TABLE II. Effects of Pyrithiamine and Pantoyltaurine and Corresponding Vitamins on Biosynthesis of Ascorbic Acid from Glucose Cycloacetoacetate (GCA) during Germination of Mung Beans.

	Fresh basis					
	mg/100 seedlings after			mg/100 g after		
Substances in germinating medium	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Nil	1.85	1.92	2.2	6.8	10.36	11.2
Glucose cycloacetoacetate (GCA)	1.46	2.8	2.1	11.48	16.8	24.6
Idem + pyrithiamine	1.36	2.23	3.51	10.88	13.38	21.06
" + " + thiamine	1.45	2.82	4.2	11.6	16.92	25.2
" + thiamine	1.53	3.25	4.4	12.24	26.0	27.65
" + pantoyltaurine	1.3	2.4	3.9	10.4	14.4	23.5
" + " + pantothenate	1.65	2.86	4.4	13.2	17.16	26.4
" + pantothenate	1.8	3.25	4.5	14.4	26.0	28.7

Concentrations: Glucose cycloacetoacetate, 100 mg/10 cc medium and 200 PPM each of pantoyltaurine, pyrithiamine, thiamine and calcium pantothenate.

enzymic biosynthesis of ascorbic acid from glucose cycloacetoacetate is indicated. These vitamins may be taking part both in condensation of glucose with acetoacetate to form GCA and in the further reaction leading to biosynthesis of ascorbic acid. In absence of these vitamins, the condensation reaction is hampered resulting in a low value of combined sugar, as reported previously, and consequently in lower output of ascorbic acid.

Summary. The anti-vitamins, *i.e.* pantoyltaurine and pyrithiamine, inhibited ascorbic acid biosynthesis from acetoacetate and glucose in germinating mung beans indicating a blocking of the condensation reaction of acetoacetate with glucose to form GCA. The corresponding vitamins reversed the inhibition by antivitamins. That pyrithiamine and pantoyltaurine inhibited synthesis of ascorbic acid in presence of GCA, and the corresponding vitamins reversed the inhibition. Cocarboxylase and CoA, of which thiamine and pantothenic acids are the constituents, are involved in both the condensation reaction of glucose with acetoacetate to form GCA and in the further metabolism of GCA to ascorbic acid.

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Received May 18, 1959. P.S.E.B.M., 1959, v101.

Ultrastructure and Site of Formation of Rabbit Papilloma Virus. (25022)

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Since its discovery in 1933(1), numerous attempts have been made to characterize physically the rabbit papilloma virus. The early conclusions from sedimentation, diffusion and viscosity measurements of purified virus protein preparation indicated an aspherical particle with a molecular weight of 47,100,000(2).

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In later studies with the electron microscope, where shadowed dispersions were examined, the virus particle was found to be essentially spherical with a diameter of 40 to 45 mμ(3,4). By using fluorescent labelled antibodies, Noyes and Mellors(5,6) have demonstrated that the virus is found only in the more keratinized cells of the epidermis. We now report electron microscopic studies of thin sections of tumor tissue and of purified ultracentrifuged pellets.

Materials and methods. Warts from Kansas cottontail rabbits which had been stored in glycerine-saline at 0 to 5° for 1 to 6 years, as well as warts experimentally induced in cottontail and domestic rabbits, were studied. With a stereoscopic microscope blocks were dissected and oriented so that thin sections could be cut which contained the dermo-epidermal junction and extended well up into the tumor. In this manner electron microscopical localization of the virus was possible. *Purification procedure.* Five g of glycerinated warts from which the dermis had been stripped were quite finely minced by chopping with razor blade on glass plate. The fragments, suspended in buffered saline (pH 7.2), were finely ground in Potter-Elvehjem homogenizer. The thick grey brei was then subjected to repeated cycles of fluorocarbon emulsification and low speed sedimentation using Vir-Tis homogenizer (45,000 rpm) and a refrigerated centrifuge (300 g)(7,8,9). Cycles were repeated until greyish discoloration was no longer present in sedimented emulsion. At this point the faintly opalescent aqueous supernatant was centrifuged at 40,000 rpm (100,000 g) for 1 hour to yield a pellet. The extreme bottom of pellet was dark, indicating presence of some melanin granules. The major part of pellet was gelatinous consisting of small freon micells. A white coat, thicker at upper side of the pellet, overlaid the gelatinous layer. Fixation with 1% buffered OsO_4 caused the white coat to turn brown. The upper thick portion of this layer was carefully isolated, dehydrated with alcohol and embedded in methacrylate. Thin sections were examined in the electron microscope.

