TECHNICAL PAPERS

The Quantitative Theory of Autoradiography Illustrated Through Experiments With P^{32} in the Chick Embryo

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In the experiments on the effects of vitamins on phosphorus metabolism in the chick embryo, our laboratory has undertaken a study of the autoradiographic technique as an excellent means of determining the distribution of P^{32} between the embryo and blastoderm. The techniques and interpretations of our results based upon some simple principles of photographic sensitometry reveal that the procedure is capable of yielding quantitative results.

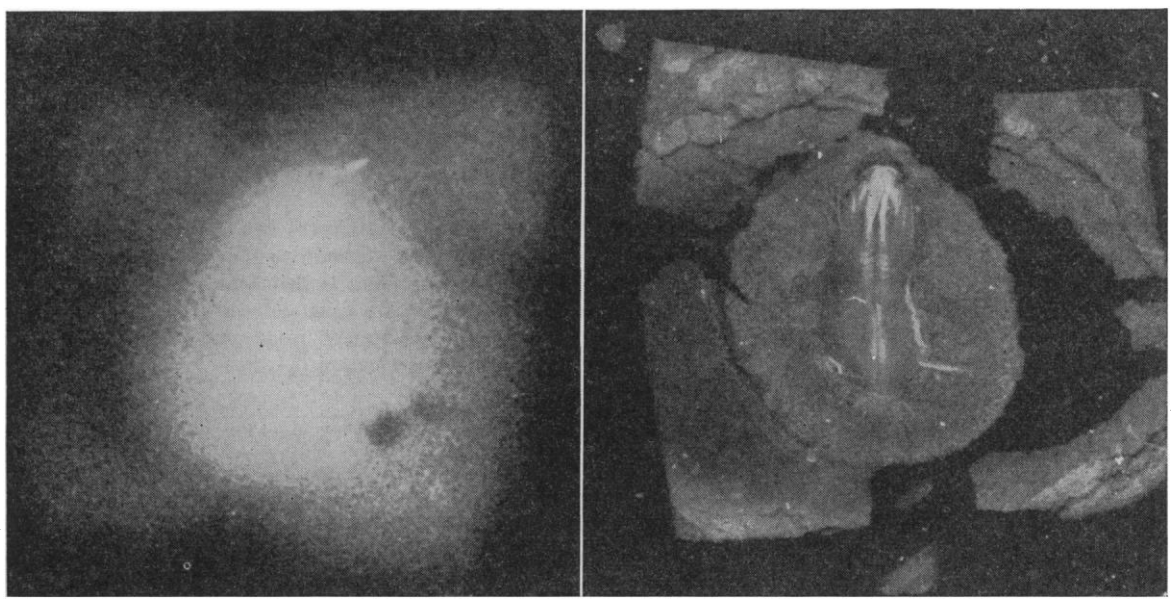

FIG. 1. The autoradiograph on the left is a mirror image of the 24-hr chick embryo on the right. Exposure was made through the glass coverslip of the mounted embryo. The dark spots on the lower right side of the autoradiograph are blemishes. The magnification in both photographs is approximately 10 ×.

Large brown New Hampshire eggs from the University of Maryland farm were sterilized on the blunt end with Lugol's solution and a small hole drilled through the shell without puncturing the outer shell membrane. A No. 27 needle on a tuberculin syringe was used to place 0.05 ml of NaH_2PO_4 in the egg white below the air chamber. The solution had an activity of 27.6 μc/ml (*i.e.* each egg received 1.38 μc).[2] These injections were performed on the afternoon of March 5, 1948. The eggs were allowed to stand for 4 hrs and were then placed in the incubator. After 24 hrs, a group was removed. The yolk was floated into warm Ringer's solution. The embryo with a portion of the blastoderm was snipped clear. The layer was washed and placed in a watch glass of warm Ringer's, where it was perfused with Bouin's solution. Fixation in Bouin's was continued for 24 hrs. The embryos were washed in 70% alcohol with a little lithium carbonate added, after which they were stained in borax carmine, dehydrated in dioxane, and mounted in Clarite.

Strips of Agfa Triple S Pan film were placed over the mounted embryos. The slides with the film were wrapped in black paper and put into a light-tight box on March 22. The film was removed on April 5, approximately 14.7 days later, and developed following standard procedure, to a gamma of one.

Good autoradiographs were obtained for all mounts—the 24-, 48-, and 72-hr embryos (see e.g. Fig. 1).

[1] We are indebted to our colleagues for many suggestive discussions of this work. The program has been assisted by the Office of Naval Research.

[2] These values were obtained by comparison with the Bureau of Standards reference sample of Radium D and E, No. 83. Our values on the intercomparison tests of I^{131} conducted by the National Bureau of Standards were consistently below the accepted value by approximately 43%. If the same ratio exists for our P^{32} measurements, the activity of the solution injected would be nearer 2.4 μc/egg.

There was a 17-day interval between the injection of the P^{32} solution and the beginning of the exposure. The equivalent activity which would have been in the whole egg at the beginning of the exposure (*i.e.* on March 22, assayed as 1.38 μc on March 5) was 0.49 μc/egg.

