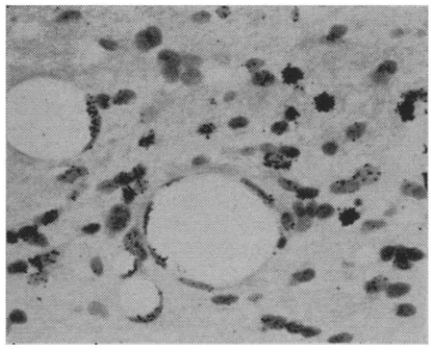

Fig. 1. Radioactive labeling of glia and vascular cells in the visual radiation of a rat in which bilateral destruction of the lateral geniculate body was combined with injection of thymidine-H^3; animal was sacrificed 2 months after operation. Stained with gallocyanin chromalum after autoradiographic exposure and development (only nuclei of glia cells stained) (about × 380).

cent solution of deoxyribonuclease in 0.003*M* $MgSO_4 \cdot 7H_2O$ to test the DNA-specificity of the retained label in the tissue.

In all sections studied, numerous glia cells showed uptake of the radioactive material and the reduced silver grains were in general localized within the nuclei of glia cells. The majority of labeled glia cells was found in the areas of the lesions (both on the injected and opposite sides of the brain) and in regions known to have intimate connections with the traumatized lateral geniculate bodies. These latter included the visual radiation, the lower layers of the visual cortex, the pretectal region, the optic tract, and the brachium of the superior colliculus. As described elsewhere (*2*) the technique proved useful in investigating the extent, kinetics and various other aspects of glial proliferation. In addition to the numerous labeled glia cells, which presumably underwent proliferation in response to the lesions, a few labeled glia cells, some labeled neuroblasts, and also labeled nuclei of some neurons were observed in brain regions not necessarily associated with the lesion area. Digestion with deoxyribonuclease removed the label. In systematic scanning of two coronal sections through the thalamus in several animals (with survival periods following lesion and administration of thymidine-H^3 ranging from 1 day to 2 months) a varying number (6 to 36 in single sections) of mildly or intensely labeled neurons and neuroblasts were observed. Most of the labeled neurons were of the stellate type; in the cortex a few labeled pyramidal cells were also seen (Figs. 1 and 2).

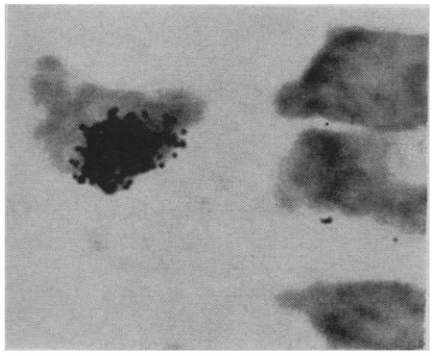

Fig. 2. Radioactive labeling of a neuron in the cerebral cortex of a rat which was sacrificed 1 month after the operation (about × 1170).

This indicates that new neurons may come into existence in the brain of adult mammals, at any rate in such forms as the rat. If the general observation is valid that mitotic figures are absent in the brain of adult mammals, these findings might suggest that the labeled neurons were formed from undifferentiated cells which divided mitotically during the period at which the administered thymidine-H^3 was available. The presence of labeled neuroblasts, mostly in fiber tracts, would support such a process of neurogenesis.

Koenig (*3*) observed the retention of some label by glia cells after administration of C^{14}-labeled adenine and orotic acid and extraction of RNA, and he suggested that this may be due to a slow turnover (that is, metabolic instability) of DNA. The plausibility of this conclusion, which was based on an indirect method of DNA labeling, was questioned by Hughes (*4*) on the basis of contrary direct evidence. The intense labeling of numerous glia cells, and some nerve cells, after as short a survival period as 1 day, also speaks against such an interpretation. The other possibility is that the uptake of thymidine observed in this study was due to some induction effect either by the lesion or by the injected thymidine. Extracerebral injection of thymidine-H^3 would be a better procedure to test whether turnover of this DNA precursor occurs in the normal brain. In fact, Schultze and Oehlert (*5*) and Messier and Leblond (*6*) reported no uptake of thymidine-H^3 by neurons after intraperitoneal injection of this substance into adult rodents. As the blood-brain barrier retards the penetration of most nucleotides, the dose of thymidine-H^3 used in these studies may have been too low to label neurons. Experiments are in progress in our laboratory to determine whether neurons take up systemically administered larger doses of thymidine in normal animals (*7*).