Results. Both the embedded tissue and embedded pellets revealed profiles of a relatively uniform class of particle having diameters of 25 to 35 mμ. The particles in the 2 types of preparation were entirely similar. Sections containing the particles are illustrated in Figs. 1 to 3.

Location of virus. Virus was found sporadically in cells from a short distance above basal layer to the surface, although it was more difficult to identify in most cornified cells. Determination of the original exact intra-cellular location was impossible because of lack of preservation of fine structure of glycerinated tissue. Viral particles were present as a pool lying in the central portion of cells among fine keratin fibers and melanin granules (Fig. 1). Cell boundaries were thick and heavily keratinized.

Structure of virus particles. The 4 different profiles in Fig. 2 may possibly result from

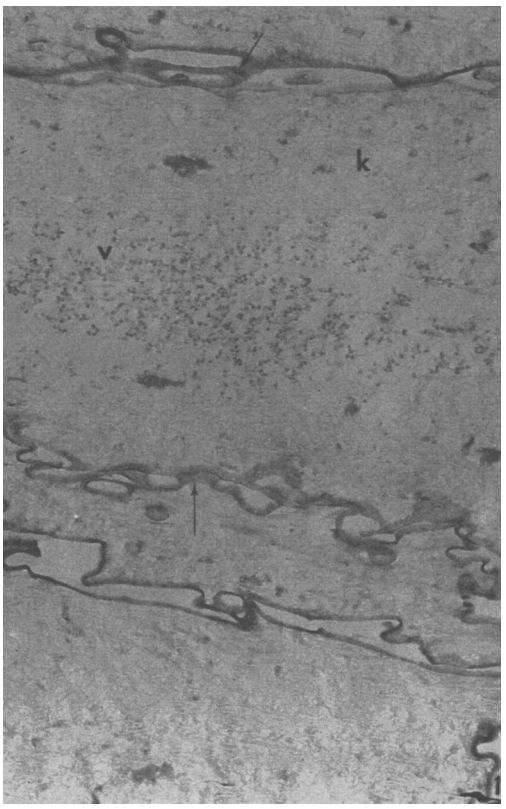
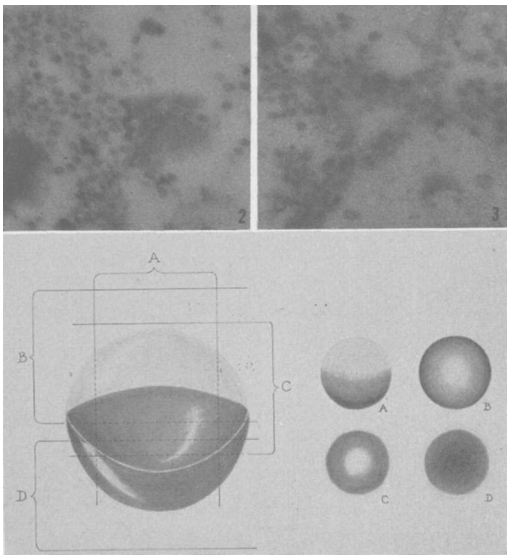


FIG. 1. Survey field showing a virus pool, v, surrounded by keratin fibers, k. Note apparent lack of infection in some cells as contrasted with large numbers of particles in others. Two of the many cell contact areas (desmosomes) are indicated by arrows. × 18,600.



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Electronmicrographs have inadvertently been reduced in size so that the fine structure has been lost. *(The editors)*

FIG. 2. Very thin section of an osmium fixed purified pellet showing variation in profiles of the virus. See Fig. 4 for explanation. × 45,000.

FIG. 3. Same as Fig. 2 except a thicker section. Particles appear to have uniform density. ×45,000.

