maternal blood taken shortly before surgery and in umbilical arterial and venous blood taken at delivery (five to eight samples were obtained for each group). Before the onset of labor, levels were low in maternal (27.6 ± 10 pg/ml) and umbilical arterial blood (11.8 ± 6.2 pg/ml). In very early labor, the maternal level was unchanged (22.7 ± 6.3 pg/ml) but the umbilical arterial level significantly elevated (36.1 ± 11.7 pg/ml). In advanced labor both maternal (45.2 ± 17 pg/ml) and umbilical arterial oxytocin levels (57.3 ± 16 pg/ml) were higher than before labor.

From these results we propose that the following sequence of events results in the initiation of labor. The concentration of oxytocin receptors increases dramatically during gestation, probably under the influence of the rising estrogen levels. Evidence for the stimulation of oxytocin receptor formation by estrogens has been found in rabbits (*13*) and rats (*4, 8*). Near term, the rapid fetal growth rate accelerates uterine distension which probably contributes to the increase in oxytocin receptors toward term, as shown in rats (*14*). Although there is no dramatic increase in circulating oxytocin at the onset of labor, the increasing concentration of receptors lowers the oxytocin threshold to the point where activation of the myometrium occurs. Simultaneously, oxytocin binds to the receptors in the decidua, stimulating prostaglandin synthesis. The released prostaglandins diffuse into the adjacent myometrium and enhance the oxytocin-induced contractions. We have shown that oxytocin-induced contractions will not dilate the cervix and lead to progressive labor unless there is simultaneous prostaglandin release (*15*). The coupling of the oxytocin receptor activation and prostaglandin synthetase activity in the decidua therefore appears to be a crucial event in the initiation of labor. Additional support for this concept is provided by the fact that both ethanol, which inhibits oxytocin release, and prostaglandin synthetase inhibitors like indomethacin, inhibit labor contractions and can be used to prevent preterm birth (*16*).

We have confirmed the fetal secretion of oxytocin at term and the arteriovenous difference in oxytocin concentrations in the umbilical cord. Although experimental limitations make it difficult to document a transfer of fetal oxytocin through the human placenta and fetal membranes, such transfer has been shown in guinea pig and baboon (*17*). The amniotic fluid at term contains considerable amounts of oxytocin (*12*), and morphological studies of the human fetal membranes at term support the view that the circulating amniotic fluid, after traversing the amnion, will continue through the intercellular canaliculi of the chorionic cytotrophoblast to reach the decidua parietalis (*18*). We therefore postulate that fetal oxytocin can provide a stimulus for the increased production of prostaglandins at the onset of labor.

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Rapid Electronic Autofluorography of Labeled Macromolecules on Two-Dimensional Gels

Abstract. *The feasibility of electronically locating and measuring tritium-labeled macromolecules directly on dried electrophoretic gels has been demonstrated. This new procedure eliminates the usual long film exposure in autofluorography and the attendant delay in processing and data reduction. An image intensifier and electronic camera tube are used to integrate the light produced by the tritium interaction with a scintillator incorporated in the gel. Preliminary results show that, compared to film, the exposure is reduced 100 to 1000 times. The response to low activity levels is improved, and spatial resolution is maintained. A proposed instrument could be used for measuring other isotopes as well as fluorescent and visible stains.*

Investigators in many biological research laboratories are examining normal and abnormal human and animal proteins with one- and two-dimensional separation techniques to produce protein maps of plasma, urine, and other materials. Projects are under way to compile a complete protein index of humans (*1*). In other laboratories recombinant DNA techniques are being used to study the basic properties of DNA and to manipulate DNA fragments so as to "engineer" organisms that can manufacture scarce substances such as insulin, growth hormone, and interferon (*2*). Still other researchers are seeking to distinguish tissues, particularly tumors, or to detect genetic diseases (*3*). Essential to much of this research and development are the sequence analysis of DNA fragments and the screening of clones for specific genes. Both techniques require the detection of radioactive nucleic acid or antibody by autoradiography (*4*).

