active site of the enzyme. This view is now strengthened by our finding that phenol liberation from the inhibited enzyme occurs in the region of pH 6 to 8, where the enzyme itself normally functions. Bearing in mind the structural similarities between chymotrypsin and trypsin, inhibition of both enzymes by DPClP may be depicted by structure I, in which the diphenylphosphoryl group is firmly bound to the serine at the active site, GH being the nucleophilic group (pK 6–7) associated with normal esterase activity. Although the enzyme is inhibited by blocking of the serine (I), at pH 8 the group GH is still free to interact with the phosphoryl group causing intramolecular elimination of 1 *M* of phenol.

The foregoing process is significant in connection with the ageing of DFP-inhibited pseudocholinesterase[5], which would now appear to be due to the elimination of one mole of isopropanol at the inhibited esteratic site of the enzyme by a similar mechanism (II). This emphasises the resemblance between the esteratic sites of chymotrypsin, trypsin and pseudocholinesterase.

(I) (II)

A detailed kinetic treatment of our reactions will be published subsequently.

Résumé. Les solutions de chymotrypsine et de trypsine inhibitées par le diphényle phosphorochloridate éliminent le phénol par un processus stoichiométrique, selon une réaction secondaire qui se produit spontanément dans la région du pH 6–8. Les auteurs émettent une proposition quant aux modalités de ce processus qu'ils appliquent au vieillissement du D.F.P.-pseudocholinestérase.

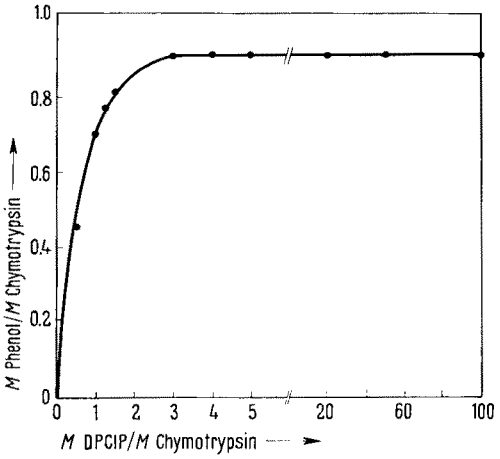

Fig. 2. Effect of DPClP/chymotrypsin ratio on amount of phenol liberated by chymotrypsin-DPClP reaction mixtures in 0.1 *M* sodium phosphate, pH 8.0 after incubation for 1 h at 20°C.

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Chemistry Department, University of Birmingham (England), April 17, 1961.

[5] F. Berends, C. H. Posthumus, I. v. d. Sluys, and F. A. Deierkauf, Biochim. biophys. Acta *34*, 576 (1959).

[6] Present Address: Institute for Enzyme Research, University of Wisconsin, Madison (U.S.A.).

[7] Present Address: Applied Chemistry Branch, Royal Military College of Science, Shrivenham (Wiltshire, England).

X-Ray Microscopy of Intestinal *Villi*

The vascular pattern of intestinal *villi* can be demonstrated by projection X-ray microscopy. The minute blood vessels of the intestinal *villus* have hitherto been studied in various laboratory animals and man either histologically[1] or by injection and clearing techniques[2]. More recently the *villus* circulation has been observed by quartz rod transillumination[3]. Although the blood supply of the gastro-intestinal tract has been studied radiologically by various workers[4,5] no study of the microcirculation of the intestinal *villus* either by contact or projection X-ray microscopy has been reported.

Renewed interest in the absorptive role and functions of the intestinal *villi* has arisen out of investigations into the relationship between intestinal mucosal changes and certain malabsorptive disorders in the human. The introduction of peroral intestinal biopsy has already resulted in the description of abnormal *villi* and a disorganised capillary pattern[6,7].

Basic knowledge of the structure and function of the intestinal *villus* and its vessels however is limited, and derives mostly from the examination of histologically fixed and sectioned material, for study of the microcirculation within the wall of the living intestine suffers technical difficulties related to inadequate optical conditions[3]. Specifically both the opacity of the gut wall and short depth of *focus* of the optical microscope have limited vascular research on the intestinal *mucosa* and its *villi*.

X-ray microscopy owing to its penetration and great depth of field allows a complete three-dimensional view of the internal vascular detail of the specimen to be seen at one time, at either low or high magnification, so that the distribution, connections, and terminal features of a large or small vascular territory can be readily integrated. Visualisation of the volume pattern of the blood vessels can be recorded stereographically if so required, by simple lateral translation of the specimen between two X-ray exposures. X-ray microscopy moreover offers the advantage that vascular research may be conducted on both fresh and living tissue.

