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15. Shale samples were dissolved in hydrofluoric acid followed by centrifuging and washing the organic residue with distilled water.
16. The dark transverse lines shown in Figs. 1, B, C, and F, are folds in the filament.
17. Separation of the filaments into at least four species is based on the comparison of size and frequency distribution of filaments in six different laminae. These species have average apparent widths of about 11, 5, 3, and 1.5 μm. In addition, filaments as wide as 40 μm and of uncertain biologic affinity are of very rare occurrence in the Newland shales. Preferential preservation of sheaths as compared to trichomes in the oscillatoriacean cyanophyte *Lyngbya aestuarii* is illustrated by R. J. Horodyski, B. Bloeser, S. Vonder Haar, *J. Sediment. Petrol.* **47**, 680 (1977). A detailed taxonomic treatment of the Newland microfossils and of similar microfossils occurring in the underlying Chamberlain Shale is in preparation (R. J. Horodyski and B. Bloeser, in preparation.)
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21. Bedding surfaces of the Newland Shales contain carbonaceous films similar to those described and interpreted as megascopic and probably eukaryotic algae from the 1300-million-year-old Greyson Shale, Belt Supergroup, by M. R. Walter, J. H. Oehler, D. Z. Oehler, (*5*). Such algae, if eukaryotic, could have been the source of the Newland sphaeromorphs. Study of the megascopic films in the Newland shales is in progress.
22. Studying shale-facies microfossils in petrographic thin sections supplements the study of microfossils in acid maceration residues as it preserves the spatial relationship of the microfossils, avoids breakage of fragile specimens, and indicates whether organic microstructures are indigenous to the rock and syngenetic with deposition of the enclosing sedimentary matrix. In addition, it provides a relatively rapid means of identifying fossiliferous horizons. Interestingly, the microfossils are evident only in petrographic thin sections oriented parallel to bedding; in sections oriented perpendicular to bedding the microfossils are not readily recognizable as biologic entities due to compression.
23. We thank J. W. Schopf and H. T. Loeblich for discussion.

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Autoradiographic Image Intensification: Applications in Medical Radiography

Abstract. *The image of an 80 to 90 percent underexposed medical radiograph can be increased to readable density and contrast by autoradiographic image intensification. The technique consists of combining the image silver of the radiograph with a radioactive compound, thiourea labeled with sulfur-35, and then making an autoradiograph from the activated negative.*

Minimizing the x-ray dose received by patients during medical examinations and maximizing the quality of the radiographs are subjects of current concern to both the medical profession and the public (*1*). Some conflict is inherent in the two objectives because higher-quality radiographs—that is, those which convey more information to the physician—usually require higher exposure levels. Recent gains in quality or exposure reduction, or both, are due to developments such as computer processing, electrostatic imaging systems, improvements in intensifying screens, and scatter rejection techniques (*2*). The experiments reported here indicate that additional exposure reductions or quality increases are possible with postdevelopment autoradiographic image intensification.

Radiographs which are normally classified as "badly underexposed" actually contain most of the information which was intended to be recorded by the original exposure. Autoradiographic intensification effectively retrieves this information by increasing the image density and contrast to readable levels. The intensification occurs on an autoradiograph made from the underexposed film after the original image silver has been chemically combined with a radioactive isotope (*3*). While the theory of autoradiographic intensification has been known for many years (*4*), the possibility of its widespread application has recently been realized through the development at the Marshall Space Flight Center of a simple, efficient radiochemical technique employing thiourea labeled with sulfur-35 (*5*). Although the procedure was developed for use in astronomical research, some of the most valuable applications may be in the field of medical radiography (*6*). These include reduction of the x-ray dose, since underexposed radiographs can be intensified after development; elimination of retakes necessitated by accidental underexposure; and maximum use of exposures with a high detective quantum efficiency (DQE), such as those made with scatter rejection devices, which can cause underexposures (*2*, *7*).

With the cooperation of scientists from Vanderbilt University Hospital, we determined the range of underexposures which can be adequately intensified by autoradiography. We found that, in general, radiographs underexposed by as much as 80 to 90 percent produce autoradiographs which compare favorably with optimum exposures. Some of the radiographs used for this research were inadvertently underexposed in routine clinical situations, and others were deliberately underexposed.

