thus very similar to that observed by light microscopy. While the staining was predominantly in the nucleus, there was a trace of lead deposition in the cytoplasm (figs 1–3). There was virtually no deposition of lead in the control preparations incubated in the presence of lead nitrate but without the glycerophosphate substrate (fig. 4). When uranyl acetate staining was followed by staining with lead citrate there was an increase in electron density of the preparations, and especially of the nucleolus and chromatin (fig. 5). There was, however, no lead staining comparable to that observed in the preparations stained for acid phosphatase (figs 2, 3). The localization of the enzyme in the nucleolus is similar in light and electron micrographs (figs 1, 2, 3) and corresponds to the light fibrillar areas (arrows) in the control preparations incubated in the absence of substrate (figs 4, 5). It would appear therefore, that the acid phosphatase of nucleolini demonstrable by light microscopy can also be visualized in electron micrographs and that the sites of the enzyme correspond to relatively electron lucent fibrillar spherical areas in the nucleolus, the fibrillar centres described by Recher et al. [5]. In summary, an acid phosphatase that is active at pH 5.0 has been demonstrated by light and electron microscopy in the nucleolini or fibrillar centres, and to a lesser degree in the nucleoplasm.

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A simple and versatile argon laser microbeam

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The use of the laser microbeam in cell biology has been well established. The first laser microbeams utilized the red wavelength (694.3 nm) of the ruby laser [1, 2], and subsequent systems have employed a mixed beam with the multi-wavelengths from the blue-green argon laser [3, 4]. The general usefulness of both the ruby and argon laser microbeams have been somewhat limiting because of either a small number of wavelengths available (only one in the case of the ruby laser), or the inability to work with many of the single wavelengths of the argon laser (primarily because of the low output of this laser). Recent developments in laser technology have permitted the construction of an argon laser microbeam with the capabilities for selection of any of the visible argon wavelengths. Such a system is relatively easy to construct, and simple to operate.

The system currently in use is diagrammatically represented in fig. 1, and photographed in fig. 2. The laser is mounted on a rectangular plywood box, above a Zeiss photomicroscope. The beam is reflected at right angles by two front surfaced mirrors that are mounted in precision optical mounts. The beam is then reflected downward at a right angle by a coated interference filter that reflects 90% of the blue-green light (wavelength shorter than 520 nm), and transmits 90% of the light longer than 520 nm. The beam next passes through a hole in the plywood frame, a 60 mm focal length lens mounted in the monocular tube of the microscope, through the vertical path of the microscope, and into an oil immersion objective (Zeiss neofluar 100×, n.a. 1.3). Light from the substage tungsten source is projected through an orange filter (transmits 10^{-4}%

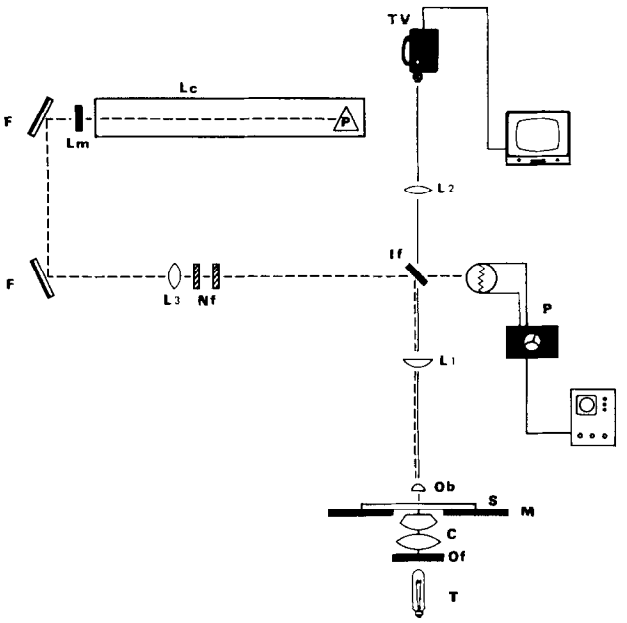

Fig. 1. Diagram of laser microbeam: *C*, condenser; *F*, front surfaced mirrors; *If*, interference filter; L_1, 60 mm focal length lens; L_2, 10 cm focal length lens; L_3, 100 cm focal length lens; *Lc*, laser cavity; *Lm*, laser output mirror; *M*, microscope stage; *Nf*, calibrated neutral density filters; *Ob*, 100× Zeiss neufluar objective; *Of*, orange filter; *P*, photodiode, with attached photometer and oscilloscope; ⧋, wavelength selector prism; *S*, specimen chamber; *TV*, television camera and monitor; *T*, tungsten light source; – – – –, laser beam; ———, sub-stage illumination.