The X-ray micrographs of intestinal *villi* presented here were taken with the Cosslett-Nixon X-ray projection microscope[8]. In this instrument two magnetic lenses form a demagnified image of a thermionic electron source upon

[1] P. E. Smith and W. M. Copenhaver, *Bailey's Textbook of Histology* (Williams & Wilkins, 1953), p. 427.

[2] L. F. Jacobson and R. J. Noer, Anat. Rec. *114*, 85 (1952).

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[4] H. B. Benjamin and A. B. Becker, J. Surg. Gynec. Obstetr. *108*, 134 (1959).

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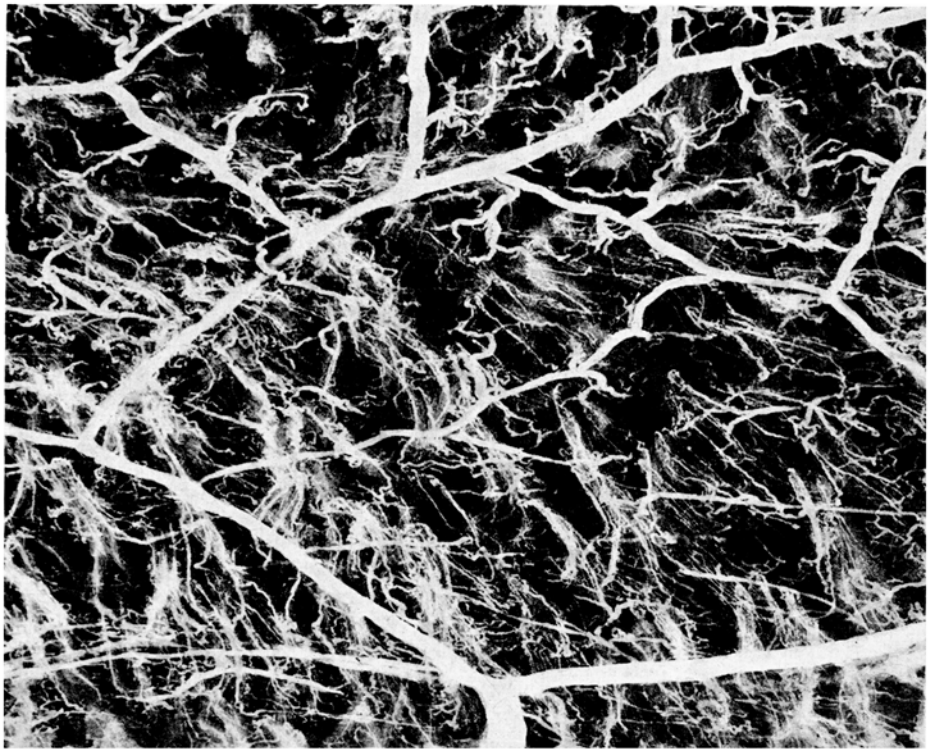

Fig. 1. Microangiogram of *ileum* showing the macromesh and micromesh vascular patterns and intestinal *villi* demonstrated by contrast medium (Micropaque). (Mag. ×55)

