COGITATIONES

Tangential Wall Stress as a Molding Force in the Development of Collateral Vessels in the Canine Heart

In 1893 THOMA[1] stated that the velocity of the blood stream inside a blood vessel determines its diameter and that the intravascular pressure, and hence the tangential wall stress determines the thickness of the wall of a blood vessel.

This statement, however, covers only the mechanical forces which may act on the walls of a blood vessel and THOMA's thesis should be completed by adding nervous, chemical and hereditary factors (LIEBOW[2]). Since 1893 the velocity of the blood stream inside a vessel has been adopted by several scientists as being the primary determinant of the development of collateral vessels in the peripheral circulation and in the heart.

Our own studies on the mechanical forces which may influence or even determine the development of arterial collateral vessels have shown that the tangential wall stress should be considered as the primary 'molding force' in the development of collateral vessels in the canine heart. It is the purpose of this communication to give a preliminary survey of the arguments in favour of the wall stress theory, which have arisen from our experimental work.

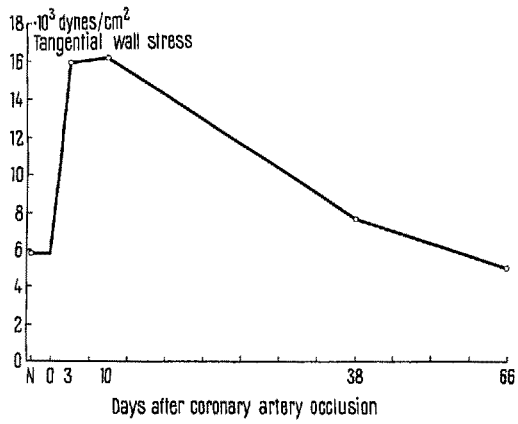
Methodology. Ameroid constrictors[3] were implanted during an aseptic operation around the circumflex branch of the left coronary artery in adult mongrel dogs of both sexes with an average body weight of 18 kg. At variable time intervals after the implantation (1, 2, 3, 4, 8 and 12 weeks) the dogs were again anaesthetized, the thorax was opened and the heart was excised. Warm barium-gelatin-suspension (50 °C) was injected into the anterior descendens artery under a controlled pressure of 100 mm Hg during 1 min. The white barium-suspension made the subepicardial vessels visible. These vessels were excised, fixed, embedded in paraffin and stained according to well accepted histological techniques.

Results and discussion. Since the pressure inside the blood vessel was known (100 mm Hg) and the fixation induced only minimal shrinking the tension acting on the vessel wall could be calculated from the vessel radius and the wall thickness, which had been measured using a calibrated projecting microscope. Tangential wall stress was calculated according to the law of LAPLACE-FRANK[4]

$$T = \frac{P \cdot R}{\delta}$$

where T means tangential wall stress (dynes/cm²), P = intravascular pressure (dynes/cm²), R = radius (cm) and δ = wall thickness (cm). Our calculations show (see Figure and Table) that in the early phases of collateral enlargement the tangential wall stress (= tension) rises considerably. The only way to counteract the increased wall tension is the increase in wall thickness. An increase in wall thickness in a maximally dilated vessel is possible only by cellular proliferation. Mitosis of the smooth muscle cells has frequently been observed by us and the increase in the vessel wall volume as a function of stimulus and time was considerable. Our new working hypothesis on the dominant mechanical influence on the development of collateral vessels would proceed as follows: Hypoxic vasodilation undoubtedly increases the tangential wall stress of small interconnecting arterioles located in the border-zone between a well perfused and a poorly perfused area. The increased wall stress is considered as triggering smooth muscle cell mitosis. Since in the early stages of the vessel wall transformation the hypoxia still persists, all newly added cellular material is used to make a further dilatation possible. That means: the vessel grows in radial direction, the wall being very thin and the wall tension still mounting. Once the vascular diameter has reached dimensions which permit adequate tissue perfusion, the hypoxia diminishes and with it the stimulus to dilation and radial growth (= end of phase 1).

Since the wall at this moment is very thin, tension is still very high and cell proliferation continues. But now this new cellular material is used to stabilize the vessel wall and the wall thickness increases again, until normal dimensions have been reached. At this moment tangential wall tension is normal again (= end of phase 2).



The time-course of the tangential wall stress of collateral vessels. Normal small coronary arteries of comparable size (N) are compared with collateral arteries at different stages of the vessel wall transformation. For the significance of differences see Table. O refers to the estimated moment of coronary artery occlusion.

Levels of significance of differences (p)

	3 days	10 days	38 days	66 days
N	3 < 0.001	6 0.001–0.01	9 0.50 –0.70	7 0.01 –0.02
3 days		10 0.80 –0.90	4 0.001–0.01	1 < 0.001
10 days			8 0.01 –0.02	2 < 0.001
38 days				5 0.001 0.01

[1] R. THOMA, *Untersuchungen über die Histogenese und Histomechanik des Gefäßsystems* (Enke, Stuttgart 1893).

[2] A. A. LIEBOW, *Situations which lead to changes in vascular pattern. Handbook of Physiology* (American Physiological Society, Washington, D.C., 1963), Section II, vol. II, p. 1251.