below 520 nm) before passing through the condenser and up into the microscope system. The image of the specimen on the microscope stage is projected through the interference filter, a 10 cm focal length lens, and into a normal resolution television camera. The image is projected on the screen of the television monitor. The 10 cm lens is necessary to adjust the focal length of the light so that the image on the television screen is parfocal with the focal plane of the microscope. The television camera is mounted on a X-Y mount permitting movement in two horizontal planes.

In a normal irradiation experiment the specimen is placed on the microscope stage and viewed on the television screen. The region of the cell that is to be irradiated is moved under a cross hair on the screen, and the laser is fired. The cell may be continually observed on the television screen, before, during, and after irradiation.

The laser is a Hughes model 3030H pulsed argon laser (see table 1 for output and wavelength characteristics). It can be used in single or multi-wavelength operation, and in single or multi-mode configuration. Wavelengths are selected by rotating a prism in the rear of the laser cavity. Mode configuration can be altered by changing the output mirror. Laser output is varied by controlling the input voltage to the laser tube, or by attenuation of the beam with calibrated neutral density filters. Pulse duration can be set at either 50 or 20 μsec by merely varying the capacitance of the system. Output is monitored with a calibrated S-5 vacuum photodiode mounted behind the interference filter and connected to a photometer and/or an oscilloscope. This permits recording of the actual energy of each pulse used in an experiment, as well as continual monitoring of the pulse shape.

Measurements on the system described above indicated that 12–15% of the laser energy was getting into the focus spot of the oil immersion objective. By addition of a 100 cm focal length lens between the laser

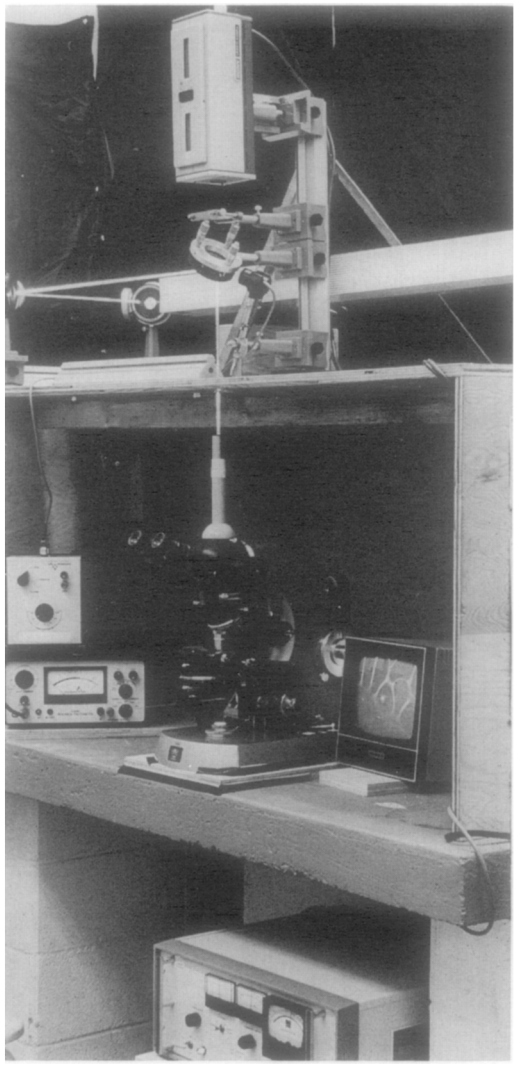

Fig. 2. Actual argon microbeam system; the neutral density filters and 100 cm lens are not included in this photo.

and the microscope (see fig. 1) the efficiency of the system was increased to 53%. The smallest effective lesion-producing spot, with or without the additional lens, is 0.5 μ [4–6].

