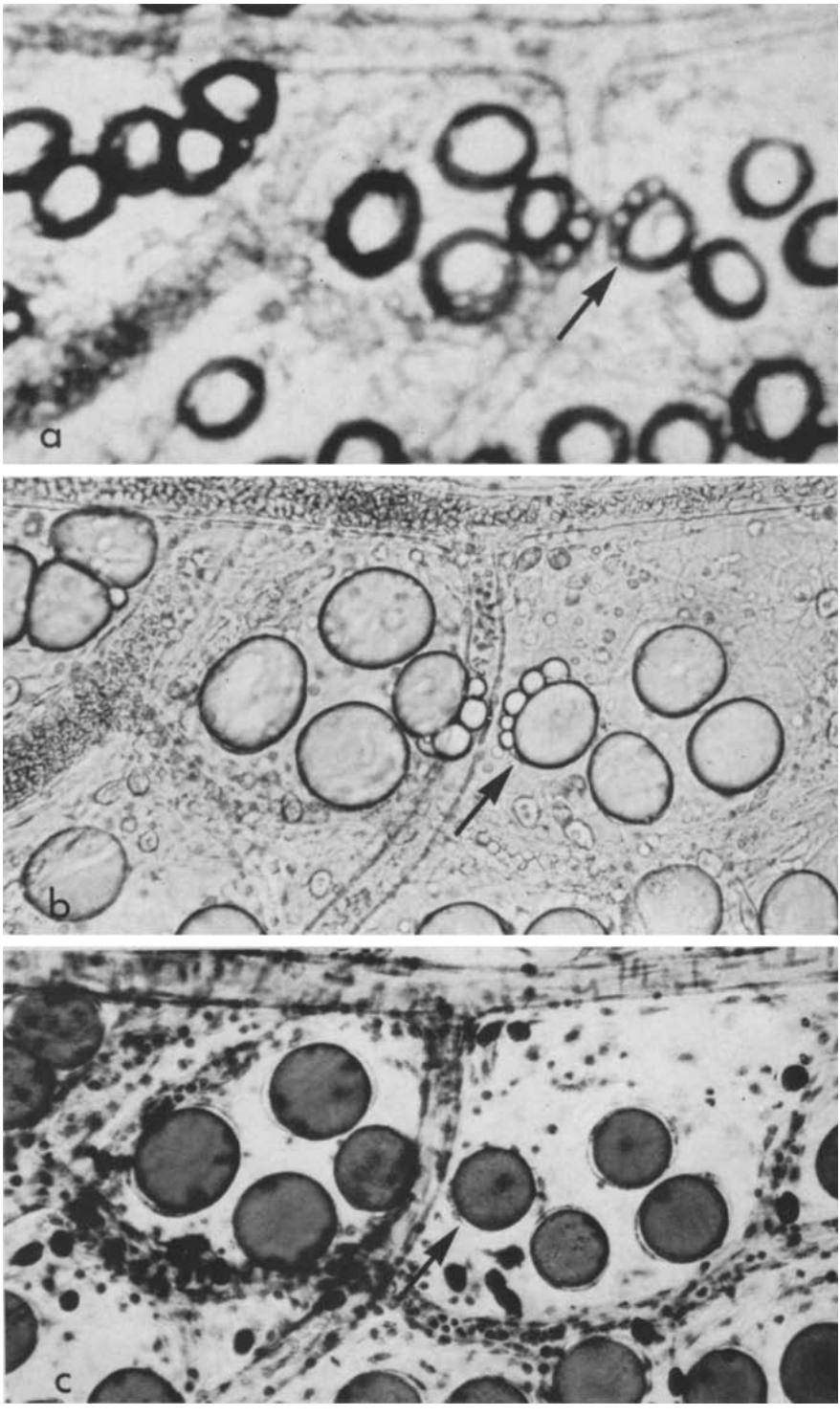

Fig. 7 a—c

7c). These abnormal fat cells were not present in all fields, and seemed to reflect injury by the turpentine, since the hypotonicity of the tap water did not induce them.

The obvious conclusion is that we are dealing with *cellular blebs* arising from fat cells. These structures, which are perhaps the commonest form of cellular injury, correspond to "blisters" of the cellular membrane. All too well known to electron microscopists (they tend to form during poor fixation), they seem to have been almost forgotten in the field of light microscopy. Yet, with the relatively poor fixation of routine tissue samples, blebs should be present by the dozen in every microscopic field. They are easily seen as "holes" when they lie within a homogeneous background, like blood plasma or thyroid colloid (the once-famous "resorption vacuoles" of the colloid are just this artefact) but when free in the tissue spaces they are singularly elusive. They certainly exist *in vivo*; Buckley produced micro-injuries in the rabbit ear chamber, and saw blebs arising and even floating away from free connective tissue cells (Buckley, 1960a, b). As far as we know, they have never been described as arising from adipocytes (Tedeschi, 1965).

