er measurements with increased precision. This technique has definite promise as an aid in studying the late-Pleistocene history of the Florida Keys and the Bahaman Banks.

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Tritium and Phosphorus-32 in High-Resolution Autoradiography

Abstract. *The sensitivity of monolayers of Ilford L-4 nuclear emulsion to β^--particles is approximately 0.20 grain per particle emitted for H^3, and 0.025 grain per particle for P^{32}. The sensitivity for thick layers is 1.3 grains per tritium β^--particle emitted within the emulsion. In electron microscopic autoradiographs the maximum resolution is approximately 0.1 micron for H^3 and 0.3 micron for P^{32}.*

The energy of the charged particle emitted by a radioactive isotope affects both the resolution and the sensitivity of autoradiographs (*1*). As the energy of a β^--particle increases, so does its range in matter, thereby decreasing the resolution. Simultaneously, the rate of energy loss at the beginning of its trajectory is decreased, down to a minimum value obtained for approximately 1-Mev particles, and the particle has a lower chance of exposing a photographic grain near the source.

Tritium, one of the isotopes most commonly used in autoradiography, emits a β^--particle of 18-kev maximum energy, the lowest of any known isotope. It offers, therefore, excellent resolution and sensitivity. The maximum range of the H^3 β^--particle in dry Ilford emulsion is approximately 1.9 μ (corrected for curved path), and its rate of energy loss at the origin of the trajectory is 3.5 kev/μ (*2*). Phosphorus-32, also commonly used, has a β^--particle with a maximum energy of 1.72 Mev. Its maximum range in dry Ilford emulsion is of the order of 2100 μ, over 1000 times longer than tritium, and the rate of energy loss at the origin for the β^--particles of maximum energy is 0.53 kev/μ, almost seven times lower than tritium. Since the maximum ranges of almost all other common isotopes fall within these extremes, it is interesting to compare the sensitivity and resolution which can be achieved with these two isotopes. Thus limits can be established which will be useful in choosing a particular isotope.

The test objects were cells of *Escherichia coli* strain C, grown in M-9 minimal medium (*3*) supplemented with Casamino acids (0.5 g/lit.) and with uridine-H^3 (*4*) for at least seven generations, or in tris-glucose medium (*5*) supplemented with phosphate-free Casamino acids (0.5 g/lit.) (*6*) and with P^{32} (*7*) as inorganic phosphate. The specific activities were adjusted according to the amount of labeling desired. Cell concentrations were determined by means of Petroff-Hausser counter. Such determinations agreed with colony counts within 10 percent. Radioactivity measurements were made on planchets in a flow Geiger counter (*8*) operated with window for P^{32} and without window for H^3 (*9*) or in a scintillation counter (*8*). The efficiency for tritium counting was measured with a standard toluene-H^3 solution (*4*).

Autoradiographs were prepared as described previously (*10*). Ilford L-4 nuclear emulsion was used for both thick and thin emulsion layers. For examination under the light microscope the emulsion was developed for 5 minutes in D-19 at 20°C. For examination with the electron microscope the emulsion was developed for 1 minute at 20°C in the physical developer described by Caro and van Tubergen (*10*).

We first tried to establish the overall sensitivity of Ilford L-4 to β^--particles from tritium. Since our interest in quantitative autoradiography has been mostly with measurements of DNA in bacteria, it was convenient to use a DNA molecule of known molecular weight as a standard. Such a molecule is conveniently provided by the DNA of the bacteriophage lambda (*11*). We have measured, in the electron microscope, the length of this DNA molecule as 17.3 $\pm$ 0.6 μ, in agreement with the results of MacHattie and Thomas (*12*). The corresponding molecular weight, 33×10^6, was verified by measuring the thymine content per phage (*11*). Fully labeled phages were produced by infecting, in the presence of labeled thymidine, thymine-requiring *E. coli* cells, themselves previously labeled for seven generations. Thus all the thymine available to the phage was labeled and its specific activity known. Since the ratios of the bases in λ DNA is known (*13*), it is easy to calculate the number of disintegrations per phage.

