possibly on thymus-derived lymphoid cells has not been reported in the literature. We concur with the aforementioned view that senescence should be regarded as a kind of protracted wasting disease but we assume that in many wasting syndromes the primary cause for the progressive diminution of the available pool of hormone-dependent, multifunctional thymus-derived lymphocytes lies in the endocrine system. Examples in support of this view are the different wasting or runting syndromes, the hormonally deficient short-lived hypopituitary dwarf mice whose life can be considerably prolonged by either hormone treatment or by injection of lymph node lymphocytes[15], and the short-lived hormone-deficient thymusless 'nude' mice[7,8]. The progressive deterioriation of this hormone-lymphoid cells relationship seems eventually to be facilitated or accelerated by many secondary causes such as infection, stress, trauma and malnutrition.

Many of these accelerating causes of senescence might derive from premature destruction of hormone-sensitive thymocytes or thymus-derived lymphocytes by hormones such as gonadotrophins and adrenal or gonadal steroids. In fact, in many conditions of stress, both emotional and muscular, the levels of STH, steroids and other pituitary trophins vary greatly[75–81]. Therefore in seeking to unravel the complex process of ageing the hormonal factors regulating cells turnover in the thymus and in the thymus-derived tissues deserve special attention.

Zusammenfassung. Es wird die Hypothese vorgeschlagen, dass Thymus-Lymphozyten (T-Zellen) neben ihren klassischen immunologischen Funktionen wichtige multifunktionelle homöostatische Kontrollfunktionen ausüben. Störungen in der Beziehung zwischen dem endokrinen System und Thymus führen zu Ausfallerscheinungen und Altern.

W. Pierpaoli and E. Sorkin[82]

Schweizerisches Forschungsinstitut, Medizinische Abteilung, CH–7270 Davos-Platz (Switzerland), 30 August 1972.

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PRO LABORATORIO

Automated Specimen Processing for Electron Microscopy: A New Apparatus

While automated specimen processing is available for paraffin embedding in light microscopy, plastic embedding for electron microscopy is still carried out manually in spite of the fact that electron microscopy is being increasingly applied to routine (e.g. diagnostic) work and that plastic embedding is increasingly used also for light microscopy employing 0.1–1 micron-thick sections. Among the problems in automating plastic embedding are: the great variety of procedures, the small size of the specimens, the requirements for cleanliness, precision and reproducibility of the procedure, the incompatibility of some chemicals with machine parts, the viscosity and pre-polymerization of the embedding resins, etc.

We now present a simple and compact apparatus which automatically dehydrates and impregnates up to 24 specimens simultaneously with up to 29 sequential changes of 10 different fluids.

Description. The main components of the apparatus (Figure 1) are: a removable support with reservoir flasks, silastic tubes closed by electromagnetically driven guillotine-type valves, a 100 ml polypropylene trough, a moving holder with 24 disposable specimen baskets (Figure 2), a 5 l container for disposed liquids, a programme-selecting panel with indication lights (Figure 3), and an electric unit with 4 switches: 'Main', 'Start', 'Step', and 'Reset'. The size of the apparatus (without flask support) is $31 \times 35 \times 40$ cm, and its weight is 20 kg.

Mode of operation. When 'Main' and 'Start' are pushed, the apparatus fills 100 ml fluid from any pre-selected flask into the trough, moves the specimen baskets up and down for a pre-selected time period, then stops the movement, empties the trough and switches over to the next step, which begins with refilling the trough from the same or another flask. Up to 29 such steps can be programmed by placing 2 pins for each step into the programme-selecting panel: 1 to determine the flask (A-K), and 1 to select the time period (0, 1, 2, 3, 5, 10, 15, 30, 45 or 60 min per step). If a time position is left without a pin, the apparatus operates on this position until the 'Step' switch is pushed. This enables the apparatus to operate on one position for a longer period without emptying the trough, e.g. overnight. Thereby, any evaporation of fluid is compensated by automatic refilling from the corresponding flask. In addition, the 'Step' switch can be pushed at any moment in order to immediately empty the trough and pass to the next step independently of the actual time setting, whereas with the 'Reset' switch the programme can be interrupted at any moment by proceeding directly to the initial zero position.