[3] J. LITVAK, L. E. SIDERIDES and A. M. VINEBERG, Am. Heart J. *53*, 505 (1957).

[4] O. FRANK, Z. Biol. *71*, 253 (1920).

This new working hypothesis has several important advantages: it explains why and when the vessel starts to grow and it explains also why and when it stops growing. Furthermore, the changes described above are in accordance with histological findings and the tangential wall stress is, under certain circumstances, a well measurable entity.

It has been shown above that the tangential wall stress can be a determining factor for both the diameter and the thickness of the wall of a blood vessel, when a chemical factor (hypoxia) forces the blood vessel to dilate.

Zusammenfassung. Die Entwicklung eines Kollateralkreislaufs wurde am Hundeherzen nach langsam erfolgendem Verschluss einer Koronararterie untersucht. An den unter Druck fixierten und histologisch aufgearbeiteten Kollateralgefässen wurde die tangentiale Wandspannung berechnet.

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PRO EXPERIMENTIS

The Preparation of Metabolically Active Suspensions of Mouse Liver Cells

The isolation of mouse liver cells has been reported by BRANSTER and MORTON[1] and by BERRY[2], the latter using the method of free cell preparation published by BRANSTER and MORTON[1]. This method involves a preliminary perfusion of the liver, in situ, followed by homogenization using a polyethylene and glass homogenizer, and finally successive filtrations through a series of filters of decreasing mesh. We wished to investigate some aspects of the nucleic acid and protein metabolism of mouse liver cell suspensions since they appeared to provide several advantages for in vitro experiments with intact mammalian cells. In the course of our initial work in the isolation of free metabolically active mouse liver cells we employed the method of BRANSTER and MORTON, as well as a number of other methods already available for the preparation of suspensions of rat liver cells[3-6]. We found that all of these methods proved to be injurious to a large proportion of the mouse hepatic tissue resulting in low yields of intact cells.

The mouse liver cells were found to be very fragile and highly susceptible to breakage during the dispersion step of the preparation, particularly when any of a number of different types of homogenizers was employed. We have developed a new method for the dispersion of liver cells which minimizes the physical treatment to the suspended cells once they are freed from the tissue.

An adult CFW mouse, fed ad libitum, is placed under ether anesthesia and the liver is perfused, in situ, via the portal vein with 15–20 ml of calcium-free Locke's solution (CFL) at room temperature at pH 7.3. This perfusion is accomplished by using a 20 ml syringe connected to a No. 24 needle shaft by a 12 inch length of polyethylene tubing. The flexible tubing aids considerably by preventing the accidental removal of the needle from the portal vein during the perfusion. The perfusion is carried out rather slowly ($1^1/_2$–2 min to completion). Sufficient pressure is maintained by means of the syringe to distend the liver. After the first few ml of fluid are administered and the liver is distended visibly, the inferior vena cava is cut to release the pressure and allow drainage. The cut is then clamped with a hemostat and the process is continued with repeated steps of pressure and release to allow adequate perfusion and complete flushing of blood from the liver.

Immediately after perfusion the gall bladder is removed and the liver excised and rinsed twice in CFL at room temperature. To provide a sufficient number of cells for metabolic studies a second mouse liver is prepared in the same manner as the above. The combined livers are rapidly weighed and then cut with fine scissors into pieces of approximately (3 mm^3) size. The tissue is then dispersed very gently by pressing it with a teflon pestle

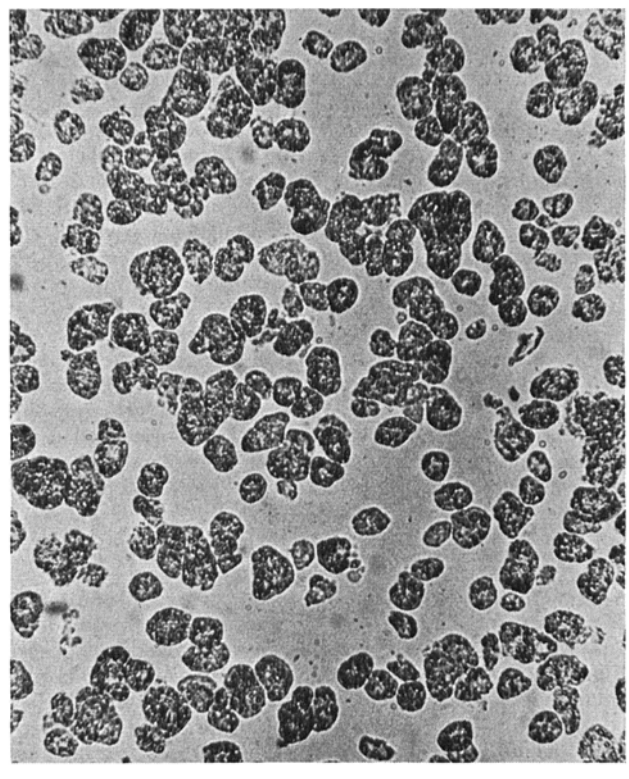

Fig. 1. Photomicrograph of mouse liver cells in suspension (× 200).

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