The underexposure limit for a commonly used film-screen system, DuPont Lo-Dose (single-coated film), was determined from experiments with radiographs of the abdominal area of an anatomical phantom. An optimum-exposure radiograph was made at 120 kV and 450 mA-sec. Underexposures were made (with no change in tube voltage or subject distance) at 100, 50, and 20 mA-sec (that is, underexposures of 78, 89, and 96 percent). Autoradiographs of the underexposed film were made and compared with the normal-exposure radiograph. The contrast and visible detail on the autoradiographs made from the 78 and 89 percent underexposures were about the same as on the normal radiograph. Figure 1 shows optimum paper prints (*8*) of the normal exposure radiograph, the 89 percent underexposure radiograph, and the autoradiograph of the 89 percent underexposure. The limiting case was the 96 percent underexposure; there was absolutely no visible image on the radiograph, and while the autoradiograph produced an image, it was of unacceptably poor quality. An example of accidental underexposure with the same film-screen system is shown in Fig. 2, where an underexposed mammogram may be compared with the corresponding autoradiograph. All autoradiographs were made on Kodak industrial films with exposure times of 2 to 12 hours (*9*).

The method of radioactivating radio-

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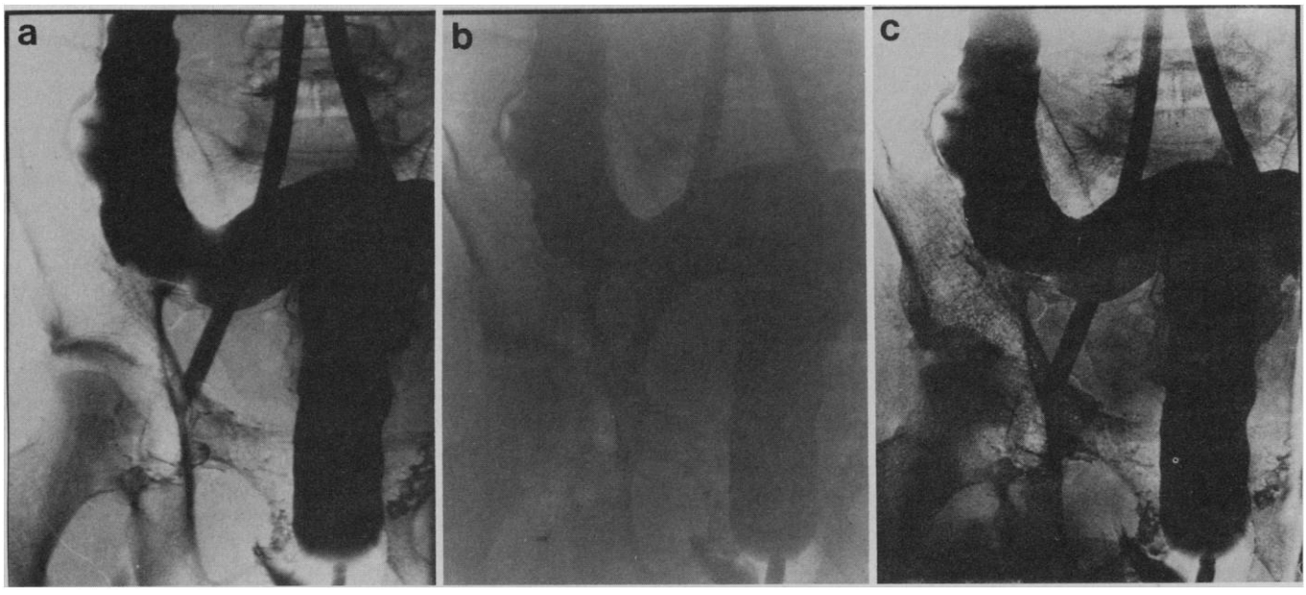

Fig. 1. Optimum prints of (a) a normally exposed radiograph, (b) an 89 percent underexposed radiograph, and (c) an autoradiograph made from the underexposed radiograph.

graphs requires special attention because of the physical nature of the films (relatively heavy emulsion, often double-coated). Successful intensification requires an efficient reaction between the photographic silver and the [^{35}S]thiourea (to form radioactive silver sulfide), and this is accomplished by smoothly agitating the film while it is immersed in the radioactive solution. We have found only one completely successful agitation method for radiographs (*10*). The film is placed inside a processing drum normally used for developing color prints, the radioactive solution is added, and the drum is continuously rotated. Commercially available devices sold with the drums accomplish this agitation automatically and efficiently. Only one side of the film is activated, and the autoradiograph reproduces only the activated image. Significant intensification can be achieved with the image from just one side of a double-coated film, but the total gain is a factor of 2 less than that obtained with a single-coated film. When deliberate underexposures are made, single-coated film is usually chosen; but even with double-coated film, autoradiographic intensification can be useful for avoiding retakes. We have found that it does not matter which side of the double-coated film is used for autoradiographs.