Special features. Self-cleaning of the apparatus is achieved after the programme is finished and the specimens are taken out by simply adding some more steps, whereby a resin solvent or another cleaner is to be filled into some of the flasks. Any of the common dehydrating fluids, resin solvents and resins which are compatible with silicone, teflon, polypropylene and stainless steel can be used, e.g. water, buffers, aldehydes, ethanol, acetone, propylene oxide, Epon, etc. The viscosity of the fluids may be as high as that of unpolymerized Epon resin and the viscous fluids can be stirred by magnetic stirrers which are placed under 2 of the flasks. Fluids are used only once and the specimen baskets are disposable.

The valves close by simply squeezing the silicone tubes (Figure 2). Therefore they cannot be blocked by viscous fluids.

The actual step in progress is indicated by a light (Figure 3), while, in addition, a counter records the completed steps. In case of a failure of the current this counter indicates the step at which the interruption took place.

The compartment which contains the trough and the valves is air-tight and separated from the electric unit. Thus highly inflammable fluids can be used without danger. The apparatus operates as well at 4 °C, e.g. in a cold room.

Results. A prototype of the apparatus has been in operation in our laboratory since January 1972. Comparative manual and automated processing yielded results which were indistinguishable. Moreover, using the advantage of automated longer dehydrating and especially impregnating times, the cutting quality of the blocks appeared to be even enhanced.

Postfixation with OsO_4-solution was completed prior to the automated processing in a separate trough which used relatively small amounts of this rather expensive and contaminating fluid (20 ml for 24 specimens). The subsequent automated processing consumed lower amounts of fluid than our usual processing.

Most striking was, however, the time saving in spite of the fact that postfixation with OsO_4 was done manually. Due to the simple and self-cleaning features of the apparatus, technicians were able to handle it after one single demonstration, and the time they spent for one complete process usually did not exceed 40 min.

Discussion. We have designed a simple apparatus which is able to reproduce exactly and reliably a wide range of different manual dehydrating and impregnating schedules, because the advantages gained by automating the tissue processing are manifold: high reproducibility, saving of time and material, relief of technicians from boring work, better impregnation due to continuous agitating, avoidance of contact with skin-sensitizing agents, cleanliness, etc. In spite of these advantages no apparatus for processing of electron microscopic specimens has been commercially available to date. Two machines with the same purpose have been described in 1967 by AIHARA et al.[1,2] and NORRIS et al.[3,4] respectively. The AIHARA machine closely resembles conventional tissue processors for light microscopy. It is programmed by a tape reader and dips up to 20 specimens sequentially into 20 different jars some of which can be heated, cooled and even evacuated. These special devices enable this rather big machine to perform especially sophisticated specimen processing, whereas our apparatus has fewer possibilities but is small, simple and easy to handle. The Norris machine uses a specimen holder agitated in a mixing chamber which can be filled from 8 different reservoirs and emptied totally or half-way, thus allowing to mix the actual amount of fluid with the newly added