The point that we would like to make here is that cellular blebbing, so difficult to observe in modern microscopic slides (especially after paraffin embedding), *is easily visible in fresh, unstained preparations*, and even with the poor optics of a microscope about 200 years old (in fact, also Buckley's experiments on cellular blebbing were made on fresh living tissues). In this respect, then, ancient microscopic technique enjoyed an advantage: the fragile blebs retain their shape in a watery medium, but after fixation and embedding they tend to shrivel and disappear.

The microscopic study of fresh, unfixed, teased tissues was still taught some 30 years ago; since then it seems to have died away. We will therefore end with a plea to resuscitate this easy and fruitful ancient approach—the "*fresh preparations*"—which is, after all, closer to life than our current slides.

To sum up our experience with ancient microscopy: the tools were poor, but the use of fresh tissue deserves to retain its place among the techniques of pathology.

We wish to thank Dr. M. Cramer, Curator of the Museum of Science, Geneva, Switzerland, who placed the ancient microscope at our disposal, and Mr. André Wiegandt, Maître Opticien, who checked it. We are also indebted to Miss Geneviève Leyvraz for Fig. 3, to Miss Lise Piguet for secretarial help, and to Mr. Jean-Claude Rumbeli for the painstaking preparation of the photographs.

Fig. 8a—c. Omentum, severely inflamed, mounted in tap water. 240 ×. (a) Ancient microscope, concave mirror. Fat cells and vessels easily recognizable, but identification of the different blood cells within the vessels is impossible. (b) Same field through a modern microscope. (c) Same field after fixation and staining