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Papova Virus Group

Abstract. The papilloma, polyoma, and vacuolating agents seem to form a natural group of tumor viruses, for which the name papova virus group is proposed. Members of the group have the following properties: 45 mμ diameter, deoxyribonucleic acid core, 42 capsomeres, absence of essential lipids, thermal resistance, slow growth cycle with multiplication within the cell nucleus, and tumorigenicity.

In the course of the work in this laboratory with papilloma (wart) virus of man (*1*), polyoma virus of mice (*2*), and vacuolating virus of monkeys (SV_{40}) (*3*), I have been made conscious of the similarities in properties between each of them and papilloma virus of rabbits (*4*). This has led me to group these viruses together in the pa'po'va virus group, the name being derived from the first two letters of each virus name: *pa*pilloma, *po*lyoma, *va*cuolating, in the order in which the viruses became known. The properties of the four members of the group are listed in Table 1. The list is less complete for

Types 1 and 2, the papilloma viruses, because work has been limited by the in vivo assay system for the rabbit virus and by the lack of any assay system for the human virus.

The size of each virus has been established by direct electron microscopic measurements both in suspensions and in thin sections (*1, 5–13a*). Electron micrographs of Type 4, vacuolating virus, are shown in Fig. 1; those of the other types are well documented in the literature cited. Capsomeres for the group fit the formula $10(n-1)^2+2$, where $n = 3$ (*6, 8, 9, 11, 13*). In preparations of each virus type, filaments have been observed having a cross section equal to the diameter of the virus particle (*7, 11, 13*).

The papova viruses contain DNA (*14–17*). The DNA in purified preparations of virus contains double helical strands as determined for Type 1 by its sharp melting behavior (*15*), and for Types 3 and 4 by their yellow-green reactions with acridine orange (*9, 17*). Polyoma virus does not react with vital dyes unless fixed, whereas vacuolating virus is penetrable to a limited degree (*9, 17–19*).

The viruses are resistant to ether and therefore do not contain lipid essential for their infectivity (*3, 20, 21*). Buoyant density as measured by ultracentrifugation in cesium chloride gradients yielded the same value, 1.30, for polyoma and vacuolating viruses (*9, 19*).

The papova viruses are relatively stable in water at 50° for 1 hour (*3, 4, 21*). However, the addition of high concentrations of $MgCl_2$ results in a marked lability of vacuolating virus, and to a lesser degree of polyoma virus (*9, 22*).

So far as is known, only a single antigenic type exists for each of the viruses listed (*3, 4, 9, 23*).

Studies of infected cells by electron microscopy, by reaction with fluorescent antibody, and by staining with acridine orange indicate that papova viruses first multiply in the nucleus and later appear in the cytoplasm (*9, 12, 13a, 24–26*). There is early involvement of the nucleolus, characterized by increased RNA, vacuolization, and virus localization (*9, 10, 12, 25*) (see Fig. 2).

Relative to other viruses, papova viruses have a slow growth cycle. After infection of cells with polyoma (*27*), or vacuolating virus (*9*), new virus begins to appear only about 24 hours later. This is also reflected in the long

Table 1. Papova virus group (see text for references).

