Observations were also made under phase contrast microscopy using a Leitz Orthoplan microscope fitted with planar objectives and Heine phase contrast condenser. For this purpose, the extract was diluted with serum-free McCoy medium to give a concentration of 10 µl/ml of medium. HeLa cells grown on coverslips were inverted over depression slides containing sponge extract in medium and observations were made over a 2-h period. Cells exposed to medium containing an equivalent amount of ethanol but without sponge extract were used as controls.

Figure 1 shows a population of HeLa cells immediately after a coverslip culture was inverted over medium containing sponge extract. After 10 min of exposure to the extract, spherical globules were observed near the periphery of the cells (Figure 2). In a few cells, the cytoplasm appeared to have fragmented and the nuclei showed prominent nuclear membranes with little nuclear detail. Marked changes were seen 1 h later (Figure 3). Cytoplasmic material appeared to be fragmented and were found to aggreagate around the nucleus in some cells, leaving a clear zone adjacent to the cell membrane. In some cells, the cell membrane appeared to have ruptured. The nuclear membrane was more prominent in some cells while in other cells it appeared to have ruptured also. Nuclear detail was lacking in most of the cells and the nucleoli were either not seen or appeared as small dark clumps. These features were also observed under the electron microscope, and a more detailed ultrastructural study is now underway[8]. No recovery was obtained when this coverslip culture was placed in sponge-free medium after the experiment.

The experiments have shown that an alcoholic extract of the sponge, *Suberites inconstans*, was cytotoxic to HeLa cells; recovery was possible only when the cells were exposed to low concentrations of the extract.

Zusammenfassung. Ein alkoholischer Extrakt des Schwammes *Suberites inconstans* erweist sich als ein Zellgift gegenüber Hela-Zellen. Wachstumsstörungen treten auf, und mikroskopisch sichtbare Veränderungen an Kernen und Membranen werden festgestellt.

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[8] C. K. TAN, C. H. TAN and Y. F. TEH, in preparation (1973).

Closed Circulation in the Rat Spleen as Evidenced by Scanning Electron Microscopy of Vascular Casts

No conclusive evidence has been available to settle the question whether the arterial blood of the spleen is directly drained into the venous sinuses or flows into the spaces of the cords of Billroth before being received by the sinuses[1]. The former 'closed' theory was originated by WEIDENREICH[2] in 1901, and the latter, 'open' theory by HELLY[3] in the next year. Although the 'closed' theory was strengthened in the 1930's by some light microscopists such as BJÖRKMAN[4], who injected various matters into splenic vessels, and KNISELY[5], who observed the spleen in living animals, the general view of modern histologists seems inclined to the 'open' theory[1]. Recent electron microscope studies on ultrathin sections of the organ have also failed to demonstrate a closed circulation in the spleen[6].

The present study was undertaken to shed light on this field of study by observing directly and 3-dimensionally the vascular casts under the scanning electron microscope.

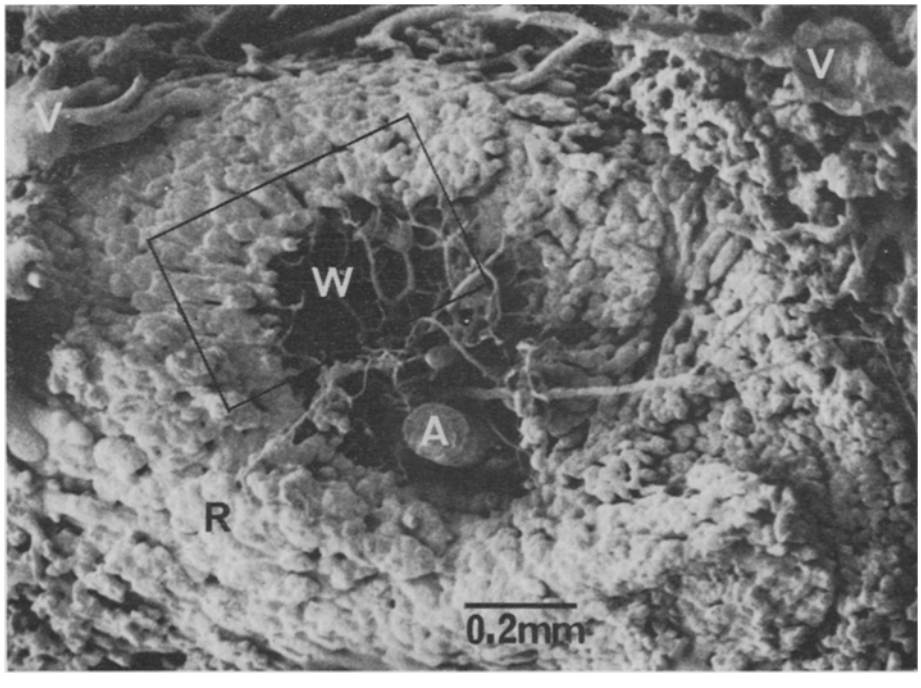

Fig. 1. A low power scanning electron micrograph showing the vascular arrangement in the rat spleen. Red pulp (R), white pulp (W), a central artery (A) and trabecular veins (V) are seen.

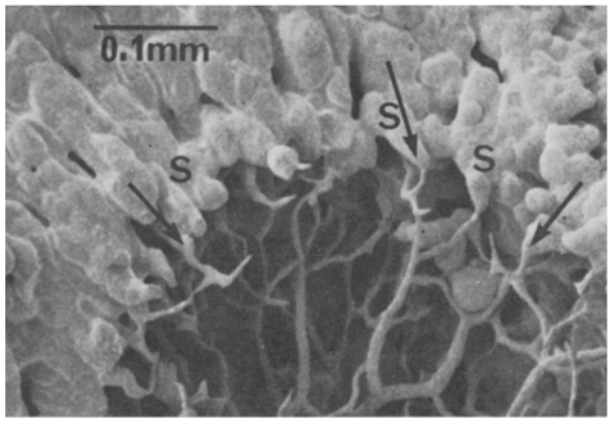

Fig. 2. Higher magnification of the boxed area in Figure 1. Note that the resin stubs representing the venous sinuses (S) communicate with the terminal threads of the follicular arterioles (arrows).