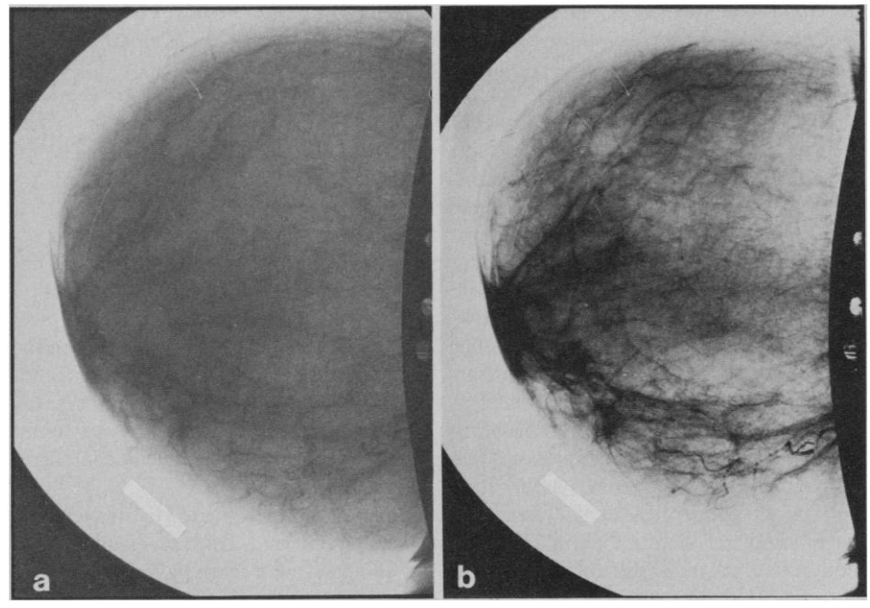

Fig. 2. Optimum prints of (a) an accidentally underexposed mammogram and (b) an autoradiograph made from the underexposed film.

Autoradiographic intensification, as a postdevelopment process, can be employed sequentially with other dose-reduction methods such as the use of intensifying screens as in the experiments reported here. The quality of radiographs can be improved through the sequential use of postdevelopment intensification with high-DQE exposures such as those made with the scatter rejection techniques of Barnes *et al.* (*7*). Radiographic quality can also be improved when the exposure time is reduced because the possibility of patient motion and subsequent image blurring is reduced. In many cases, the sharpness of the image will also be increased since a smaller focal spot results from the lower tube current.

In summary, an autoradiographic intensification technique which can be used on a routine basis has been developed, and its possible use with medical radiographs is reported here. Good autoradiographic images can be obtained even when the original radiograph has been underexposed by 80 to 90 percent. Possible applications for medical radiography include optimum use of high-DQE techniques, reduction of x-ray dose, and rescue of accidentally underexposed radiographs.

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8. We made the best possible print from each film. However, the appearance of the prints differs from that of the films in two cases: (i) the image of the underexposed radiograph is enhanced by printing on high-contrast paper; and (ii) the background fog of the autoradiograph is higher than that of the optimum exposure, and this does not show on the print.
9. Industrial x-ray film was used only because it was readily available in the laboratory. The speed of the various types of Kodak x-ray film decreases in the order AA, M, and R; the time required to make an autoradiograph increases by about a factor of 2 as the speed of the receiver film decreases.
10. The agitation problem does not preclude automation of the process because a similar technique can be incorporated in the processing machine. Also, we have not tried gaseous burst agitation.

11. I thank G. Rao and A. Brill of Vanderbilt University Hospital for supplying the films and participating in the evaluation; R. Wagner, G. Barnes, E. Kerkes, and R. O'Dell for discussions and help; and D. Speich for the photography.

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Fasting Decreases Triiodothyronine Receptor Capacity

Abstract. *Fasting decreases the ratio of hepatic nuclear to serum triiodothyronine (T_3) by diminishing the binding capacity of nuclear T_3 receptors. In combination with the lower serum T_3 concentration caused by fasting, the decrease in receptor content results in a marked decrease in nuclear T_3-receptor complexes. The changes in T_3 receptor content and circulating T_3 in fasted animals appear to be independent synergistic adaptations for caloric conservation in the fasted state. Unlike changes in hormonal level, the modification of nuclear receptor content provides a mechanism that may protect cells with a low caloric reserve independently of the metabolic status of the whole animal.*