Trait	Type 1 papilloma, rabbit	Type 2 papilloma, human wart	Type 3 polyoma	Type 4 vacuolating
Size (mμ)	40 to 50	40 to 50	40 to 50	40 to 50
Capsomeres (No.)	42	42	42	42
Filamentous forms occur	Yes	Yes	Yes	Yes
Nucleic acid: type	DNA		DNA	DNA
Nucleic acid: strandedness	Double		Double	Double
Lipid	Nonessential		Nonessential	Nonessential
Buoyant density			1.30	1.30
Thermal stability at 50° C in H_2O	Relatively stable		Relatively stable	Relatively stable
Thermal stability at 50° C in $MgCl_2$			Labile	Exceedingly labile
Antigenic varieties	One		One	One
Growth cycle (hr)			24	24
Multiplication site	Nucleus	Nucleus	Nucleus	Nucleus
Natural host	Rabbit	Man	Mouse	Monkey
Latent and chronic infections common	Yes	Yes	Yes	Yes
Tumorigenic	Yes	Yes	Yes	Yes

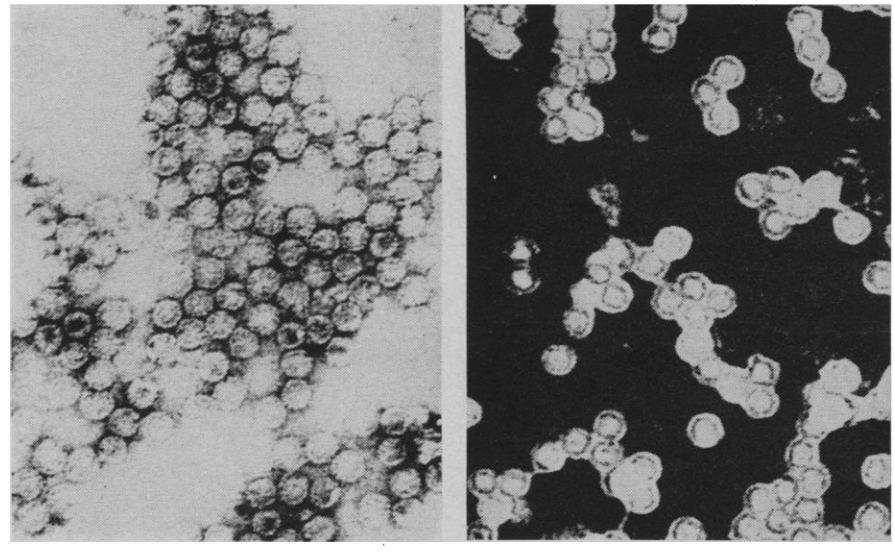

Fig. 1 (above, left and right). Vacuolating virus (SV_{40}), papova virus type 4. Electron micrographs (about × 98,000) of preparations stained with potassium phosphotungstate (above left: print of original negative showing capsomeres on virus surface) and with uranyl acetate (above right: print of reversed negative showing core as well as capsid of the virus).

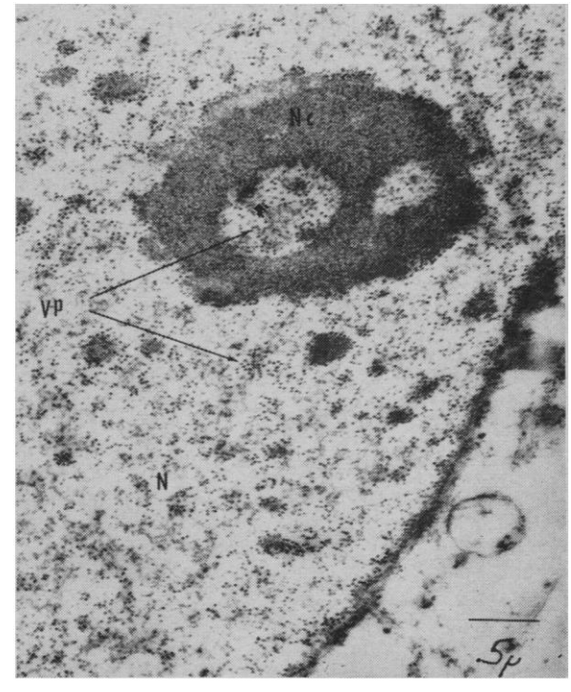

Fig. 2 (right). Ultrathin section of portion of the nucleus *N* of a cercopithecus monkey kidney cell 4 days after infection with vacuolating virus. Numerous virus particles *VP* are present throughout the nucleoplasm and within the nucleolus *Nc*.