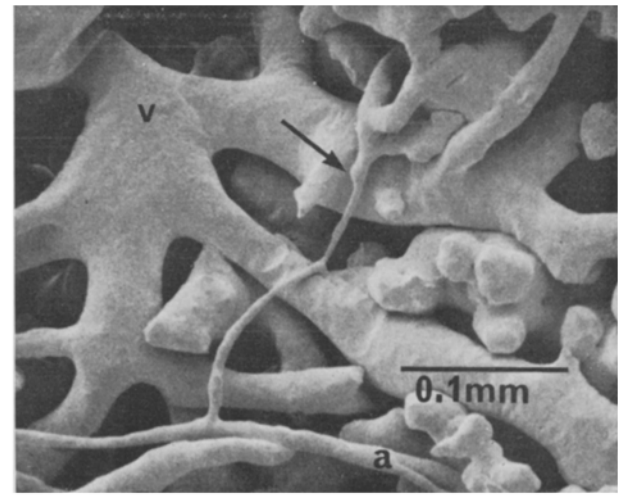

Fig. 3. A scanning electron micrograph showing the connection (arrow) between a terminal twig of the penicillar artery (a) and sinuses. A branching trabecular vein (v) is seen.

The methodological exploration was recently made by one of the authors and reported elsewhere[7]. Plastic casts of the vascular bed in adult rat spleens were made by injection from the thoracic aorta of methacrylic methyl ester mixture. After polymerization of the injected resin, the tissues of the animal were removed by maceration in a concentrated NaOH solution and rinsing in water. The casts were carefully dissected under the binocular, and appropriate parts to be observed were exposed. The specimens were then coated with gold in a vacuum evaporator and observed in a JSM-U3 type (JEOL) scanning electron microscope with an accelerating voltage of 5 kV.

A survey view of a portion of specimen (Figure 1) shows a structural unit of the vascular bed in the spleen. The round dark area (W) corresponds to a lymph follicle or white pulp, and the outer white mass (R) to the red pulp. The loose, coralliform structure (V) surrounding the latter reproduces the trabecular veins. The central artery is represented by the cylinder (A) located eccentrically, in the follicle. It issues 2 types of branches. The more numerous and thinner ones are follicular arterioles which ramify within the follicle to terminate at its periphery. The less numerous and thicker ones are penicillar arteries which pass deep into the red pulp (Figure 1).

A closer view of the resin mass in the red pulp (Figure 2) shows that it consists of irregular-shaped stubs which are mainly elongated radially and are connected with each other by constricted portions. It is obvious that the stubs represent individual venous sinuses. The narrow spaces between the stubs must correspond to the cords of Billroth.

At the central margin of the red pulp, the resin stubs or sinuses are arranged in a palisade and each of them receives a terminal twig of the follicular arterioles at its conical end (Figure 2). The penicillar arteries enter the red pulp and divide into their terminal twigs in the outer zone of the red pulp, and each of them is also connected with the conical end of a sinus (Figure 3). The connection of the arterial end and the sinus reminds us of the calyx of an egg-plant. A visualization closest to these figures may be the diagram presented in 1936 by KNISELY[5] who observed microscopically the circulation in the living spleens of mice, rats and cats with the aid of a specially devised illumination. This method of KNISELY could not produce a photographic record and his view was supported only by a few later authors[8].

The present scanning electron micrographs seem to indicate unequivocally that every arterial end directly communicates with the sinus in the rat spleen. If there were an arterial end opening into the cord of Billroth, this might be reproduced by a resin thread expanded into an irregular, perhaps angular form representing the labyrinthic meshes in the cord. This was never found in our specimens. Studies are now in progress to elucidate whether the closed circulation now evidenced in the rat may also be the case in other species of mammals, including man.

Zusammenfassung. Rasterelektronenmikroskopische Beobachtungen der Methakrylatausgüsse der Blutgefässe von Rattenmilz ergeben, dass jedes Arteriolenende unmittelbar mit einem Sinus verknüpft ist. Man bekommt nirgends ein Bild, das eine offene Endigung der Arteriolen in die Milzstränge andeutet. Dieses Ergebnis stützt die «geschlossene» Theorie der Milzgefässe, die neuerdings von der «offenen» Theorie überschattet scheint.

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1 F. TISCHENDORF, in *Handbuch der Mikroskopischen Anatomie des Menschen* (Eds. W. v. MÖLLENDORFF and W. BARGMANN; Springer-Verlag, Berlin, Heidelberg, New York 1969), vol. VI/6, p. 575.
2 F. WEINDENREICH, Arch. mikrosk. Anat. EntwMech. *58*, 247 (1901).
3 K. HELLY, Arch. mikrosk. Anat. EntwMech. *59*, 93 (1902).
4 S. E. BJÖRKMAN, Acta med. scand., Suppl. *128*, 191, 1 (1947).
5 M. H. KNISELY, Anat. Rec. *65*, 23 (1936).
6 J. S. BURKE and G. T. SIMON, Am. J. Path. *58*, 127 (1970), L. T. CHEN and L. WEISS, Am. J. Anat. *134*, 425 (1972).
7 T. MURAKAMI, Arch. histol. jap. *32*, 445 (1971).
8 H. M. PECK and N. L. HOERR, Anat. Rec. *109*, 447 (1951).