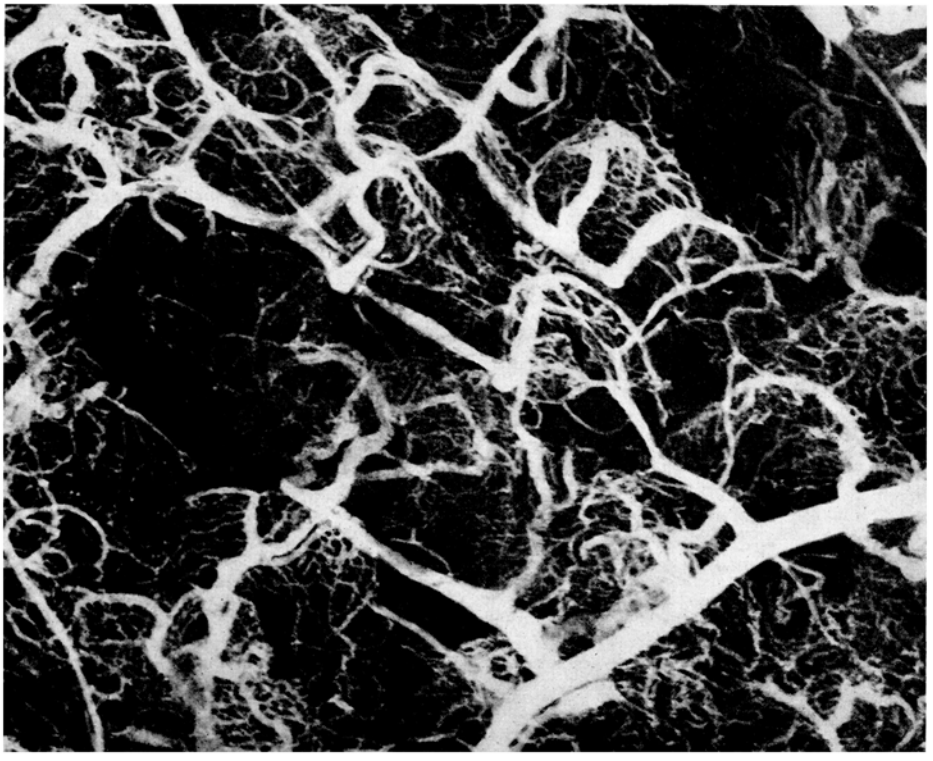

Fig. 2. Microangiogram of *duodenum* showing the origin of the arterioles and venules within the *villi*, and surrounding capillary network. Note the convergence of the axial veins of the *villi* upon the mucosal veins. (Mag. ×100)

a thin metal foil which serves both as X-ray target and window of the evacuated tube. A point source of X-rays about 0.5 to 1 μ in diameter is thus produced, and used to project an image of the specimen, which is placed close to the source in air, on to a distant fluorescent screen or photographic plate. The resolving power of the instrument is approximately equal to the X-ray source diameter, while X-ray magnification is determined by the ratio of the target-specimen and target-plate distances, both of which are variable.

The microangiograms presented (Figures 1 to 5) were obtained by successive irrigation of the small intramural vessels of the rabbit intestine with body warm solutions of a plasma extender with anticoagulant action (Dextran sulfate) and a contrast medium of colloidal dimension (25% Micropaque). This was achieved by infrarenal retro-

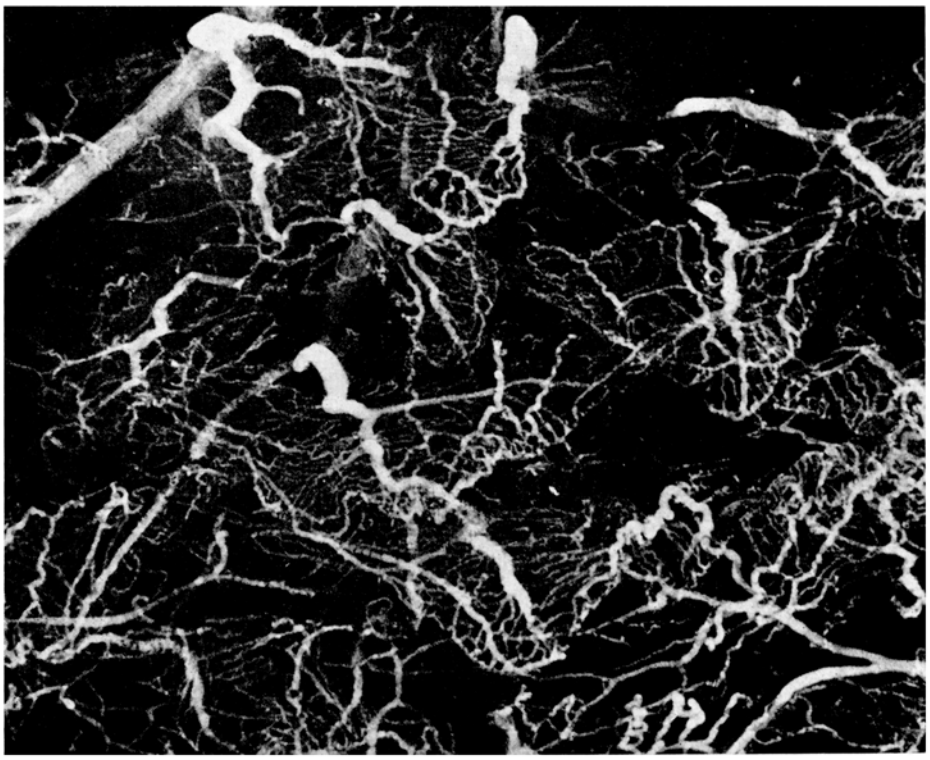

Fig. 3. Microangiogram of *jejunum* showing that the capillary network of the *villus* is derived from both the *villus* arteriole and subjacent submucous *plexus*. The large axial venules draining the *villi* are a striking feature. (Mag. ×96)