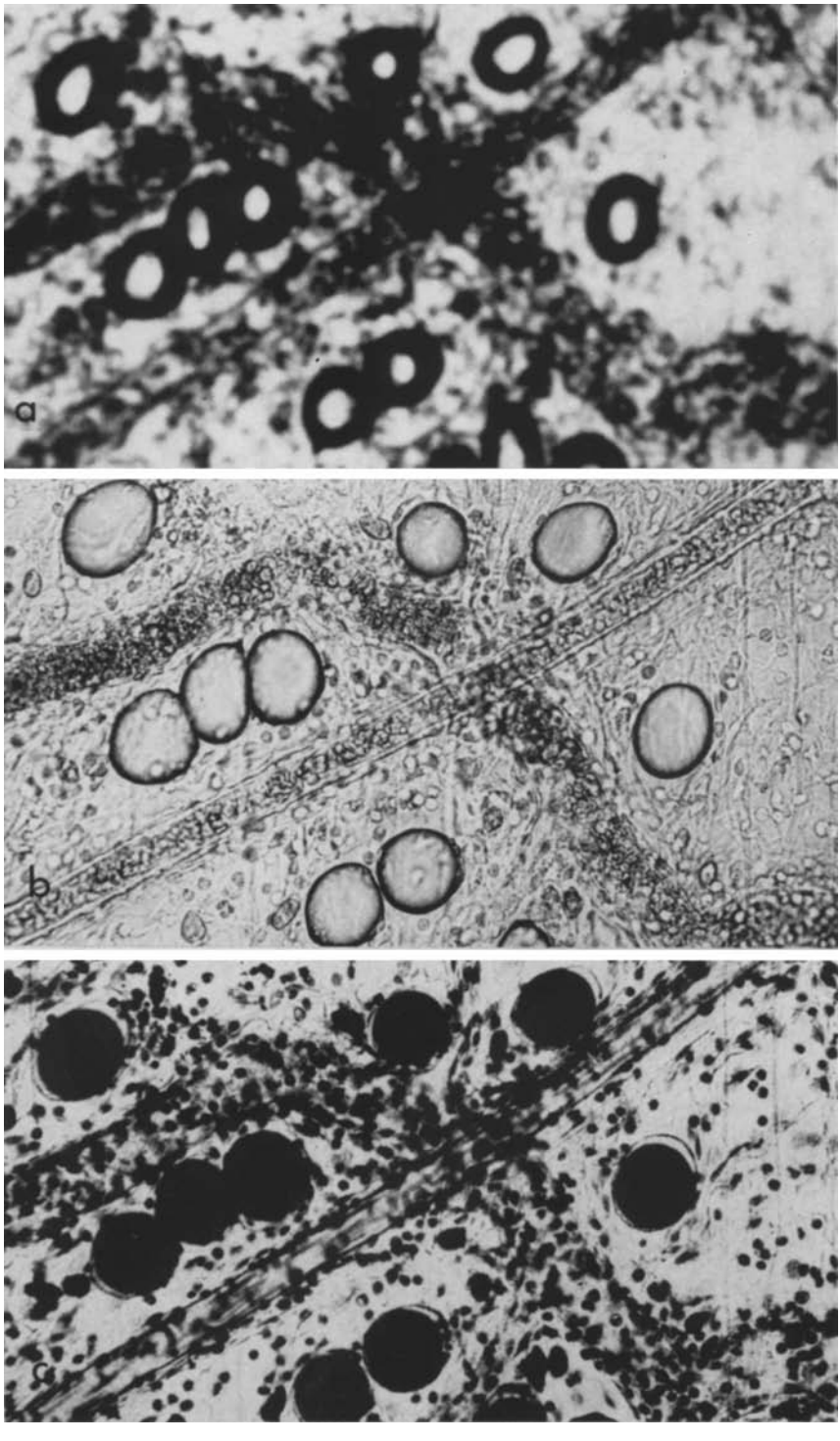

Fig. 8 a—c

References

Ackerknecht, E. H.: A short history of medicine. New York: Ronald Press Co. 1955

Baker, H.: The microscope made easy. Preface of the third ed. London: Dodsley 1744

Belloni, L.: Spunti di patologia osteo-articolare nel "de Sedibus" di G. B. Morgagni. In: Convegno internazionale sugli aspetti moderni della morfologia in biologia e in medicina. Padova 1961

Belloni, L.: Micrografia illusoria e "animalcula". Physis **4**, 65–73 (1962)

Belloni, L.: Il Primo Ventennio della Microscopia (Galilei 1610—Harvey 1628). Dalla Microscopia alla Anatomia Microscopica dell' Insetto. Clio Medica **4**, 179—190 (1969)

Belloni, L.: The repetition of experiments and observations: Its value in studying the history of medicine (and science). J. Hist. Med. **25**, 158–167 (1970)

Bichat, X.: Traité des membranes en général et de diverses membranes en particulier. Paris: Richard, Caille et Ravier, An VIII (1799)

Bradbury, S.: The quality of the image produced by the compound microscope: 1700–1840. Historical aspects of microscopy, edited by S. Bradbury and G. L'E. Turner. Cambridge: W. Heffer & Sons Ltd. 1967

Buckley, I. K.: Tissue injury by high frequency electric current: Observations with the Sandison-Clark ear-chamber. I. Injury to small blood vessels. Aust. J. exp. Biol. **38**, 195–210 (1960). II. Injury to small groups of cells. Aust. J. exp. Biol. **38**, 211–226 (1960)

Clarke, E.: Modern methods in the history of medicine. London: Athlone Press 1971

Clay, R. S., Court, T. H.: The history of the microscope. London: C. Griffin & Co., Ltd. 1932

Cohnheim, J.: Ueber Entzündung und Eiterung. Virchows Arch. path. Anat. **40**, 1–79 (1867)

Fontana, F.: Traité sur le vénin de la vipère, sur les poisons Américains, sur le laurier-cerise et sur quelques autres poisons végétaux. — On y a joint des observations sur la structure primitive du corps animal, différentes expériences sur la reproduction des nerfs, et la description d'un nouveau canal de l'oeil. Florence, 1781 (2 vol.).

Hughes, A.: A history of cytology. London-New York: Abelard-Schuman 1959

Lister, J. J.: On the improvement of achromatic compound microscopes. Phil. Trans. **120**, 187–200 (1830)

Malpighi, M.: De Omento, Pinguedine et Adiposis Ductibus... Londini: Typis M. F. Impensis R. Littlebur, R. Scott, Tho. Sawbridge, G. Wells 1686

Morgagni, G. B.: De Sedibus et Causis morborum per Anatomen indagatis. Venezia 1761

Otto, L.: Vergleiche zur optischen Leistung historischer Mikroskope. Mikroskopie **20**, 189–195 (1965)

Otto, L.: Zur Kenntnis der zusammengesetzten Mikroskope des Giuseppe Campani (1635–1715) in Rom. Mikroskopie **21**, 306–313 (1966)

Otto, L.: Beispiele zur Entwicklung der Abbildungsgüte des Mikroskops. Wissenschaftl. Z. der Humboldt-Universität zu Berlin, Math. Nat. R. **XVII**, 793–798 (1968)

Rather, L. J.: Addison and the white corpuscles: An aspect of nineteenth-century biology. London: Wellcome Institute of the History of Medicine 1972

Tedeschi, C. G.: Pathological anatomy of adipose tissue. In: Handbook of physiology, sect. 5 (adipose tissue), p. 141. Washington: Amer. Physiol. Soc. 1965

Virchow, R.: Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre. Berlin: A. Hirschwald 1858

Zanobio, B.: L'immagine filamentoso-reticolare nell'anatomia microscopica dal XVII al XIX secolo. Physis **2**, 299–317 (1960)

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