(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.

References

1. MAYNEORD, W. V., MARTIN, J. H., and LAYNE, D. A. *Nature*, **164**, 728 (1949).
2. BALDWIN, G. C., and KLAIBER, G. S. *Phys. Rev.*, **73**, 1156 (1948).
3. FRIEDLANDER, G., and PERLMAN, M. L. *Ibid.*, **74**, 442 (1948); LAWSON, J. L., and PERLMAN, M. L. *Ibid.*, 1190.

Manuscript received June 19, 1952.

Developmental Forms of Vaccinia Virus[1,2]

William H. Gaylord, Jr., and Joseph L. Melnick

Section of Preventive Medicine and
Department of Microbiology,
Yale University School of Medicine,
New Haven, Connecticut

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.

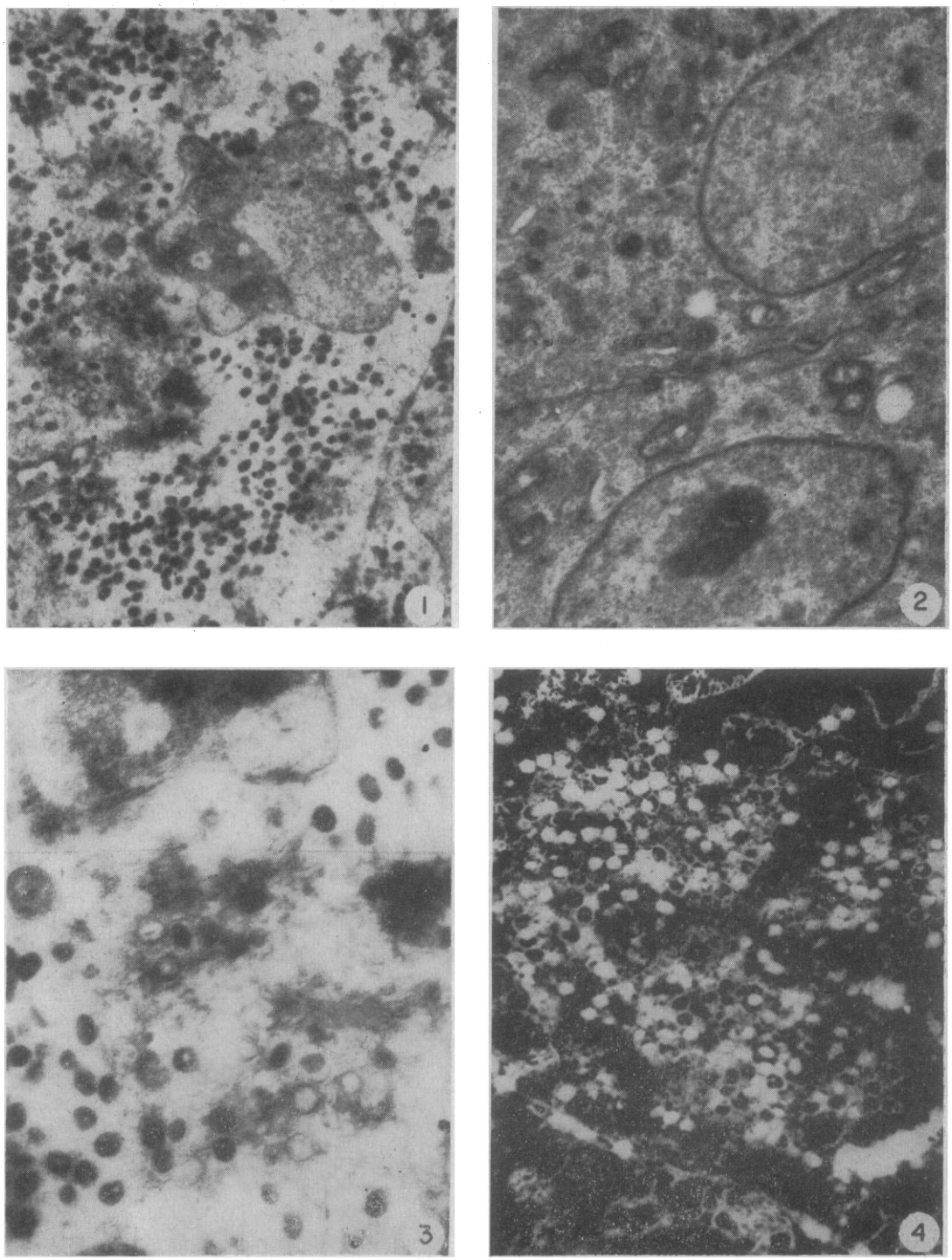
The ectoderm of 72 hr membranes was heavily infected from the surface to the basal layer, but no virus was seen in the mesoderm or entoderm. Mitochondria were numerous in both infected and control cells, occurring as rounded, swollen forms in the ectoderm, as long filaments in the fibroblasts of the 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–