After purification (*11*) the labeled phages were used to infect a culture of *E. coli* C, chosen because of its simple spherical shape. Infected bacteria, rather than phages, were used to provide a visual marker for the location of the phages on the slide. Ambiguities due to occasional clusters of background grains were thus avoided. Moreover, we were interested in measuring directly the efficiency obtained for DNA in bacteria, and high-resolution autoradiography showed (*14*) that soon after injection the λ DNA enters the bacterial nuclear region.

The average grain count per phage was obtained by fitting the observed grain count distribution over the labeled cells to a Poisson distribution (*15*). This average grain count increased linearly with time of exposure. The rate of increase, for thymidine-H^3 with a specific activity of 8.6 c/mmole, was 0.47 grain day^{-1} phage^{-1}. The calculated rate of emission of β^--particles was 16 per day per phage. The efficiency was thus 41 percent (0.41 grain per β^--particle emitted within a bacterium). The efficiency in terms of particles entering the emulsion was higher than 41 percent, because 50 percent of the β^--particles went toward the glass slide rather than the emul-

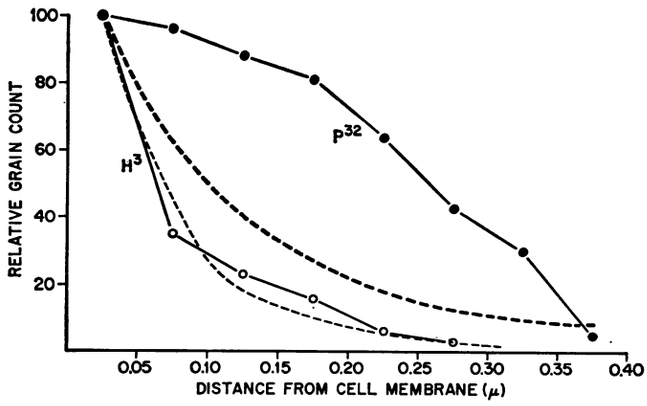

Fig. 1 (above). Distribution of grain counts versus distance from the cell membrane for cross section of labeled *E. coli* in electron microscope autoradiographs, developed in L-4 emulsion. Light dashed line, H^3-theoretical; open circles, H^3-experimental; heavy dashed line, P^{32}-theoretical; solid circles, P^{32}-experimental.

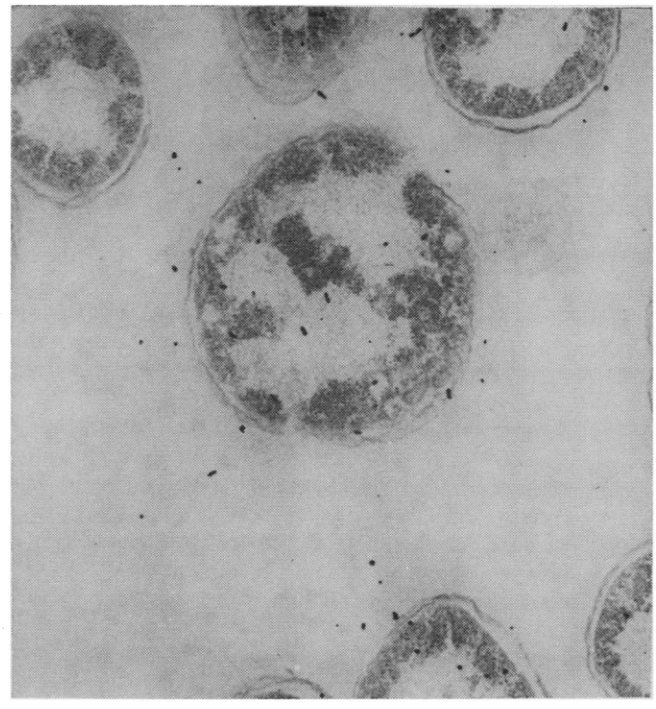

Fig. 2 (right). Electron microscope autoradiograph of *E. coli* labeled with P^{32}. Physical development (*10*); many of the grains are found over the cell or close to it. The label is presumed to be present in all the major bacterial structures. (× 50,000)

sion, and because some of the particles were absorbed within the bacteria themselves. This absorption was measured in a scintillation counter by comparing counts obtained from labeled bacteria with those given by a hot trichloroacetic acid extract from the same cells. We also measured it (*14*), using Ilford K-5 emulsion as a detector, by growing cells in a known, small concentration of leucine-H^3 until the leucine was exhausted from the medium and by comparing the grain count with the amount of label taken up per cell. All estimates of β^--particle absorption within cells agreed, ranging from 35 to 40 percent and averaging 38 percent.

