then placed on the muscle and covered by the fascia, which is closed loosely by catgut ligatures. The skin is sutured with nylon thread leaving only the metallic needle head above the skin surface (Fig. 3). The catheter is filled with sterile saline and the valve opening covered with a polyethylene cap.

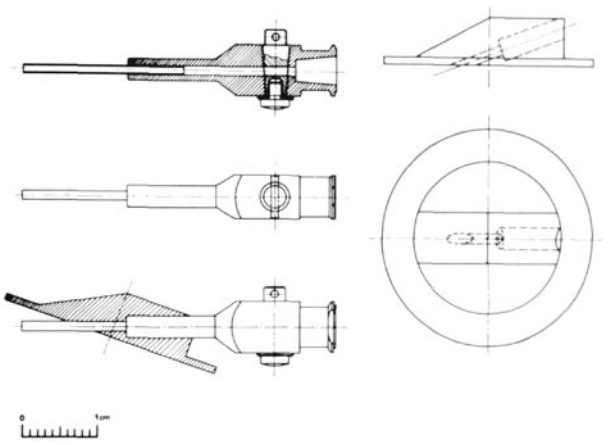


Fig. 2. Working draft of the syringe needle with stopcock valve and of the polyamide ring with crossbar.

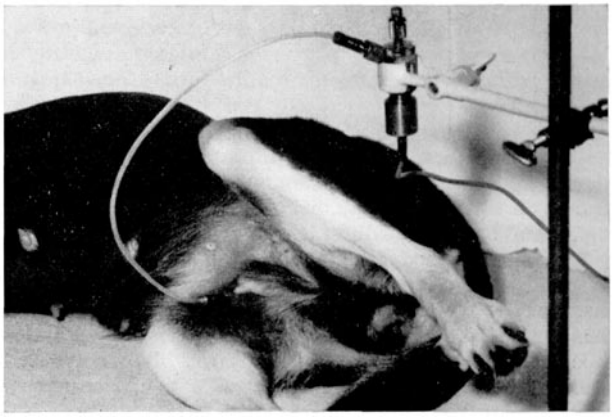


Fig. 3. Unanaesthetized dog with implanted aortic catheter during recording of aortic pressure pulses. This photograph was taken 10 months after implantation of the catheter.

The length of the catheter may be chosen at will. For our hemodynamic studies we found it most suitable that the tip of the catheter should lie between the origin of the renal arteries and the aortic bifurcation. During the first few days penicillin and streptomycin are given routinely. As a rule the wound heals within two weeks and thereafter the animal is ready for experimental study.

Postoperative procedures. Blood clotting presents no problem. The catheter is flushed with saline at the beginning and end of each experimental period. To open and close the valve we use a special key (Figure 1).

Apart from a protective bandage no special care is necessary. Infection, either local or general, has not occurred although connecting the catheter to the recording apparatus is not done under strictly aseptic conditions. As of now we have prepared over 20 dogs with the described procedure and used them in circulatory studies for many months. One of the dogs in current use has had a functioning catheter for more than one year. Death, when it occurs, is usually caused by perforation of the aorta due to chronic traumatization of the aortic wall by the tip of the catheter. Autopsy shows obliteration of the lumen of the cannulated femoral artery. The aortic part of the catheter is often found to be incorporated into the vessel wall, only the last few inches lying free in the lumen. Embolic episodes during life were not recognized in contrast to earlier experiments in which catheters were implanted in the carotid artery.

Applicability. We have used this method of aortic catheter implantation for repeated measurements of aortic pressure pulses as well as for the determination of cardiac output with the dye-dilution method. For the latter purpose aspiration and reinfusion of the arterial blood was performed under strict aseptic conditions. The dye was injected by a second catheter of the same type implanted into the right jugular vein, the tip lying in the right atrium.

Zusammenfassung. Es wird die Herstellung und Implantation von Aortenkathetern beim Hund beschrieben. Die Methode erlaubt wiederholte, kontinuierliche blutige Messung des Aortendrucks sowie die Bestimmung des Herzminutenvolumens am wachen Tier.

H. Thoenen, A. Hürlimann, and W. Haefely

Abteilung für experimentelle Medizin, F. Hoffmann-La Roche & Co., AG, Basel (Switzerland),
December 14, 1964.

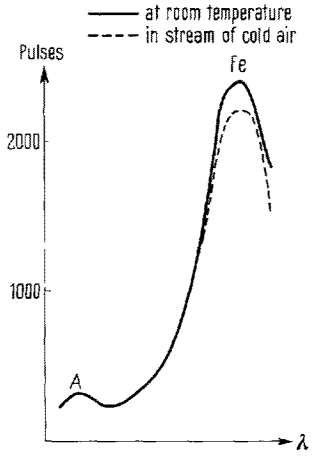
Quantitative Measurements of Microscopic Changes in the Vascular Bed

A method for measuring microscopic changes in vascularity could find many research applications, as standard methods are not accurate enough. Quantitative X-ray fluorescence microanalysis has made it possible to measure vascularity changes in vivo within areas 50–750 μ across. The method is applicable to surface areas or membranes.