In the projects and techniques mentioned above, autoradiography or autofluorography is used in which x-ray film is exposed for hours or days in order to visualize the distribution and the amounts of labeled macromolecules. The

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activity is quantified by visual estimation or by densitometry. In some laboratories the film is optically scanned by mechanical or television techniques which transmit the data to a computer for analysis and comparison (*5*). In addition to the long exposure time, other difficulties associated with the use of film arise from nonlinearity, reproducibility in manufacture and development, fogging, and dynamic range. The last factor sometimes requires a series of progressively longer exposure to encompass the strongest and the weakest spots, thereby further increasing the time needed to acquire data.

The activities of two-dimensional gels labeled with ^{3}H, ^{14}C, ^{35}S, and ^{32}P have been directly detected with one- and two-dimensional spark chambers and proportional counters (*6*, *7*). The low energy of the beta particles from some isotopes requires that very thin windows be used or that the gel be placed inside the counter, with the risks of contamination of the counting gas and distortion of the electric field due to charging of the gel. In these counters the beta particles must leave the gel to be detected, and thus there is a loss of efficiency at lower energies (*7*). Another way is to scan the gel with a point or slit detector, using a Geiger-Müller tube or scintillation counter. The gel can also be cut into small pieces which are counted individually in a liquid scintillation counter (*8*).

Autofluorography is a technique in which a scintillant is incorporated into the gel (*9*). This allows the weak beta particles from ^{3}H, for example, to be detected in situ by the light they produce, since the light, unlike the beta particles, can penetrate the gel and interact with the film. Bonner and Laskey (*9*) reported that, with autofluorography, ^{3}H activities of 50 disintegrations per second in a band 0.1 by 1 cm (~ 5 dps/mm^2) and 8 disintegrations per second (~ 0.8 dps/mm^2) could be detected in 24 hours and 1 week, respectively—a sensitivity improvement of several hundred times over that with autoradiography. Laskey and Mills (*10*) have reported an increase in the sensitivity as well as an improvement in the linearity of autofluorography by preexposure of the film. The amount of exposure must be optimized to obtain linearity. Too much exposure produces increased sensitivity at low activity levels with a downward curving response at higher levels. Too little exposure gives an improved response that is concave upward, resembling the curve without preexposure. At the optimum preexposure for linearity, they obtained a sensitivity increase of four times over that of autofluorography without preexposure. At the optimum preexposure for sensitivity, the increase was ten times but with nonlinearity at higher activity levels.

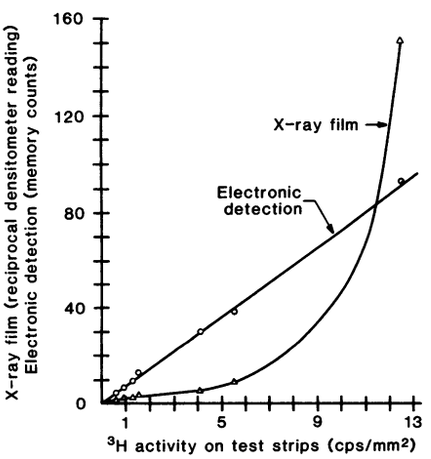

Fig. 1. Comparison of x-ray film and electronic autofluorography of ^{3}H-labeled calibration strips of gels containing the scintillant PPO. Exposures for the x-ray film were 48 hours at 23°C and for the electronic detection 34 minutes (sum of 17 2-minute exposures). The autofluorography was carried out by E. P. Lester.

For electronic detection, the low light levels (10 to 1000 photons) require the sensitivity of a photomultiplier tube. Furthermore, if the entire gel is to be measured at the same time with a spatial resolution comparable to that of film, the detector must be an area device that is the equivalent of a hundred thousand or more detectors operating simultaneously. To eliminate the extreme light amplification and signal-to-noise ratio necessary to count each scintillation, as in a liquid scintillation counter, the detector should have an integrating property as film has. This property would make it possible to take time exposures and to store the integrated images of the radioactive spots electronically in the detector before readout. Given the photomultiplier-equivalent sensitivity, total area capability, and integrating property, the time needed to acquire data with such a detector could be reduced by orders of magnitude over the time needed for film.

