the abdominal cavity. In all, Kupffer cells throughout the liver contained large amounts of ThO_2. With the exception of the 15-day specimen, there were free loaded macrophages in all vessels of the liver, and some granules of ThO_2 in parenchymal cells. The spleen had, in every case, considerable numbers of ThO_2-charged macrophages located within distinct sinuses. The lungs contained cells with ThO_2 in capillaries, in the tissues outside the capillaries, free in the air passages, and free in the lumina of larger blood vessels. The kidneys had the same appearance as those of the frogs described above.

The stomach and gut differed from those of the frogs in showing very few loaded macrophages in the lumen or in the wall, and in having no appearance whatever of discharge of ThO_2 through the epithelium. The same was observed in relation to the external skin. There was no sign of passage of ThO_2 into the lumina of the glands, and very little ThO_2 in subcutaneous macrophages. The epithelium of the cloaca, however, although without ThO_2 in or beneath the surface layer, was thickly covered with mucus containing vast numbers of heavily loaded macrophages, as well as fragments of such cells and free granules of ThO_2. The cloaca of the frogs was not studied.

In the one *Desmognathus* (lacking both lungs and gills), ThO_2 was distributed in exactly the same manner as in the adult *Ambystoma,* except, of course, for the lung. Otherwise, the difference between the two was strictly one of degree. The kidney and cloaca had much heavier and denser amounts of ThO_2 in *Desmognathus.*

Larval forms: These in general agree with the adults in the distribution of ThO_2 observed. The liver could not be distinguished from that of adults on the basis of thorium distribution. The larval spleens contained some large masses of Thorotrast granules free in sinuses, along with larger quantities in macrophages. The gut contained some ThO_2 in macrophages in the walls and lumen, but none of the larvae gave evidence of transfer of Thorotrast through the epithelium.

The kidneys differed slightly from those of adults in showing as a constant feature granules of material that seemed to be ThO_2 within cells of the proximal convoluted segments of the tubules, apparently being discharged. Larval skin had the appearance of adult skin. The *R. catesbiana* larva had both gills and lungs, but the larval urodeles had gills only. The *Rana* lung had not yet begun to function in respiration. In all four larvae, the gills were observed to contain filled macrophages in capillaries, in the tissues outside the capillaries, and on the external surface of the gill epithelium, either sticking directly to the latter or trapped on it in attached flakes of mucus. The lungs of the *R. catesbiana* tadpole contained ThO_2 in macrophages in capillaries and in the tissues outside the capillaries, but none in the lumen of the lung.

In no specimen studied was there any significant amount of ThO_2 in skeletal muscle. Isolated macrophages located between muscle fibers contained a few granules only.

The presence of ThO_2-filled macrophages in both portal and hepatic veins suggests, as more comprehensive data have done for the mammal, that filled macrophages move through the liver, presumably largely from the spleen.

The amphibian lung and gill observations further suggest that whichever of these is active in respiration is also responsible for the release of a part at least of the ThO_2 introduced into the body.

Furthermore, the presence of granules of ThO_2 free and in macrophages in various parts of the kidney—i.e., glomeruli, lumina of tubules, cells of the proximal convoluted segment, and also in the cloaca—suggests that the kidney is at all times active in the removal of circulating Thorotrast. The one *Desmognathus,* lacking both lungs and gills, showed a much higher degree of saturation in the kidney than other specimens and much more ThO_2 in the cloaca. This indicates that in the absence of lungs and gills the kidney is the chief organ of disposal of ThO_2. This is in contrast to the situation in mammals, in which the kidney removes a very small part of any ThO_2 injected, and the lung, absent in *Desmognathus,* is the chief organ of Thorotrast removal.

That the skin, which serves also for respiration, may serve as an organ for disposal of macrophages carrying ThO_2 is suggested by the finding of such cells in the lumina of skin glands in one of the frogs. However, the rarity of the observation (one frog, no urodeles) indicates that this route is not an important one.

Further investigation of this problem in a more quantitative manner would be highly desirable for comparison with the situation in mammals.

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Radioactivity Induced in Yeast by 100 Mev X-Radiation[1]

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Mayneord, Martin, and Layne (*1*) have shown that 24 mev of x-radiation induces a small amount of radioactivity in tissue, ascribed to production of O^{15} and C^{11} by (γ,n) reactions.

In view of the fact that Mayneord *et al.* were working near the thresholds of these reactions, and that maximum yields are expected at much higher voltages

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(2), it was deemed desirable to extend this study to 100 mev, well above all known threshold and resonance levels.