Fasting decreases the serum concentration of 3,5,3′-triiodothyronine (T_3), concomitantly increasing the concentration of 3,3′5′-triiodothyronine (reverse T_3) (*1*). The latter has little or no calorigenic activity (*2*). The reduction in serum T_3 is probably a mechanism for caloric conservation in the fasted state. However it is the T_3-receptor complex rather than T_3 itself that is required for a hormonal effect, and a decreased concentration of serum T_3 does not necessarily imply fewer T_3-receptor complexes. The number of such complexes is determined both by the concentration of T_3 available for binding and the number and association constants of T_3 receptors. Several hormones diminish the concentrations of their own receptors (down regulation), thus tending to neutralize the biological effects of sustained changes in their concentration (*3*). This may also be true of T_3. Down regulation of the pituitary nuclear T_3 receptor by exposure to T_3 has recently been reported (*4*). Of several putative T_3 receptors (and sites of action), the nuclear receptor is the best characterized and thus far is the only receptor for which a clear correlation between occupancy and biological action has been established (*5–7*). The purpose of the present study was to determine whether fasting affects the nuclear T_3 receptor. We report that fasting decreases the ratio of hepatic nuclear T_3 to serum T_3 by decreasing the nuclear content of T_3 receptors.

Female Sprague-Dawley rats weighing between 150 and 250 g were used. Rats of similar weight were paired and placed in individual cages with free access to water. Food was withheld from one of the pair. For studies of nuclear T_3 uptake in vivo, [^{125}I]T_3 in dilute rat serum was injected into the femoral artery. This procedure and subsequent exsanguination through the abdominal aorta were carried out under light ether anesthesia. The rats were exsanguinated 30 minutes after injection. This is sufficient time for T_3 to equilibrate with rat nuclei (*8*). The livers were removed and chilled on ice. All subsequent procedures were carried out at 0° to 4°C. A weighed portion of liver, dissected free of fibrous tissue, was homogenized in ten volumes of 0.25*M* sucrose in 20 m*M* tris buffer, *p*H 7.85, containing 1 m*M* $MgCl_2$. The homogenate was centrifuged for 10 minutes at 600*g* and the pellet resuspended by gentle homogenization with 0.5 percent Triton X-100 in the sucrose solution. The Triton treatment was repeated once more and the final pellet was washed and resuspended in sucrose. Portions of the nuclear suspension were taken to determine radioactivity as well as DNA (*9*). The portion taken for determination of radioactive content was counted in an automatic well-type gamma scintillation counter without further preparation. For determination of DNA, a separate portion of the nuclei was washed three times in tris buffer to remove sucrose. The DNA was measured by the method of Ceriotti (*10*). The solubilized T_3 receptor was extracted from nuclei by the method of Samuels *et al.* (*11*). Total serum T_3 was determined by solid phase immunoassay (*12*) of unprecipitated serum. Serum portions were precipitated with 10 percent trichloroacetic acid before counting to separate [^{125}I]T_3 from [^{125}I]iodide.

Triiodothyronine binding studies were carried out in vitro by incubating the nuclear extract with tracer quantities of [^{125}I]T_3 and separating bound from free T_3 with dextran-coated charcoal (*13*). The charcoal was prepared by mixing 300 mg of Norit-A and 30 mg of dextran-15 in 100 ml of 0.4*M* KCl in sucrose buffer, *p*H 7.85. A 0.5-ml portion of extract containing [^{125}I]T_3 was added to 1 ml of dextran-charcoal in an ice bath, vortexed, and allowed to stand for 15 minutes. The charcoal was separated by centrifugation and the decanted supernatant and charcoal were counted in a gamma scintillation counter to determine bound and free [^{125}I]T_3.

Rats fasted for 5 days remained active and appeared to be in good condition. The results of the experiments in vivo are summarized in Table 1. With fasting, serum T_3 concentrations decreased on the average by about 50 percent. Liver weights decreased with a relative conservation of DNA so that the DNA/liver weight ratio increased proportionately. The ratio of hepatic nuclear to serum

Table 1. Effects of a 5-day fast on the nuclear/serum ^{125}I ratio after injection of [^{125}I]triiodothyronine.

Measure	*N*	Control rats	Fasted rats	*P*†
Weight change (g)	6	+21.7 ± 6.6*	−75.8 ± 17.1	<.001
Serum T_3 (ng/dl)	5	48.2 ± 10.0	24.4 ± 13.7	<.025
Liver weight (g)	6	9.8 ± 1.3	5.5 ± 0.9	<.01
Liver DNA (mg/g)	6	0.97 ± 0.10	2.0 ± 0.28	<.001
Nuclear/serum ^{125}I (cpm/μg DNA)/(cpm/μl serum)	6	0.94 ± 0.11	0.65 ± 0.18	<.005

*Mean ± S.D. †Paired *t*-test.

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