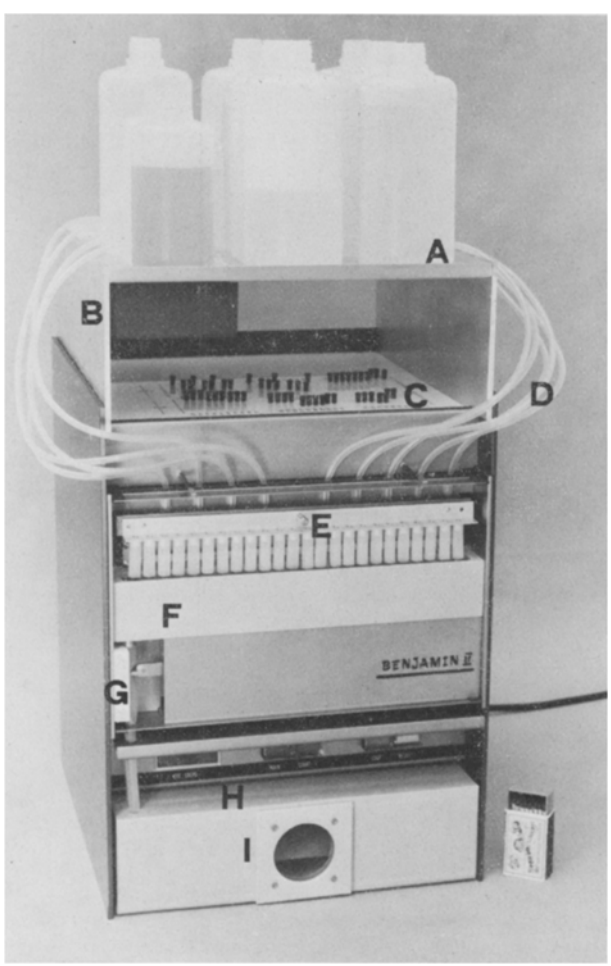

Fig. 1. General view of the apparatus, showing the main features: reservoir flasks and removable support (A), stirrer (B), programme-selecting panel (C), disposable tubes (D), holder with specimen baskets (E), trough (F), outlet valve (G), switches and counter for effected steps (H), container for disposed fluids with level indication (I).

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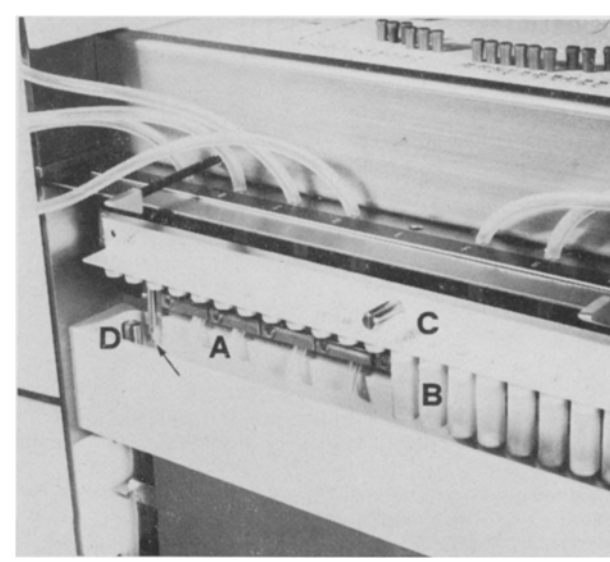

Fig. 2. Detail of the trough: guillotine valves with tubes (A), specimen baskets, made from teflon and with grids of stainless steel at the base (B), basket holder (C), fluid level control (→), overflow (D).