It can thus be estimated that, for β^--particles reaching the emulsion, the overall efficiency would be 132 percent, or 1.3 grains per particle.

Another kind of information of some relevance to the methods of quantitative autoradiography, is the distribution of grains in the tracks from H^3 β^--particles. We measured this distribution by using weakly labeled bacteria and a short exposure time, so that the probability of having two decays within one cell was low (it was about 2 percent of the labeled cells, and since the cells were fairly large the double decays could usually be easily distinguished from tracks). A correction for background was introduced by counting grains over unlabeled cells. Out of 800 registered β^--particles, 78 percent gave one grain; 16.5 percent, two grains; 5 percent, three grains; and 0.5 percent, four grains. On this basis 100 β^--particles would give 128 grains, in agreement with the efficiency of 132 percent found above. Since in one case we were considering actual decays and in the other only those decays which produced at least one grain, it seems that practically all β^--particles reaching a thick emulsion give one or more grains. If, therefore, a crystal of L-4 emulsion is hit by a β^--particle in the energy range of tritium, the chance that a latent image will be produced is very high, approaching 100 percent.

Some experiments were performed to establish the sensitivity of a monolayer of L-4 emulsion. Films of L-4 emulsion, forming a monolayer of photographic grains, were applied by the loop method (*10*) over labeled bacteria and placed on glass slides. The area covered by the film included an electron microscope specimen screen for a check of grain distribution. The rate of emission of β^--particles per cell was determined in a flow Geiger counter. After suitable exposure the average grain count per cell was measured. Under the conditions used, background was negligible.

The sensitivities thus measured can only represent orders of magnitude, since the monolayers of grains are neither perfect nor perfectly reproducible. Accordingly the values obtained showed variations of 30 to 50 percent. For H^3 the maximum efficiency was of the order of 20 percent (0.20 grain per particle). Tracks were practically nonexistent. For P^{32} the efficiency was reduced to 2.5 percent, approximately. Tracks of four, five, or more grains were found on rare occasions. It seems therefore that, at the origin of the trajectory, the sensitivity of L-4 emulsion is approximately 8 times greater for H^3 than for P^{32}.

The values are quite reasonable. For H^3, if the efficiency of developable grains per hit were 1, the efficiency per decay would be roughly 25 percent (50 percent of the decays go in the wrong direction, and the emulsion contains about 50 percent, per volume, of silver halide); the value of 20 percent found is in good agreement.

For P^{32} one can consider that an electron at minimum ionization (1 Mev) produces 26 grains/100 μ in L-4 (*16*). Since a β^--particle going through an emulsion such as L-4 with a concentration of silver halide of 0.5 and a grain diameter of 0.13 μ will cross 5.78 grains/μ (*17*), approximately 0.045 grain is exposed per hit at minimum ionization. One would except on this basis an efficiency of 0.011. We find 0.025, which is not surprising since an appreciable fraction of the β^--particles from P^{32} is in a low energy range and gives therefore a higher probability of exposure.

We discussed previously (*1*) why a monolayer of silver halide grains will give optimum resolution, for a given emulsion. We find now, that with such a monolayer of L-4, the efficiency for a β^--emitter will range from 0.2 to 0.02 depending on the energy. Similar results might be expected for other nuclear emulsions with equally high sensitivity of the individual crystals. Since material suitable for electron microscopy must be thin, and therefore the amount of radioactive material included correspondingly reduced, sensitivity is one of the major points to be considered when planning an experiment.

We have calculated and demonstrated experimentally that the resolution obtained for a point source of H^3, using L-4 monolayers, is about 0.1 μ (*1*). Such calculations can be extended to grain-count distributions around a uniformly labeled circular area. The distribution calculated for H^3 is shown in Fig. 1, in which the expected grain counts are plotted against distance away from the circumference of a labeled circle 1 μ in diameter. Experimental points were obtained by measuring grain counts around cross sections of bacteria approximately 1 μ in diameter and labeled uniformly with tritiated uridine.