Computations based on analysis of yeast and on physical data for cross sections (3), resonance energies (2), and x-ray spectrum indicated that the dose rate in this material that is due to radioactivity would be of the order of $2 \times 10^{-5} \times$ the dose rate that is due to direct absorption of the x-radiation.

Blocks of yeast were irradiated with x-rays from the 100 mev betatron. Samples were counted by means of a special device built by one of us (G.C.B.) for the study of short-lived activities, with which counting could begin 0.2 sec after a precisely timed irradiation. The findings were:

1. No short-lived activities of $0.5 < T < 125$ sec are induced.
2. The resulting activity is due to O^{15}, C^{11}, and N^{13}. Their initial proportions, 1.00, 0.013, and 0.16, are in agreement with the above computations for the counting geometry employed.
3. Type K film placed in the yeast recorded less than 0.005 r after an irradiation to a total dose of 3050 r, the film exposure being such as to collect 60% of the total delayed dose. This is obviously a negligible fraction of the original dose.

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Developmental Forms of Vaccinia Virus[1,2]

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The mode of vaccinia virus reproduction is unknown. Wyckoff (1) has described certain bodies in vaccinia-infected cells, thought to be associated with virus production, which were in the size range of, but less opaque than, elementary bodies (mature, infective virus particles). Gaylord, Melnick, and Bunting (2) recently pointed out that vaccinia elementary bodies appear as discrete particles only in large, and presumably old, inclusions, whereas elementary bodies in small inclusions were obscured by a dense matrix. The observations here correlate these findings and extend them to show a relationship between the less opaque bodies and mature virus.

Thin sections of chick chorio-allantoic membrane infected with vaccinia were prepared for electron microscopy, according to the method of Palade (3), using osmium tetroxide buffered at pH 7.3, with veronal-acetate for fixation. One drop (0.05 ml) of virus suspension was inoculated on the membrane of 15-day eggs and harvested after 72 hr. Nonspecific lesions, caused by introducing a like amount of an isotonic salt solution on 15-day membranes, were excised after 72 hr and used for controls. The tissues were imbedded in methacrylate, which was not removed from sections except for comparison studies. Some sections were flattened by floating on warm (60° C) water; others were suitable for observation immediately.

The ectoderm of 72 hr membranes was heavily infected from the surface to the basal layer, but no virus was seen in the mesoderm or entoderm. Mitochondria were numerous in both infected and control cells, occurring as rounded, swollen forms in the ectoderm, as long filaments in the fibroblasts of the mesoderm, and as short, thick filaments, often fragmenting, in the entoderm. They were easily distinguished from mature virus because of their larger size, irregular shape, and low electron density. Elementary bodies appeared as dense, uniform, discrete particles in great numbers throughout most of the cytoplasm. In very thin sections the hollow interior of the thick-walled mature virus particle previously described (2) was readily visible.

The cytoplasm in the central perinuclear region of many infected cells was more dense than normal (Fig. 1). Such areas were usually well demarcated and are considered to be the matrix observed in formalin-fixed sections (2). Within these areas round or oval-shaped bodies were seen (Figs. 3, 4) that had the dimensions of mature virus or were slightly larger in diameter. The bodies were always less dense than mature virus and presented a variety of internal structures. Some appeared to be empty circles bounded by a delicate membrane (Fig. 6); in others a small dot of dense material appeared (Figs. 3–6, 8). The dense inner body was present in sizes ranging from the barely visible to a diameter approaching that of mature virus and was located either centrally or eccentrically. At other times the whole body was filled with material of low electron density (Fig. 7). In such cases the limiting membrane was still visible. Many of the bodies were half or only partially filled with the low density material, and in these forms the denser small mass could sometimes be seen (Fig. 8). At times, when the internal body approached the size of an elementary body, it was also "empty," giving the appearance of concentric circles (Fig. 3).

All the above-described bodies are considered by us to be virus developmental forms in different stages of maturation. They were regularly seen in infected cells and never in control cells. They occurred most frequently in the perinuclear matrix area of a cell, and less frequently in areas where mature virus was highly concentrated. Their size was of the order of magnitude of mature virus, being somewhat larger. Mature virus was oval-shaped, with major axes of 200–

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[2] The data for this paper form part of a dissertation to be presented by William H. Gaylord, Jr., in partial fulfillment of the requirements for the Ph.D. degree, Yale University.