tinely determined using the Biuret method. In conducting the experiment 6 animals were used for each time period and F-test analysis of variance was used to test for significance.

Results. All of the rats given 25 IU PMS on the 24th day of age ovulated during the night of the 26th. At 12.00 h on the 26th day, rats given PMS showed full uterine ballooning. At 24.00 h there was vaginal opening and ovulation as indicated by tubal ova. Although tubal ova were noted, no attempt was made to count them.

The specific activity of the enzyme (LDH units/mg protein) is shown in Figure 1. There was no significant difference ($p < 0.05$) between the 4 consecutive hours of 6, 12, 18 and 24, but there was an abrupt increase in the activity at 30 and 36 h (1,823 $\pm$ 287 and 1,168 $\pm$ 253 versus 172 $\pm$ 108 at 0 h; $p < 0.01$). The activity started to decline again at 42 h. The activity observed at 48 h was not significantly different ($p < 0.05$) from that observed at 48, 54, 60, 66 or 72 h. It is also clear from this Figure that there were no significant changes in LDH activity in the control.

The LDH activity (LDH units/ml) in the plasma of animals injected with PMS compared with animals which were not treated is presented in Figure 2. It is clear from this figure that there was an increase in LDH activity of blood at 36 h. This increase was significantly higher ($p < 0.01$) than the activity at the other hours (298 $\pm$ 46.7 versus 121 $\pm$ 18.5 at 0 h). Variations among the control show no significant variations along the period of the study.

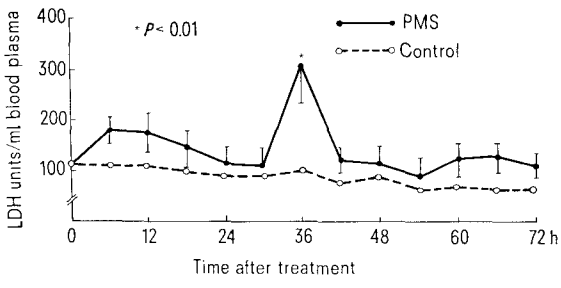

Fig. 2. Effect of 25 IU of PMS injected in the immature rat (24 days old) on blood LDH activity. Each point represents 6 animals and the standards errors are shown by the vertical lines.

Discussion. Studies on the molecular mechanisms of hormone action have indicated two general patterns. One pattern applies to the polypeptide hormones which act primarily through the activation of adenyl cyclase with the subsequent formation of cyclic AMP[5], and the second pattern applies to the steroid hormones which act primarily at the gene locus to initiate the synthesis of specific species of RNA[6]. The hormonal stimulation of LDH is not so easily delineated, but a conclusion might be drawn from the analogue results on estrogen level.

Recent studies indicated that estradiol levels reach maximum at 42–52 h after PMS injection in the immature rat and then decrease dramatically[7]. It is possible that lower levels of estrogen are able to induce LDH activity to its maximum, around 36 h. The direct effect of PMS in inducing LDH activity is unlikely. Human chorionic gonadotropin administration to the immature rats treated with PMS increased the amount of CAMP formed several fold in less than 1 h[8], which, if it were the mediator, should increase LDH activity immediately. Moreover, estrogen is known to act to induce mRNA for the formation of different enzymes[6].

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PRO EXPERIMENTIS

The Micro-Focal X-Ray Unit and its Application to Bio-Medical Research

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Summary. The micro-focal X-ray unit is a modified Cosslett and Nixon X-ray microscope of greater operational stability and flexibility. Its combination with a closed circuit television system provides a quick method of obtaining a point source of X-rays to examine the detailed structure of organs and biological specimens.

The term X-ray microscope has been used to describe apparatus which uses one or two electron lenses to focus an electron beam onto a target, from which the X-rays are emitted. The principles of this form of apparatus were first described in 1939[2,3]. The apparatus was developed by Cosslett and Nixon in the 1950's and early 1960's[4–8]. Since that time the machine has been used to study a wide variety of biological tissues[9]. However, the material examined has been restricted to small specimens; such as insects[4] or to specimens of a few millimetres thick, or thin sections[10]. This restricted application of the machine is due to the limitations in its desgin.

The micro-focal X-ray unit described herein is a modified X-ray microscope which has been designed to overcome the limitations of the Cosslett and Nixon machine. Also the application of the machine to bio-medical research has been increased by the incorporation of a closed circuit television system.