[K. O. Smith and L. Jordan, Baylor Virus Laboratory]

incubation period of 1 to 2 weeks required to develop polyoma (*28*) and vacuolating (*9, 22*) virus plaques.

Each of the viruses listed produces latent and chronic infections in its natural host, and each is tumorigenic. In the case of polyoma, the virus is neoplastic for species other than the mouse (*29*). Vacuolating virus has been found to produce tumors (sarcomas) when injected into newborn hamsters, with recovery of virus from the neoplasm several months after the injection (*30*). However, there have been no demonstrations as yet of tumorigenic activity in the natural primate host.

When the properties of the four agents are examined, it is apparent that they fall into a natural group for which the name, papova virus group, is proposed. The cardinal features of the viruses within the group are similarities in the size, morphology, and buoyant density of the viruses; the presence of double-stranded DNA; the absence of essential lipid; relative thermal stability; slow growth cycle characterized by multiplication within the nucleus and involvement of the nucleolus. Papova viruses produce latent and chronic infections in their natural hosts, and all are tumorigenic in their natural or other host species, or both (*31*).

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Antibody Production in Human Malaria as Determined by the Fluorescent Antibody Technique

Abstract. No reliable serological test has been available in the past to follow the course of antibody production in malarial infections. The indirect method of immunofluorescence was utilized in this investigation to study antibody response to sporozoite-induced *Plasmodium vivax* infections in two human volunteers. Malarial antibody was demonstrated approximately 3 weeks after infection, and has persisted thus far for 121 days. These preliminary results suggest that this method of serological testing is specific and provides a sensitive means of titrating antibody produced in malarial infections.

At the present time there is no accepted serological test which is adaptable for routine use in the investigation of antibody production in malarial infections. Attempts made in the past to obtain specific complement fixation and precipitation reactions with sera from patients infected with malaria have met with only limited success (*1*). Since the fluorescent antibody technique has been used successfully in the specific staining of malaria parasites (*2*), our investigations were undertaken, utilizing this technique to follow the course of antibody production in human volunteers after sporozoite-induced infection with *Plasmodium vivax*.

Two white male volunteers, ages 40 and 41, were each bitten by 13 *Anopheles freeborni* mosquitoes heavily infected with the Venezuelan strain of *P. vivax*. Thin and thick blood smears were examined daily for parasites which were demonstrated on the 14th day after sporozoite inoculation and persisted for 25 days (patient M.C.) and 48 days (patient C.R.). Because of moderately severe clinical illness, the course of this disease was modified by intermittent antimalarial chemotherapy (Fig. 1).

Sera for antibody studies were obtained at approximately weekly intervals and frozen until ready for use. Flocculation Venereal Disease Research Laboratory tests were performed at the same intervals on all sera and remained negative throughout the course of infection. The indirect fluorescent antibody technique (*3*) was employed with thin blood films from the volunteers infected with *P. vivax* as the antigen. The blood smears were prepared when high parasite counts were present, and contained a variety of morphological forms ranging from young trophozoites to mature schizonts. A heterologous system was always used, with blood films and sera obtained from different patients. The blood smears were air dried, and stored unfixed at room temperature, 4°C, −20°C, and −50°C. The smears stored at −50°C proved most satisfactory, showing a minimum of background fluorescence after storage up to 15 weeks. Fluorescein isothiocyanate conjugated horse anti-human globulin (Sylvana Chemical Co.) was absorbed twice with rabbit liver powder, and once with rabbit bone marrow powder. Merthiolate in a dilution of 1:10,000 was added to the conjugate which was stored at 4°C until ready for use. The same lot of conjugate was used throughout, and an optimal dilution was employed to produce maximum fluorescence of the parasites with minimum fluorescence of the red blood cell ghosts. In certain preparations nonspecific fluorescence of the white blood cells occurred, but the amount was less than the specific fluorescence of the malaria parasites and did not interfere with the interpretation of the stained slides.

The blood smears were dehemoglobi-