In a test, we used a gel containing ^{3}H-labeled proteins and test strips of gels loaded with ^{3}H-labeled leucine. Earlier, in preparation for x-ray film exposure, the scintillant 2,5-diphenyloxazole (PPO) had been incorporated in the gels. The gels were exposed to a camera system consisting of an image intensifier and a secondary electron conduction camera tube (*11*). The system is capable of integration times of several minutes. The scanning electron beam, which reads the integrated signal from the camera tube target, was turned off during the integration. Precautions were taken to avoid stray light that would mask the light from the scintillator. At the end of the integration time, the stored image of the gel was scanned, digitized with an 8-bit analog-to-digital converter in 0.33 second, and stored in a computer-controlled digital memory of 512 by 512 12-bit words. The

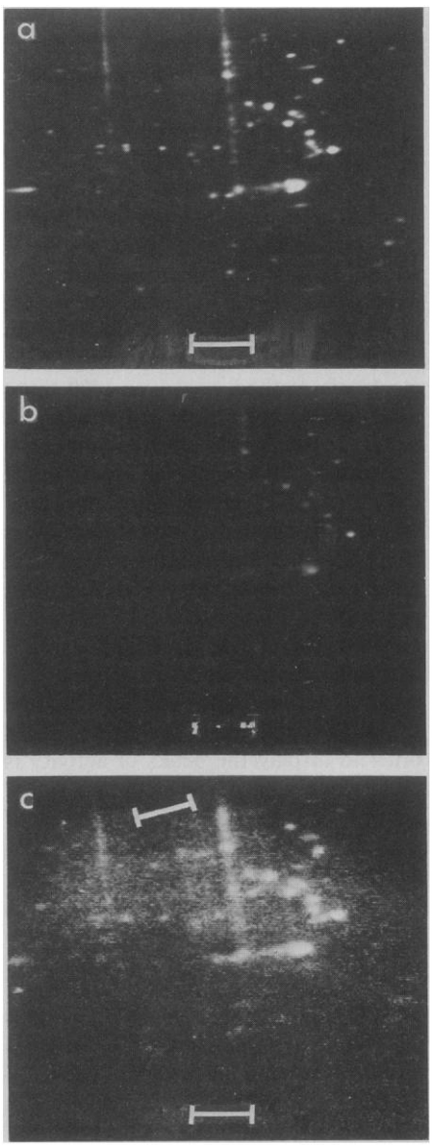

Fig. 2. X-ray film and electronic autofluorography of the same portion of a dried polyacrylamide gel containing electrophoretically separated ^{3}H-labeled proteins and the scintillant PPO. (a) Print of x-ray film, exposure 48 hours at 23°C. (b) Print of x-ray film, exposure 4 hours at −80°C. (c) Electronic image, exposure 2 minutes. This image was reconstructed from digital data and photographed from a television monitor. The electronic camera resolution for point light images was 0.5 mm. Improved response of electronic detection at low activity level (Fig. 1) includes weak peripheral intensities, resulting in spots that are enlarged as compared to film. The bright spot at the extreme left center is an electronic artifact. Scale, 1 cm. The autofluorography was carried out by E. P. Lester.

intensity of any point in the image could be determined from the number at the memory address of the point. The entire image could be displayed on a television monitor for inspection and photographing.

Preliminary results are shown in Figs. 1 and 2. Figure 1 is a comparison of the response of the electronic technique to that of the x-ray film as measured on our densitometer. The same ^{3}H- and scintillant-containing test strips were used for both curves. The improved linearity of the electronic response curve is evident. A comparison of the film image from a dried polyacrylamide gel containing electrophoretically separated ^{3}H-labeled proteins and PPO with the image generated from digital memory and photographed from the television screen is shown in Fig. 2. After an electronic exposure of 2 minutes, the images are comparable in visible detail and resolution, although the exposure time for the electronic method was over 1400 times less. Easily recognizable and measurable features were visible after 1 minute.