[1] Aided by grants from the Fluid Research and James Hudson Brown Memorial Fund of Yale University School of Medicine.

[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.



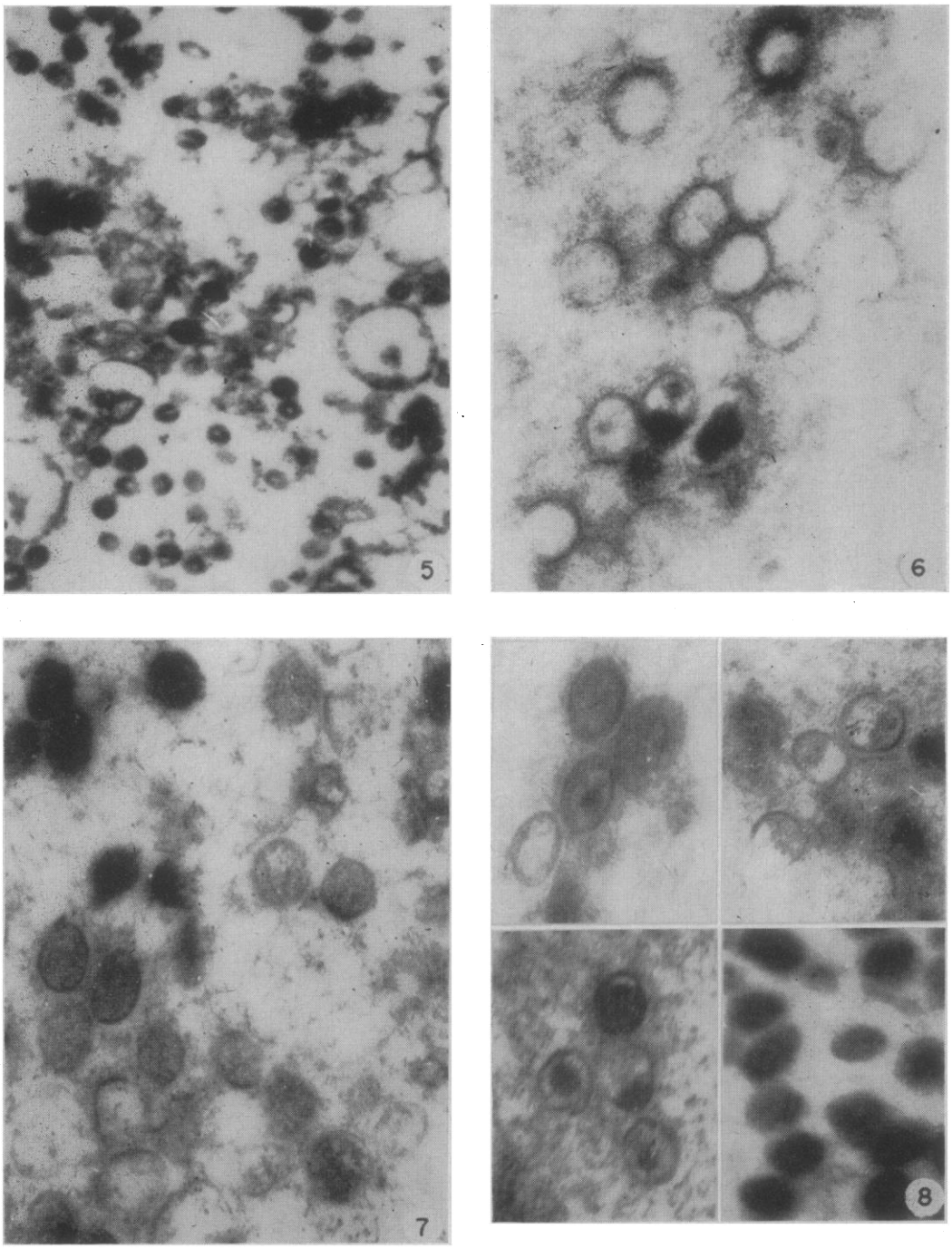
FIGS. 1–4. 1—Ectodermal cell infected with vaccinia virus. Area at lower left of nucleus is matrix within which "developmental" forms predominate. Mature virus appears as black particles. × 8000.