The various assumptions which had been made in the calculations of resolution for tritium are no longer valid with P^{32}. For all practical purposes the range of β^--particles from P^{32} can be considered infinite. There is probably very little change in the path of the particles near the origin due to the passage through silver bromide; the probability that a grain hit by a particle will be exposed is no longer 1, as we have seen before. If we make the simplifying assumptions that, with P^{32}, the particles are not absorbed or deflected and that the probability of exposure of a grain hit by a β^--particle remains constant over a fairly long range, then the probability of exposing a grain in a monolayer becomes exclusively dependent on the solid angle from which it is seen by the source.

The calculated distribution, while not as favorable as for H^3, would still give very good resolving power. We find, however, that, whereas the experimental points for H^3 fit the theoretical curve quite well, those for P^{32} do not. The actual distribution is much broader than expected. One possible explanation is that the probability that a grain, hit by a β^--particle, be exposed increases with the distance from the source. This is to be expected, but it does not seem that the energy loss in a path of 0.3 to 0.4 μ in tissue or emulsion is sufficient to affect the results significantly. The discrepancy is therefore unexplained.

The situation for P^{32}, while clearly worse than for H^3, is not hopeless (Fig. 2). For a total of 650 grains counted over circular cross sections of bacteria about 1 μ in diameter, 61 percent of the grains were over the cells, 75 percent within 0.1 μ of the cell, and 87 percent within 0.2 μ. It is therefore clear that structures of this size could be resolved fairly easily in tissue sections. By comparison with the results for tritium (Fig. 1), the resolution obtained can be set roughly at 0.3 μ.

Because of its short half-life, high specific activities can be obtained with P^{32}, overcoming, to some extent, the low sensitivity of the emulsion to it. It seems therefore that the inferior resolution and sensitivity obtained with P^{32} do not preclude its use in high-resolution autoradiography. Since practically all the isotopes used in biology emit β^--particles of lower energy than P^{32}, it can be safely predicted that they will give results falling somewhere between P^{32} and H^3, and that in high-resolution autoradiographs with L-4 emulsion they will give a sensitivity of 2.5 to 20 grains per 100 β^--particles and a resolution of 0.1 to 0.3 μ, depending on the energy spectrum of the β^--particle emitted.

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Reduction of Trimethylene Dipyridyl with Illuminated Chloroplasts

Abstract. *Chloroplasts photochemically reduce 1,1′-trimethylene-2,2′-dipyridylium dibromide and concurrently form adenosine triphosphate. Reduced trimethylene dipyridyl in darkness will reduce spinach ferredoxin,* Clostridium pasteurianum *ferredoxin, nicotinamide-adenine dinucleotide phosphate, and other viologen-type dyes.*

The present status of the terminal reaction sequence in photosynthetic electron transfer by plant chloroplasts can be summarized in the following scheme (*1*) in which reduced nicotinamide-adenine dinucleotide phosphate (NADPH) is the terminal product.

$$\text{Photochemical system} \cdots > \text{Spinach ferredoxin} \cdots \blacktriangleright$$

$$\text{NADP reductase} \rightarrow \text{NADPH}$$

There has been considerable speculation regarding the nature of the components prior to the reduction of spinach ferredoxin (*1, 2*). With the important demonstration that the standard electrode potential (E'_0) at *p*H 7.55 of spinach ferredoxin is near that of the hydrogen electrode (*3*) it was supposed that spinach ferredoxin is reduced either directly by the photochemical system or by way of an unknown component with an E'_0 below that of the hydrogen electrode. Prior to this report the compound with the lowest redox potential reduced by chloroplasts was methyl viologen, with an E'_0 of −446 mv (*4*). Data will be presented on the photochemical reduction with spinach chloroplasts of 1,1′-trimethylene-2,2′-dipyridylium dibromide with an E'_0 of −550 mv and the concurrent formation of adenosine triphosphate.

The standard reaction mixture con-