FIG. 4. Schematic diagram illustrating the possible effect of plane of section on appearance of assumed non-homogenous viral bodies. To give the profile represented in A, the plane of section is vertical in the large sphere and the beam passes horizontally. To give profiles B, C, and D, the sphere is sectioned horizontally at levels indicated.

different aspects of the same particle as illustrated in Fig. 4. A particle's appearance is affected by its vertical position in the section, the extent being dependent on thickness of the section. It is difficult to estimate thickness of sections by interference colors when they are under 50 mμ, because sections thinner than this all appear grey when viewed with white light on a liquid surface(10). In studying serial sections of vaccinia virus(11), one virus particle (235 mμ diameter) required 7 or 8 sections to traverse it. This indicated an average section thickness of 30 to 33 mμ. It is likely that the section represented in Fig. 2 has a thickness less than the diameter of the particles, whereas that of Fig. 3 is probably greater. The particles of Fig. 2 show more knife distortion, which is characteristic of very thin sections, than do those of Fig. 3. The difference in appearance of the various particles of Fig. 2 can be explained by assuming a non-homogenous density in the spheres as illustrated in Fig. 4. It is therefore likely that all the profiles illustrated are different aspects of very similar particles. In the thicker section of Fig. 3 most particles appear to have a uniform density because more of them lie wholly within the section.

Discussion. The profile diameter most frequently observed in our sections is about 33 mμ. This measurement is significantly less than those reported in the literature where measurements were made on air dried or frozen dried dispersions. Electron microscopy does not permit a true measurement of biological substances in their natural state and it is likely that most of the discrepancy results from the methods of preparation. The same kind of discrepancy occurred with other viruses. Influenza virus was about 100 mμ(12) in dispersion and 65 mμ in fixed sections(13). Similarly the mouse mammary carcinoma agent was about 130 mμ to 150 mμ in unsectioned, air dried preparations(14), whereas in embedded and sectioned preparations its average size was only 100 mμ(15). The unsectioned bodies may appear large because of flattening and because of a coat of protein or other material adhering to them. On the other hand, alcohol dehydration and methacrylate embedment cause appreciable shrinkage of sectioned specimens.

In attempting to locate the site of viral development a rather thorough search was made of tissues fixed immediately after removal from both wild and domestic rabbits. So far, however, we have been able to identify the viral bodies only in tissues that were soaked several months in glycerine. In the freshly fixed papillomas granules and particles were so abundant that identification of the virus was difficult and uncertain. Soaking in glycerine dissolved away enough cellular material to leave the virus distinctly visible. It has been well established that storage in 50% glycerine preserves viral activity even for several years, but a great deal of cellular contents appears to be lost, leaving the virus readily visible. All of our preparations have been tested and found to be biologically active. A quantitative correlation of the activity with particles and the effect of preparative procedure on activity in both wild and domestic

rabbits will be reported elsewhere.

Summary. Thin sections of rabbit papillomas reveal pools of dense virus particles in only highly keratinized cells. Viral bodies were readily distinguishable only in papillomas which had been soaked in glycerine-saline for some time. Viral bodies in fresh warts were masked by keratin, melanin and other granules. The bodies are spherical with a non-homogeneous density. They appeared to vary from 25 to 35 mμ in diameter, the most frequent size being 33 mμ. Particles in tissues soaked 1 year or 6 years were identical and equally active in test animals. Sectioned pellets of virus purified by a fluorocarbon process revealed the same particles as those seen in the tissue.

Since submitting this paper we have found the virus in fresh papillomas from cottontail rabbits. It is first seen in the nucleolus, but later fills the entire nucleus and may form crystalline-like arrays.

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Received May 19, 1959. P.S.E.B.M., 1959, v101.

A New Virus of Ducks Interfering with Development of Malaria Parasite (*Plasmodium lophurae*). (25023)

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The avian malaria parasite *Plasmodium lophurae* has been maintained for 14 years in my laboratory by weekly transfer in young ducklings of infected blood. In Feb. 1958 my technician, Mary Chichester, and I noted that in one passage the infections lost their synchronicity(1) and failed to develop with usual severity, and the parasites appeared abnormal. On a subsequent passage parasitemias remained very low but the ducklings died. Since these deaths could not have been a result of malarial infections it appeared likely that some other infective agent had been present in a duckling used for passage of malaria and that this agent had been enhanced in virulence by rapid passage. The following experiments were therefore undertaken to separate this agent from the malaria parasites, to characterize the agent, and to study the effect of introducing the agent into ducks simultaneously infected with *P. lophurae*.

Experimental. 1. *Recovery of P. lophurae free from contaminating agent.* Blood was taken from a duckling showing 17 parasites, many of them abnormal, per 100 red blood cells, and mixed with one-tenth its volume of physiological saline containing heparin at 27 mg/100 ml. When the heparinized blood was inoculated to other ducklings these died in 5 days with parasitemia of 1/100 red cells. When blood cells were separated from plasma by centrifugation, washed with centrifugation 3 times in 0.85% sodium chloride solution, and finally resuspended in saline to original vol-