2—Portions of two control cells. × 8000.

3—Matrix area from cell in Fig. 1 taken at greater magnification. Close examination, especially of original plates, reveals more than 25 virus developmental bodies imbedded in matrix. × 18,000.

4—Matrix area of a cell from which methacrylate had been removed. Many stages of developmental bodies can be seen. Negative print, palladium-shadowed. × 11,000.

250 mμ and minor axes of 110–150 mμ. The less opaque bodies were usually circular, with diameters of 250–290 mμ. Oval shapes attained long axes of 400 mμ. They presented a remarkable degree of uni-



FIGS. 5–8. 5—Area showing developmental bodies in different stages, as well as mature virus particles. The latter have greater electron density and "hollow" interiors. × 16,000.
6—Higher magnification of virus developmental bodies, showing a predominance of "hollow" forms. × 37,500.
7—Developmental bodies, several filled with homogeneous material of low electron density. × 42,000.
8—Virus developmental bodies in different stages of growth. Upper left, body filled with homogeneous, low-density material, one of which has a denser internal structure. Upper right, partially filled bodies. Lower left, larger internal structures, presumably a more advanced stage of development. Lower right, mature virus particles from another area of the same cell. All × 40,000.

formity, unlike mitochondria or other normal cellular constituents or of the cross-sectioned endoplasmic reticulum described by Palade (*3*).

It would appear that each "developmental body" gives rise to a single mature virus particle (classical elementary body). The smaller internal mass seems to

develop in size, at the expense of the less dense material (Fig. 8), to emerge as a mature particle. The picture of mature virus forming from larger, less dense bodies imbedded in a matrix material is similar to the situation described by Banfield, Bunting, Strauss, and Melnick (*4*) for molluscum contagiosum. In retrospect, some of their micrographs may be reinterpreted in the light of the present findings. Close re-examination of their original plates (*4*) reveals that in molluscum contagiosum the "holes" in the cytoplasmic matrix contain dense bodies ranging from the barely visible to the size of mature virus particles, with the entire "virus developmental" body seemingly encased in a fine membrane. We have also observed similar developmental forms (precursors of mature virus) in cells infected with ectromelia virus, another member of the pox group.

References

1. WYCKOFF, R. W. G. *Proc. Natl. Acad. Sci., U. S.*, **37**, 565 (1951).
2. GAYLORD, W. H., JR., MELNICK, J. L., and BUNTING, H. *Proc. Soc. Exptl. Biol. Med.*, **80**, 24 (1952).
3. PALADE, G. E. *J. Exptl. Med.*, **95**, 285 (1952).
4. BANFIELD, W. G., *et al. Proc. Soc. Exptl. Biol. Med.*, **77**, 843 (1952).

Manuscript received June 18, 1952.