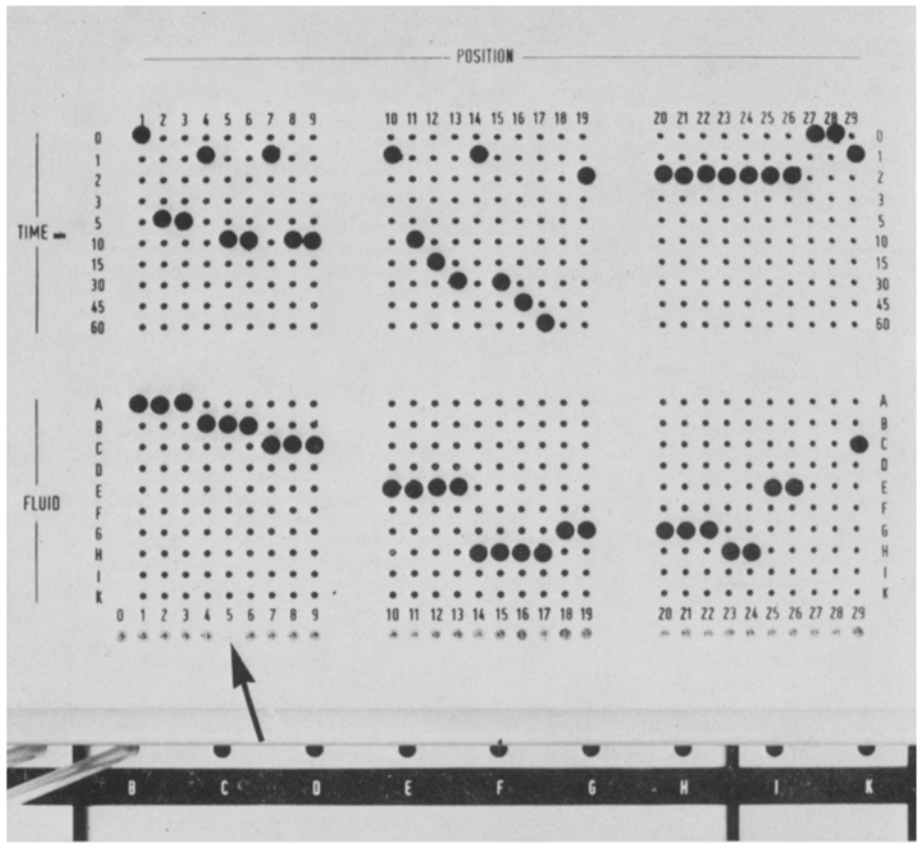

Fig. 3. Programme selecting panel. According to the programme selected in this illustration, the apparatus will perform the following steps: 1. step will be omitted due to time setting 0; 2 fluid A for 5 min; 3. fluid A (fresh) for 5 min; 4 fluid B for 1 minute (e.g. short rinsing); 5. fluid B for 5 min, etc. The light (→) indicates the actual step in progress (in this instance step No. 5). At step No. 18 the apparatus will remain operating (time positions without pin) until the switch 'Step' (not visible) is pushed in order to switch over to the following steps for self-cleaning.

fluid. Except for the mixing procedure, our apparatus operates on a similar principle. However, it has some different features: specimen baskets and tubes are disposable, containers of any size can be used as reservoirs, there is no need of pumps, and the reproducibility of the programmes is achieved by fixed time settings and premixed fluids.

Zusammenfassung. Eine neue, einfache Maschine zur automatischen Entwässerung und Einbettung elektronenmikroskopischer Präparate wird beschrieben. Bis zu 24 Präparate werden gleichzeitig in einer Wanne bewegt, deren Inhalt bis 29mal aus 10 verschiedenen Behältern entsprechend einem vorwählbaren Programm ausgewechselt wird. Die Maschine reproduziert zuverlässig und genau zahlreiche bisher von Hand ausgeführte Verfahren. Sie ist klein, einfach zu bedienen, kann über Nacht arbeiten und spart so viel Zeit.

C. Haudenschild and H. Tschirky

Department of Experimental Medicine,
F. Hoffmann-La Roche Co. Ltd.,
CH-4002 Basel (Switzerland),
28 July 1972.

PRO EXPERIMENTIS

Optical Method for Measuring Water Flow with Automatic Recording

Flow of water across biological and synthetic membranes is a major feature of their permeability characteristics. Neurohypophyseal hormones can drastically change the water permeability of structures like distal nephron and amphibian epithelia. These latter membranes have been widely used both as a model of the mammalian kidney and as a convenient in vitro system to study water transport across polar epithelia.

Measurements of water flow across toad bladder and frog skin have always presented technical difficulties. Three main types of technique have been proposed: 1. gravimetric methods[1-3], 2. a constant volume technique with automatic recording[4], and 3. a volume flow technique employing an horizontal pipette[5]. The movement of a meniscus in an horizontal pipette or calibrated capillary is an almost ideal technique to measure water