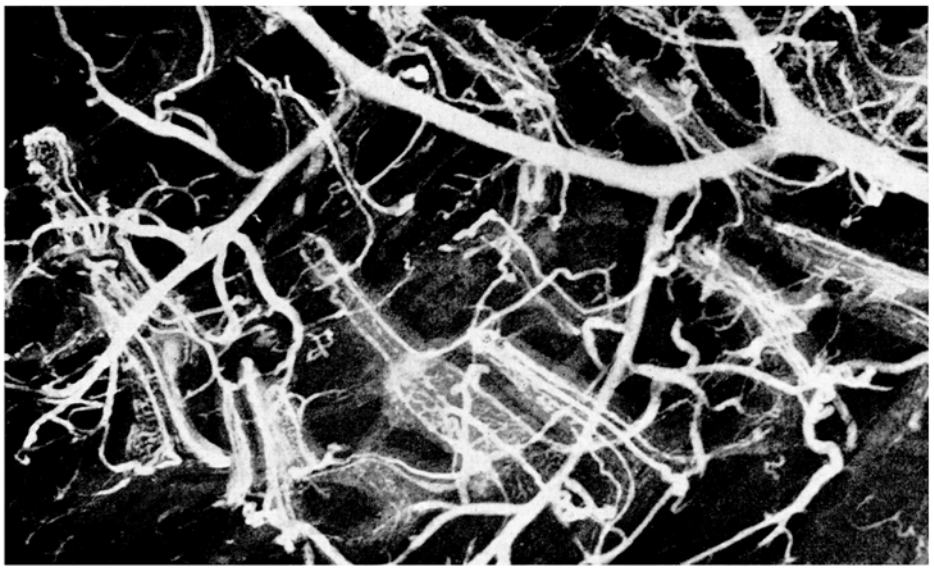

Fig. 4. X-ray micrograph of *jejunum* showing that some *villi* are supplied by two arterioles (see lower right). The *villus* arterioles are more slender than the corresponding venules. (Mag. × 95)

grade intubation of the abdominal *aorta* with fine polyethylene tubing (2.15 mm o.d.), and recovery by similar tubing introduced into the inferior *vena cava* immediately below the heart. Operative procedures were carried out on heparinised and anaesthetised animals, and irrigation effected with a pressure head of 1 m. Contrast medium was introduced soon after blanching of the small intestine was observed. Segments of *duodenum*, *jejunum*, and *ileum* were then removed and comparative studies made of formalin fixed, air dried, and fresh material, over the X-ray projection microscope. Microangiographic studies of exteriorised living small bowel are currently in progress in this laboratory.

The bowel loop or exsected portion thereof was positioned across the target assembly of the X-ray microscope, which lies within the polepiece of the upper or objective lens of the instrument. A thin aluminium foil target (4 μ thick) used with an accelerating voltage of 10 kV provided good resolution and adequate penetration for vascular studies of the opened bowel, while a thicker copper foil target (5–10 μ) and higher accelerating voltage of 15 to 25 kV provided better penetration for studies of the intact bowel.

The X-ray micrographs were recorded with a simple metal box camera, using varying target plate distances ranging from 0.5 to 2.5 cm. Detailed projection micrographs or microangiograms of the smallest blood vessels within the bowel wall were readily obtained using either standard lantern slide plates (Kodak Contrasty) or high resolution plates (Ilford), while working under atmos-