Because of the increased sensitivity at low radioactivity levels, the picture generated from our digital data shows larger spots than the film autofluorograph but more nearly represents the actual sizes of the spots. Film, because of its threshold, can give an exaggerated appearance of resolution by suppressing weak peripheral activity and emphasizing the peaks. Manipulation of the digital data in order to display the peaks of activity is easily done with our system and would have produced a picture more like that of the film. We chose to display all the data down to background level. Since the resolution of our camera and recording system was better than 0.5 mm for a point image, smaller spots than those in the photograph could have been seen. We conclude that the resolution (minimum spot size and separation) was determined by the electrophoretic technique.

We believe that these results point the way to a new instrument for rapid and improved quantitation of low-energy, beta-emitting isotopes used in chromatography and electrophoresis. Furthermore, isotopes such as ^{55}Fe, ^{125}I, or ^{51}Cr, which emit Auger electrons, or fluorescent x-rays should be amenable to this technique (*12*). Higher energy beta particles, such as those from ^{32}P and electron-capture gamma rays, could be detected with interposed phosphors and conversion layers. The greatly increased sensitivity should permit the use of much less radioactivity, which would lower the cost and ease the problem of radioactive waste disposal. In addition, as has been pointed out by O'Farrell (*13*), smaller initial amounts of protein should improve the resolution of closely spaced spots. Alternatively, the speed with which the distributions can be visualized suggests that one might be able to observe the movement of the macromolecules by taking a sequence of photographs constituting a time-lapse motion picture of the pattern development. Drift rates should be easily determined from such digital pictures. Since light is the agent that enables one to detect the particles, the system can be adapted for the quantitation of fluorescence and visible light absorption introduced by selective staining (*14*).

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Human Liver MAO-A and MAO-B Separated by Immunoaffinity Chromatography with MAO-B–Specific Monoclonal Antibody

Abstract. *A monoclonal antibody was used to prepare immunoaffinity columns that efficiently bind monoamine oxidase B activity but not monoamine oxidase A activity from detergent extracts of human liver mitochondria. The only discrete polypeptide component that eluted from affinity columns with potassium thiocyanate migrated in sodium dodecyl sulfate–polyacrylamide gels with an apparent molecular weight of 59,000, as expected for human monoamine oxidase B. These results support the hypothesis that there is an intrinsic structural difference between monoamine oxidase A and B and demonstrate that immunoaffinity chromatography can physically resolve the two enzyme species in liver extracts.*

Monoamine oxidase (MAO) (E.C. 1.4.3.4) is an integral protein of the outer mitochondrial membrane (*1*) and plays an important role in the degradation of dopamine, norepinephrine, epinephrine, and serotonin (*2*). In most tissues, MAO consists of a mixture of two catalytically distinct forms, A and B, each expressed in characteristic proportions (*3*). MAO-A is inhibited by the irreversible active site inhibitor clorgyline and oxidizes serotonin (*4*), while MAO-B is inhibited by the related compounds deprenyl (*5*) and pargyline (*6*) and oxidizes phenylethylamine and benzylamine (*5*). These inhibitors mimic substrates of the respective MAO forms and bind covalently to a cysteinyl-flavin residue in the active site subunit of MAO (*7*). Studies with [^{3}H]pargyline-labeled enzyme have shown that the flavin-adenine dinucleotide–containing subunits of rat MAO-A and -B migrate in sodium dodecyl sulfate (SDS)–polyacrylamide gels with apparent molecular weights of 55,000 and 60,000, respectively (*8*). Similarly, human MAO-B from platelets and MAO-A from placenta are reported to have molecular weights of 64,000 and 67,000, respectively (*9*), or 60,000 and 64,000 (*10*).

One approach to studying molecular differences between the two MAO species is to investigate the ability of antibodies to distinguish them. Results of studies with conventional antiserums have been equivocal (*11–13*). Since pub-

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