Several features of this system make it more desirable than earlier argon microbeams. The laser itself is the most powerful pulsed argon laser commercially available. With a total peak power of 35 watts pulse (compared to 1.5 W of our prototype system) as much as 1 050 μJ can be delivered to an area of a μm or less. In addition, the high power of the mixed wavelength beam permits selection of any of the single visible wavelengths for irradiation. The high power of this laser also makes available the ultraviolet wavelengths as a mixed beam, or singularly as a monochromatic beam. With a total of 14 W available at the 514.5 nm wavelength, it should even by possible to work with the second harmonic of this wavelength (257.2 nm).

Another improvement over the earlier argon systems is the use of an interference filter in place of a synchronized rotating mirror. This modification considerably simplifies the operation, circuitry, and general construction of the system. In addition, the image projected on the television screen is of better quality, and a direct energy measurement can be made on each pulse of light that passes through the system.

The general simplicity of construction, ease of operation, versatility with respect to available wavelengths, output, and responding organelles [7], should make this instrument useful and generally available to a large

Table 1. *Laser characteristics*

Wavelength (nm)	Maximum output, W (multimode) 50 μsec pulse
528.7	1.75
514.5	14.0
501.7	3.5
496.5	3.5
488.0	7.0
476.5	3.5
457.9	1.75
351.1	4.64
363.8	3.20
379.5	0.16

Total visible, 35 W.
Total UV, 8 W.
Pulse repetition, 1 pulse/sec. to 200 pulse/sec.

number of biologists. Indeed, the monetary output for such a system may be minimal, depending upon the needs of the investigator (construction of a complete system may range from $1 200 to $15 000).

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Frequency of "Y chromatin body" in human skin fibroblasts in tissue culture, and its relation to growth phase

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It has been shown recently by Pearson, Bobrow & Vosa [3] that the presence of a Y-chromosome in a cell can be demonstrated in interphase by fluorescence microscopy after staining with an aqueous solution of quinacrine dihydrochloride ("Atebrin", G. T. Gurr). In 25 to 50 % of cells in buccal smears from normal males the Y-chromosome showed as an intensely fluorescent body with a diameter of approx. 0.25 μm.

As the staining properties of the Y-chromosome in males and that of the heterochromatic X-chromosome in females seem different with Atebrin [2] as well as with quinacrine mustard [1], we found it worthwhile investigating if the frequency of the Y chromatin positive interphases varies with growth phase, as has been shown to be the case with the frequency of the X chromatin positive (Barr positive) interphases [6].

Material and Methods

Primary cultures of skin fibroblasts were established as described elsewhere [4]. The experiments were performed with cultures in the third passage in Leighton tubes [5]. Cultures from a normal male and a normal female were grown simultaneously. The growth curve was determined by counting the cells in three cultures from each person each day using a celloscope (Model 202, AB Lars Ljungberg, Stockholm). The frequency of cells with a Barr body in the female cultures was determined by counting 100 interphases each day in a Feulgen light green stained culture. As regards the staining of the Y chromatin body we used the following procedure. The cultures were fixed in methanol for 60 min, stained with an 0.5 % aqueous solution of Atebrin for 5 min, destained in running tap water for 3 min, embedded in McIlvaine buffer (pH 4.1) and thereafter counted as soon as possible. The frequency of cells with a Y chromatin body was determined by both of us, except on day 1 of expt 197 when the counts were performed by one of us only. Both of us counted the number of cells with a Y chromatin-like body among 100 cells in each of four cultures per day: two from the normal male and two from the normal female. The countings were done blindfold.

A Zeiss standard microscope with incident light from a 'HBO 200' mercury vapour lamp was used. The excitor filter was a 4 mm 'BG 12' and a 500 nm filter was used as barrier. All countings were done with an oil immersion objective (× 100).

Results

After a couple of preliminary experiments the two experiments illustrated in fig. 1 were performed. The figure shows the cell number per culture each day of the experiments for the normal male and female together with the frequency of Y chromatin positive cells in the male and the frequency of X chromatin positive cells in the female. It was possible by counting Y chromatin-like bodies to distinguish blindfold between male and female cultures in all cases but one: in one of the male cultures from expt 198, day 1, none of the 100 cells counted had a Y chromatin body. The average for the four male cultures on that day was 6.8 per 100 cells (fig. 1). The