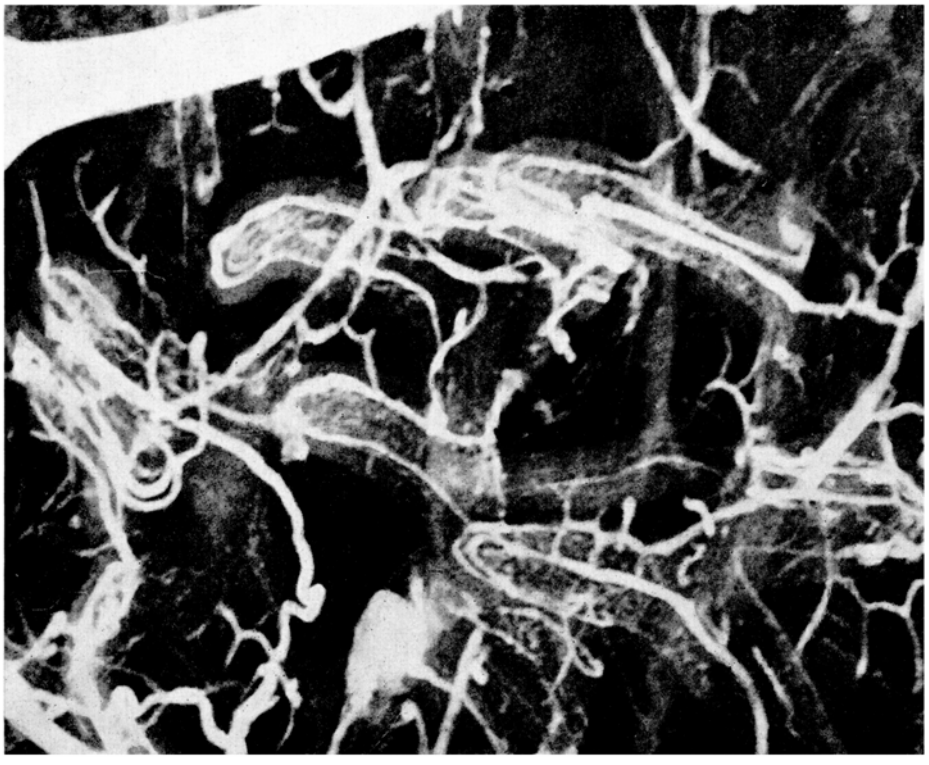

Fig. 5. X-ray micrograph of a cluster of jejunal *villi* showing arterio-venous anastomoses at the tips of several *villi*. The capillary network of the *villi* is not well filled with contrast medium. The *villus* arterioles are more slender than the corresponding venules. A *villus* tip is seen end-on at the left. (Mag. ×193)

pheric conditions. Since the magnification is dependent upon the target-specimen and target-plate ratio, both the size of the vascular field and degree of magnification could be varied at will. Contact and projection studies of frozen sections (150 μ) were also recorded for purposes of comparison.

X-ray projection micrographs of the small intestine wall show the branches of the *vasa recta* or mural trunks entering the *submucosa* to ramify and anastomose with similar branches from neighbouring arteries to form the submucosal arterial network or *plexus*. Small mucosal arteries are seen to arise from both the main and subsidiary anastomosing branches of the submucosal *plexus*. As many as 10 to 14 mucosal arteries have been observed passing from a long secondary anastomotic arcade to supply adjacent *villi*.

These mucosal arteries follow a long or short course before breaking up into a leash of two, three or more fine arteries or arterioles to supply as many or fewer *villi*, so that a single or double branched vessel may be seen entering the base of a *villus*. These *villus* arteries or arterioles eventually terminate in the capillary bed of the *villus* and an apical arterio-venous anastomosis.

At higher magnification both the capillary network and apical arterio-venous anastomosis of the *villus* can be distinctly seen, as well as the manner in which the venous capillaries join the relatively large axial venule that drains the *villus*. The convergence of three or more such venules from neighbouring *villi* upon the subjacent mucosal vein is also readily followed.

The general vascular pattern and relation of the submucous *plexus* and its branches to the intestinal *villi* can be visualised in a single print such as Figure 1, which is a low power microangiogram of the whole thickness of the ileal wall. The basic vascular characteristic of the gut as earlier stated[9] and since confirmed[3,5] is the mesh pattern. Inside the large macromesh of the submucous *plexus* there exists a further network of smaller vessels or micromesh from which the arterioles supplying the capillaries arise, as may be seen in this and succeeding Figures.

Medium power microangiograms, such as Figure 2 depicting part of the duodenal wall, reveal the mode of termination of the mucosal arteries, origin of the arterioles and venules within the *villi*, as well as the surrounding capillary network. The capillary network of the *villus* is clearly derived from both the *villus* arteriole and the subjacent submucous *plexus*. The axial venules draining the *villi* are a striking feature, as is their convergence upon the mucosal veins (Figures 2 and 3). In some instances the venule of the *villus* appears to be marginal, and a number of *villi* are supplied by two arterioles (Figure 4).

Micrographs at higher magnification, such as Figure 5, which shows a cluster of jejunal *villi*, confirm the existence of an arterio-venous anastomosis at the tip of the *villus*. This would appear to take the form of a short circuit to bypass the fine capillary network of the *villus* when digestive processes are less active.

Comparison of the plates shows the variation in form of the *villi*, which may be low and broad, fingerlike, or triangular, as between the upper and lower ends of the small intestine. Density counts of *villi* could be readily determined from X-ray projection micrographs.

Zusammenfassung. Röntgenmikroskopische Studien an Darmzotten des Kaninchens zeigen das Gefässmuster der Zotten nach Injektion mit einem Kontrastmedium.

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Department of Anatomy, Dalhousie University, Halifax (Nova Scotia, Canada), May 2, 1961.

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