[1] Acknowledgments. This work has only been possible through the kindness and personal generosity of R. V. Ely. I wish to thank Mr J. Ralph and Mr D. Haig of Eltron International for their help with the C. C. T. V. system and Mr K. Fitzpatrick for preparing the photographs.

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One of the major limitations of the Cosslett and Nixon machine was that the foil target was mounted on top of the objective lense, which also formed the end window of the electron beam. The vulnerability of the foil to perforation through overheating required the accelerating voltages to be restricted to 30 kV or below. In the present unit the transmission target is replaced by a variable solid target unit[11]. This comprises a series of rings of different elements (W, Cu, Cr, Al) mounted on a water or oil cooled spindle inserted through the side of the target unit. The selection of any target element can be made while the equipment is in operation. The target assembly is rotatable so as to present a multiplicity of surfaces and so avoid pitting and contamination. The electron beam strikes the target at about 45° to its surface, the X-rays are emitted at right angles to the beam through a thin aluminium window (Figure 1). This arrangement provides greater stability and permits the operation of the machine at higher accelerating voltages than were possible with the Cosslett and Nixon machine.

The seond major defect of the Cosslett and Nixon machine was that the electron gun was of the 'hot' type, that is the outer casing of the gun was under tension during operation of the apparatus. Because of this, the position of the tungsten filament in the gun could not be altered for purposes of aligning the electron beam within the column, while the apparatus was operational. This has been overcome by replacing the electron gun with one of 'shock-proof' design[9], in which the outer casing is insulated from the filament mounting. This enables the filament to be moved while the tube is in operation and the optimum vertical position of the filament to be selected. As the gun can be moved in the horizontal plane by adjusting screws, the correct positioning of the filament in the horizontal axis can be acheived, thereby removing the necessity, present in the Cosslett and Nixon machine, of 'lining up' the anode plate and the electromagnetic lenses with the electron beam.

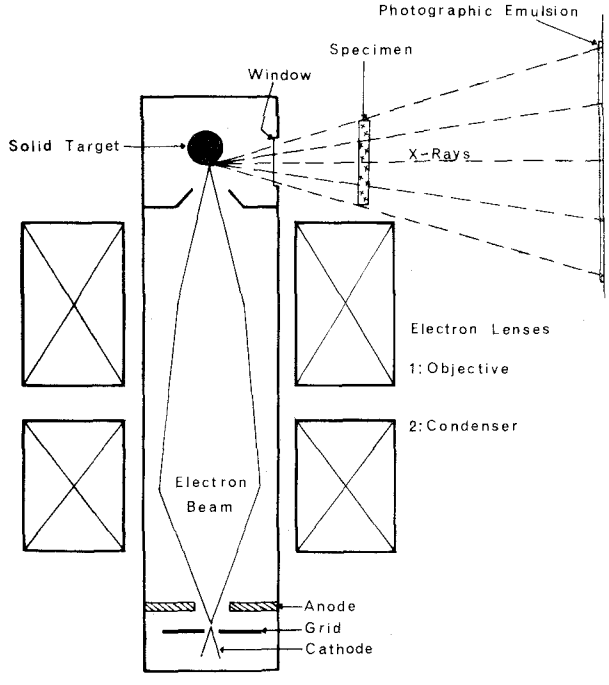

Fig. 1. Diagram of the micro-focal X-ray unit. The electron lenses form a reduced image of the cathode at the target; the X-rays emitted project an enlarged image of the specimen onto the photographic plate or screen.