A focused X-ray beam from an X-ray microscope is passed through an aperture system consisting of two molybdenum electron microscope apertures. The aperture close to the X-ray source has the smallest diameter. The other aperture serves as a scatter trap and defines the size of the area analysed. The specimen is placed on top of this aperture. X-ray fluorescence is generated in the specimen and radiates in all directions. A sector of this radiation is then analysed by a proportional counter and a multichannel pulse height analyser. This system can discriminate between the different wavelengths of the fluorescent radiation. On an oscilloscope, a curve is seen in which each peak is due to one element and its height is proportional to the quantity in the specimen. The

method can be used to determine amounts as small as 10^{-10} g.

Changes in the vascular bed in the web of a frog's feet in vivo in areas 200 μ across, have been examined by determining changes in iron present in different conditions. The total amount of iron in the area analysed is measured for a period of 1/2 min. Most of the iron analysed is due to the hemoglobin present in the erythrocytes, and variations in the serum iron are relatively unimportant.



The experiments are performed in air with the specimen fixed in relation to the proportional counter. A peak representing argon in the air is used as a reference. Expression of the results in ratios Fe/A makes them independent of variations in intensity of the primary beam. If a very small drop of iron solution of known concentration and volume is analysed, the results can be expressed in g per analysed area (LONG and RÖCKERT[1]). The curve shown (Figure) illustrates the change within a circular area of 200 μ diameter measured at room temperature and during exposure to a stream of cold air.

Résumé. Une méthode d'analyse quantitative du fer dans l'hémoglobine a été développée, utilisant la fluorescence aux rayons X. Elle est appliquée à des tissus vivants pour l'enregistrement de changements très faibles dans la circulation sanguine dans des fragments microscopiques.

H. RÖCKERT

Department of Histology, University of Göteborg (Sweden), September 4, 1964.

[1] J. V. P. LONG and H. O. E. RÖCKERT, Third Internat. Symp. X-ray Optics and X-ray Microanalysis, Stanford 1962 (Academic Press, 1963).

A Novel Mass Culture of *Entamoeba histolytica* on Nutrient Agar

BOECK and DRBOHLAV[1] in 1925 accidentally discovered pathogenic amoebae in a flagellate culture from infected stool material. The culture medium consisted of a solid slant with a liquid overlay. Completely liquid culture media were then introduced and later an essentially synthetic medium by HANSEN and ANDERSON[2].

In 1958 a hartmannellid (?) free-living amoeba macroscopically contaminating a blood-agar culture of *Staphylococcus albus* was isolated by the author. This amoeba, subsequently re-isolated on several occasions from human stool material, proved to be of great value in the assay of amoebicides[3]. The present work – an offspring of the work of 1958 and the following years – witnesses the entry of *Entamoeba histolytica* into a new age, the age of solid media.

Methods. Sterile nutrient agar containing 0.2% dextrose and 0.7% table salt is used throughout. Plates or slants are overlaid with an egg white-agar-rice powder-amoeba-blood mixture, prepared as follows: Sterile egg white is warmed to 55–60°C in a water bath and 7 cm³ of melted nutrient agar, cooled to about 60°C, is added to 30 cm³ of the warm egg white and well homogenized with it. 6–8 g of sterile rice powder are then added and well dispersed with the aid of sterile glass beads. A boiled saline suspension of the free-living amoeba mentioned above, obtained from 10–15 Petri dish cultures, is added in addition to 5–10% defibrinated human blood. Nutrient agar previously prepared in the Petri dishes or tubes with a homogeneous mixture is overlaid. Tubes are put in a slanting position. In a few moments the overlay will not trickle on tilting the plate or holding the tube in the upright position. The medium is now ready for use.

Mucus and blood from four cases of acute amoebic dysentery were inoculated in the centre of the plate medium. Two more cases of dysentery containing *Trichomonas hominis* and pus cells but no amoebae were also inoculated in the centre of the plates. The inoculated media were immediately incubated at 37°C under anaerobic conditions using alkaline pyrogallol as an oxygen absorbing agent. Subcultures were made every 48–72 h.

Results. Entamoeba histolytica, morphologically indistinguishable from amoebae found in acute dysentery, grew luxuriantly in four out of four cases of acute amoebic dysentery. *Trichomonas hominis* grew in clusters in two out of two stools infected with this flagellate.

Résumé. Une nouvelle méthode de culture de *Entamoeba histolytica* et *Trichomonas hominis* sur plaques d'agar est employée ici avec succès.

K. A. YOUSSEF[4]

Ministry of Health, Cairo (Egypt), July 27, 1964.

[1] W. C. BOECK and J. DRBOHLAV, Am. J. Hyg. *5*, 371 (1925).
[2] E. L. HANSEN and H. H. ANDERSON, Parasitology *39*, 69 (1948).
[3] K. A. YOUSSEF, Exper. *20*, 463 (1964).
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