Crown Gall Suppression by Anti-Auxin[1]

Paul E. Waggoner and A. E. Dimond

The Connecticut Agricultural Experiment Station, New Haven

The continued depression of auxin level in plants, following exposure to ionizing radiation (*1*), has been suggested (*2*) as a mechanism by which radiation suppresses crown gall caused by *Agrobacterium tumefaciens*. If this suggestion is correct, then other agents that lead to auxin destruction should suppress galls.

This prediction was tested by exposing carrot roots inoculated with *A. tumefaciens* (*3*) to maleic hydrazide (MH), an agent that increases enzymatic destruction of indolacetic acid (IAA) (*4*). A carrot root was surface-sterilized, cut transversely into 12 slices ½ cm thick, and a slice placed apical-end down on 2% water agar in each of 12 Petri plates. Similar slices from 3 other roots were also placed in each plate. The surfaces of all slices were inoculated at the same time with an aqueous suspension of *A. tumefaciens* (Riker's strain A-6). The 4 slices in a plate were transferred at the times shown in Table 1 to another plate, where the agar solution contained a 2.67×10^{-3} *M* concentration of the diethanolamine salt of MH.[2] After incubation at room temperature for 12 days, the galls on the slices from each root were ranked according to size (Table 1).

Evidently crown gall development is suppressed by MH applied as late as 168 hr after inoculation. Similar results were obtained in experiments using plants growing in the greenhouse. When the roots of tomato plants were dipped in 5.24×10^{-3} *M* solution of MH salt 8 days after, or at the time of, inoculation, only small galls developed, whereas large galls developed on untreated plants.

TABLE 1

RANK OF GALL SIZES ON CARROT ROOT SLICES TRANSFERRED TO A 2.67×10^{-3} *M* SOLUTION OF DIETHANOLAMINE SALT OF MH

Transfer, hr before (−) or after (+) inoculation	Root			
	1	2	3	4
−168, −120, −24				
0, +4, +8	0*	0	0	0
+ 24	0	0	4	0
+ 96	1†	2	1	0
+168	2	1	2	0
No. MH	3, 4‡	3, 4, 5	3, 5, 6	1, 2, 3

* No gall.
† Smallest gall on root 1.
‡ Contaminated.

The effect of MH upon multiplication of the pathogen was determined by growing it in a 2% glucose and 0.2% yeast extract medium containing concentrations of the MH salt up to 28.8×10^{-3} *M*. No relation between turbidity and MH concentration was evident after 24 hr at room temperature.

These experiments show that MH, which is known to increase enzymatic destruction of IAA (*4*), suppresses gall formation by affecting tumor enlargement, not by affecting alteration of normal to tumor cells or by affecting pathogen multiplication. In these respects MH suppresses galls in the same way as does ionizing radiation. The hypothesis that radiation suppresses galls by depressing the auxin level has thus led to a prediction borne out by experimentation. Whether the hypothesis accounts for the entire radiation effect remains to be seen.

References

1. SKOOG, F. *J. Cellular Comp. Physiol.*, **7**, 227 (1935).
2. WAGGONER, P. E., and DIMOND, A. E. *Am. J. Botany* (in press).
3. DE ROPP, R. S. *Ibid.*, **37**, 352 (1950).
4. ANDREAE, W. A. Mimeo. Abst., Am. Soc. Plant Physiol. Meeting, Ithaca, N. Y. (1952).

[1] Research conducted under contract AT(30-1)-580 with the Atomic Energy Commission.

[2] The MH was supplied by the Naugatuck Chemical Division, U. S. Rubber Company.

The Effect of Triphenyltetrazolium Chloride on Oat Embryo Respiration[1]

Glyn O. Throneberry and Frederick G. Smith

Department of Botany and Plant Pathology, Iowa State College, Ames

The reduction of tetrazolium salts has been widely

[1] Journal Paper No. J-2115 of the Iowa Agricultural Experiment Station, Ames. Project 1083. This study was made in part under authority of the Agricultural Marketing Act of 1946 (RMA, Title II) and was carried out in cooperation with the Grain Branch, Production and Marketing Administration, U. S. Department of Agriculture.