The above modifications limit the estimated diameter of the X-ray source to the order of 3–5 μm and 5–10 μm for the equipment operating at voltage ranges of 5–30 kV and 30–50 kV respectively. As the spacial resolution within the X-ray beam corresponds to the size of the source, it follows that there is a reduction in the resolving power of the X-ray unit proportional to the increase in the accelerating voltages applied. The method of column alignement, formerly a tedious procedure in which the electron beam was focussed either onto a fluorescent or aluminised screen, has been considerably facilitated through the introduction of closed circuit television. The television camera comprises a fibre optic scintillation coated high resolution vidicon tube. The X-ray focus is determined by positioning a grid holder containing fine meshes (32, 100, 200, 300 and 400/cm) in front of the target. As the target is at earth potential, the meshes are placed at 5 mm distance from the focal spot. The shadow image cast by the mesh onto the scintillation screen of the vidicon is viewed on a monitor and the necessary adjustments to the lens focussing controls can be made to focus the electron beam. Figure 2a shows the 32, 200, 300 and 400/cm meshes photographed from the television monitor at a final magnification of 530 obtained by placing the port of the vidicon 12 cm away from the target thereby providing an initial magnification of 25. This is obtained from the ratio of the source to image and source to object distances, the initial magnification is then increased by a factor of 22 produced electronically by the television system. Figure 2b shows the same meshes recorded on a moderately fine grained X-ray film placed in the X-ray beam so as to obtain an initial magnification of 60. Both these recordings were made with the X-ray apparatus at an acceleration voltage of 20 kV and a current of 0.5 mA. The 6 and 12 μm bars of the 400 and 200/cm meshes respectively are visible on the monitor and radiograph, indicating that at an acceleration voltage of 20 kV the micro-focal X-ray unit has a special resolution of at least 6 μm. The lack of clarity of the 400/cm mesh in Figure 2a is not representative of the actual image on the monitor; the poor resolution is a function of the distortion associated with photographing the image from the curved surface of the cathode tube as well as the limitations of the photographic technique.

This type of vidicon represents an advance over other forms of X-ray sensitive vidicon in that it can operate at much lower excitation levels. This is due to the fibre optic disc being coated with a recently developed rare earth phosphor (type P 43; Eltron International, U.K.). The excitation level of the phosphor is 0.1 kV at a wavelength of 1.2 Å[12]. The use of the scintillation coated vidicon with the micro-focal X-ray unit provides a means of focussing the equipment from voltages as low as 10 kV, when the port of the vidicon is placed at a distance of 12 cm from the X-ray source.

With the micro-focal X-ray unit and television system, it is possible to examine a wide range of biological material by interposing the specimen between the ports of the variable solid target unit and the vidicon, at a target to specimen distance that gives the required initial magnification. The shadow image produced is recorded either on magnetic tape by connecting the television camera to a suitable video-tape recorder, or on X-ray film by re-

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placing the camera with a plate holder and film. As both specimen and recording surface are open to air, this makes the selection for examination of a region of special interest in the specimen much easier, as well as enabling the specimen to be tilted or translated between exposures, to obtain stereo-pair radiographs for stereoscopic viewing. The spacial separation within the different specimen layers produced by three dimensional radiography, combined with the virtually unlimited depth of focus allows the machine to be used to ascertain the relative position of the structural elements in an opaque object without destruction. With a large primary magnification fine grained emulsions are no longer required, and the use of relatively cheaper, coarse grained X-ray films reduces the length of exposure time from several minutes to several seconds.

In recent studies the apparatus has been used for the detailed examination of the structural organisation of bone[13–15] and tissue blood supply. In both these fields of study the micro-focal X-ray unit has definite advantages over the technique of light microscopy. The penetration and depth of focus obtained in the stereo-projection microradiographs permits a complete three dimensional examination of the internal structures or vascular detail of the organs at high and low magnifications. Because this technique is non destructive organs and tissues can be used subsequently for histology. Nevertheless, the results of projection microradiography are generally sufficiently precise to obviate wax-plate reconstructions. From the stereo-projection microradiographs the trabecular density in bone and the volume patterns of blood vessels within the whole or part of the organ, e.g., the glomerular density within the kidney, can be measured. An illustration of the results obtained from one of the microangiographic studies is illustrated in Figure 3, recorded with the X-ray machine operating at 20 kV and 0.5 mA. Figure 3a is a projection microangiograph of part of the rat ureter, and Figure 3b shows approximately the same region of the specimen as visualized on the television monitor. Two arteries can be seen running down on either side of the ureter, branches of which supply it; other branches supply the surrounding adipose tissue in which lies a third artery to the left of the ureter. With radiographs of this type, illustrated in Figure 3a, vessels of major calibre can be traced to capillary level. Although the smallest vessels visible on the television monitor are 35 μm in diameter, in the radiographic plates the smallest