Although our initial concern has been with a procedure for obtaining autoradiographs with P^{32} in the chick embryo, the interpretation of our results leads directly to a simple quantitative theory which may have wider applicability in autoradiography with isotopes of not too long half-lives.

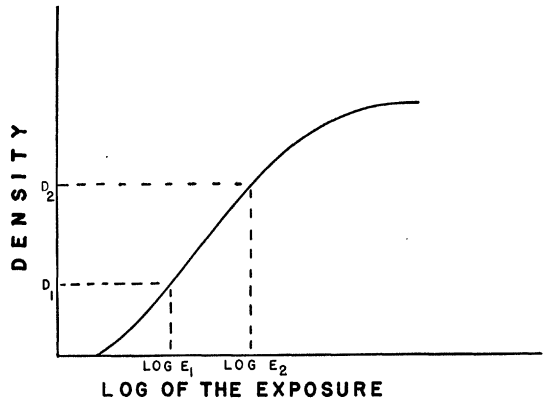

FIG. 2. An idealized Hurter and Driffield curve. The exposure is usually expressed in meter-candle-sec.

In photographic sensitometry, the Hurter and Driffield (H & D) characteristic curve expresses the relation between the density and the logarithm of the exposure. An idealized H & D curve is reproduced in Fig. 2. The slope of the linear portion is the gamma (γ) of the emulsion. This slope is not only a function of the emulsion but depends upon the developer, temperature, and time of development.

From two points on the linear portion of the H & D curve we have

$$\gamma = \frac{D_2 - D_1}{\log E_2 - \log E_1}. \tag{1}$$

In autoradiography, a reasonable definition of exposure is

$$dE \propto I_0 e^{-\lambda t}\, dt,$$

where I_0 is the initial activity of the radioactive substance in μc or counts sec^{-1} and $e^{-\lambda t}$ is the decay factor for the radioactive isotope present in the sample. The formulation leads to

$$E = KI_0 \int_0^t e^{-\lambda t}\, dt = \frac{KI_0}{\lambda}(1 - e^{-\lambda t}) = E_{max}(1 - e^{-\lambda t}), \tag{2}$$

where t is the time the film is in contact with the radioactive material and K is a factor independent of time but dependent upon the geometry, the film characteristics, and the emanations from the radioactive substance. If I_0 is expressed in counts sec^{-1}, then for E to be in meter-candles-sec, K would have the units of (meter-candles-secs) $counts^{-1}$. Thus, K converts counts or disintegrations to the usual sensitometry unit.

Substituting the definition of E from equation (2) into equation (1) yields

$$\gamma = \frac{D_2 - D_1}{\log I_0(2) - \log I_0(1)},$$

where I_0 (1) designates the initial intensity in the region marked (1) where the density is D_1 with corresponding definitions for the other symbols. Thus, if the film is developed to a known γ and the density of two regions on the linear portion of the H & D curve measured, we would have the relative amounts of the radioactive isotope deposited in the two regions.

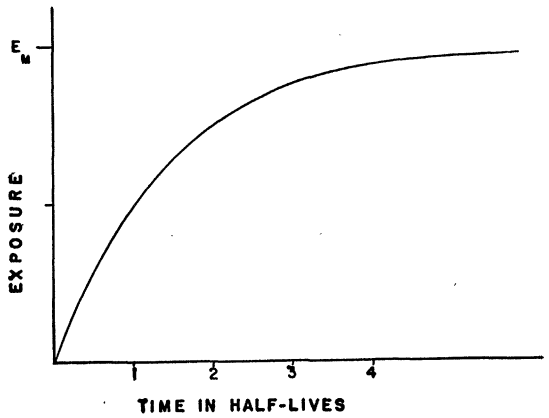

FIG. 3. A graph of the exposure as a function of half-life under the assumptions of this paper. E_m is the maximum exposure.

The graph of equation (2) in Fig. 3 is most instructive, for it relates the exposure to the time in contact. The most interesting point is that contact for a half-life results in an exposure equal to half that which would be received on indefinitely prolonged exposure. This is easily seen from equation (1), when

$$\gamma = \frac{D_2 - D_1}{\log E_m - \log E_{m/2}} = \frac{D_2 - D_1}{\log 2}.$$

Thus, a very long exposure would lead to a difference in density of only 0.3 γ.

In the autoradiograph of Fig. 1, the intense middle section of the embryo has a density D_2, and the peripheral section, the region of the blastoderm, has a density D_1. These densities were read on a Weston Photographic Analyzer, model 877, yielding $D_2 = 0.79$ and $D_1 = 0.33$. The background of the exposed film was taken at density zero. With these values, equation (1) gives

$$I_0(2) = 3\, I_0(1).$$

That is, the region of the embryo has about three times as much radioactive phosphorus per unit area as the blastoderm. At this stage of development, the thicknesses of the two regions are comparable; hence we may conclude that the embryo is concentrating the available phosphorus at a fairly high rate.

Further experiments on direct film calibration with P^{32} are in progress in our laboratory.