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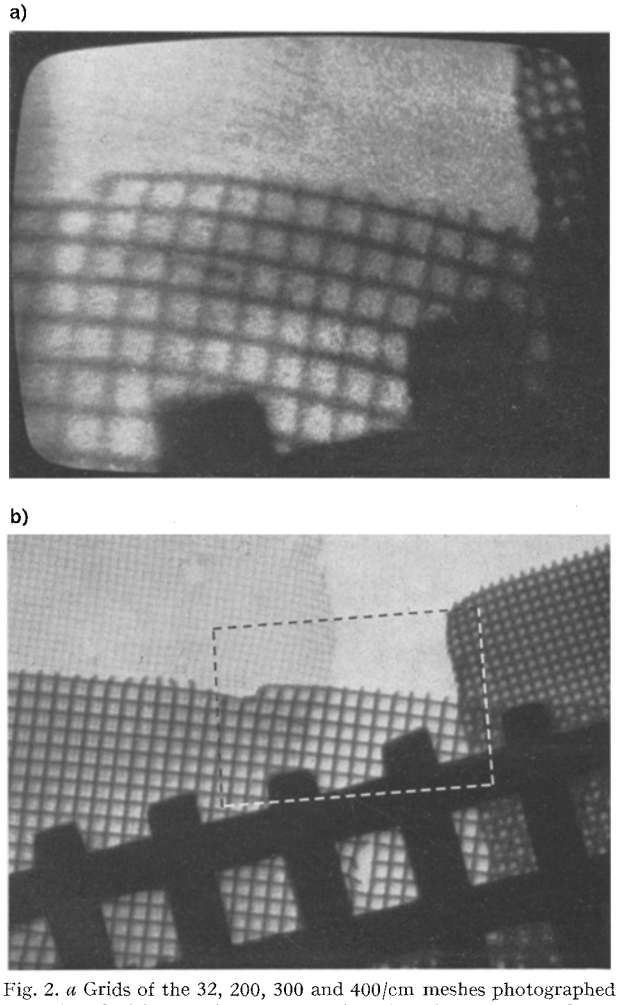

Fig. 2. *a* Grids of the 32, 200, 300 and 400/cm meshes photographed from the television monitor at a magnification of × 530; reproduced at × 240. *b* A print from the projection microradiograph of the same grids; the area demarcated is the same viewed by the television camera. Initial magnification × 60; reproduced at × 105.

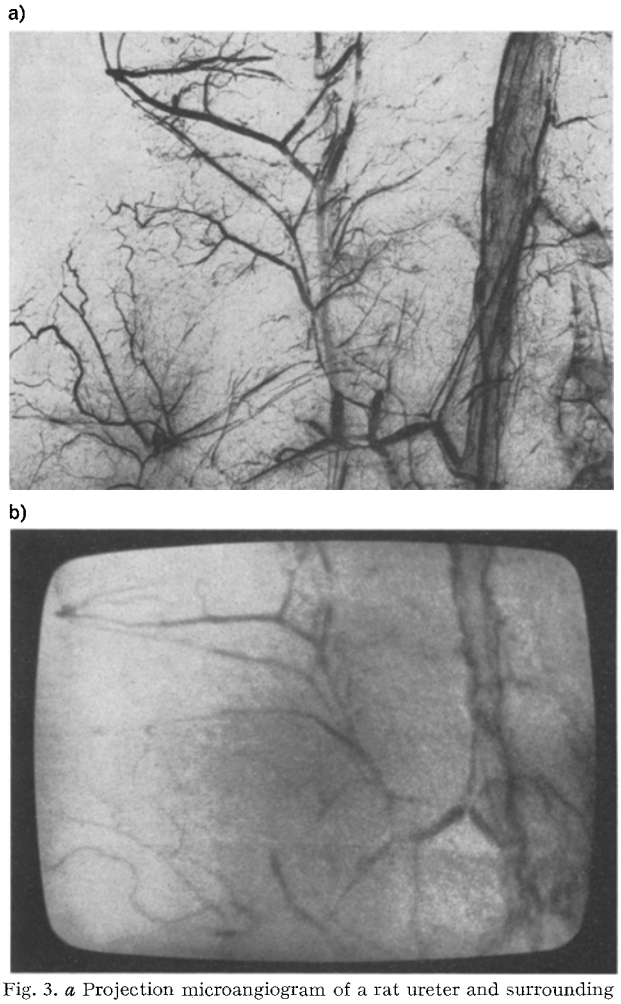

Fig. 3. *a* Projection microangiogram of a rat ureter and surrounding adipose tissue. Initial magnification × 13; reproduced at × 12. *b* Approximately the same region of the rat ureter photographed from the television monitor. Initial magnification × 26; reproduced at × 13.5.

vessels that can be demonstrated are 5 μm in diameter.

At present the equipment is being developed for in vivo dynamic recording of blood flow patterns through a variety of organs such as bone, kidney, intestine and skin. Previously this type of radiographic study has only been possible with high voltage X-ray generators and fine grained photographic emulsions[16]. The micro-focal X-ray unit allows fine structural detail to be recorded on relatively coarser grained emulsions and has the additional advantage that it produces less radiation damage to the tissues.

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An Inexpensive Cuvet for Spectrophotometry of Samples in 5 μl Volumes

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Summary. A simple cuvet requiring about 5 μl of sample is described. Such a cuvet is easy to build and suitable for measurements in ordinary spectrophotometers. The measurements compare favourably in accuracy and reproducibility with those obtainable with standard macrocuvets.

The routine control of the quality and quantity of macromolecular preparations from biological samples quite often implies spectrophotometrical determinations.

In particular, this applies to ribonucleic acid, phenol extracted from tissue or cultured cells, which routinely is tested for purity by its UV-spectrum. Furthermore the RNA, if sufficiently pure, is evaluated quantitatively in terms of its optical density units at 260 nm. In those preparations where the RNA is further processed, e.g. fractionated by electrophoresis, these controls are usually performed by taking an aliquot of the final RNA solution and reading it after appropriate dilution in standard quartz cuvet usually requiring from 0.10 ml of sample (e.g. Zeiss MT 2).

In some cases, and particularly often in neurochemistry, one must deal with tissue samples in the milligram range resulting after extraction in a few microliters of RNA, at a concentration suitable for subsequent electrophoretic analysis[2,3]. In these cases, even with the use of commercially available but expensive micro-cuvets ranging down to working volumes of 20 μl (e.g. Zeiss MR 1 D), the UV-determinations mentioned above are hardly possible to perform.

In this report we describe how to build simply an inexpensive cuvet suitable for UV-spectrophotometry of samples in the range of 5 μl in the ordinary Zeiss PMQ II or PMQ 3 spectrophotometers.

The cuvet system consists of the cuvet proper and a brass casing, both being easily produced in the workshop of the laboratory (Figure 1). The cuvet proper is made from commercially available bored glass tubings (Chance Brothers Ltd Glassworks, Birmingham, England), which are cut at lengths slightly above the desired optical path-length. The cut ends are ground and polished to exact dimensions in a lathe on which a grinding disc is mounted to the chuck[4]. A small piece of quartz glass (manually cut) is glued onto one of the polished ends (low viscosity cyanoacrylate adhesive IS 03 Locktite, Dublin, Ireland). The brass casing consists of a socket in which the cuvet proper is placed and a screw on which another small quartz glass is fitted. This quartz glass forms the other window of the cuvet when socket and screw are joined. To compensate for possible errors in parallelism between the flat end of the screw and the polished end of the cuvet proper, a rubber packing ring is fitted between the screw and the quartz glass. Tightness of the cuvet is thus ensured.

Filling the cuvet is suitably made with the cuvet in vertical position, either within or outside the socket, by

Accuracy of readings at 260 nm in the MT 2 and in the microcuvet on repeated fillings (12 times) of the same RNA solution as in Figure 1, but diluted 1:1

Cuvet	Mean absorbance	SD
MT 2	0.530	0.0016
Microcuvet	0.263	0.0026

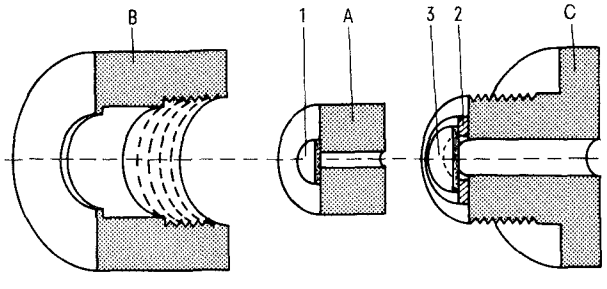

Fig. 1. Sectioned and exploded view of the cuvet and casing. A) Cuvet proper constituting a hollow conventional glass cylinder sealed at one end with a quartz glass [1]. External diameter 6.5 mm, internal diameter 1.0 mm, length 5.00 mm. B) Cuvet socket in brass with threading for knurled screw (C). External diameter 12 mm, internal diameter snuggly fitting the cuvet (A). Length 10 mm. The depth of the non-threaded part of the socket is smaller than the length of the cuvet proper so that the quartz glass of the knurled screw gets in touch with the cuvet when joined. C) Hollow knurled screw (bore appr. 2 mm in diameter) with glued rubber packing ring [2] onto which a quartz glass [3] is fitted.

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