cations were observed, the frequency of tetraploid cells was 10 times higher in the Down's syndrome (30% *vs.* 3%). The reason for this is not clear. It was believed that the increased tetraploidy and endoreduplication observed with cultures from the frozen tissue fragments might be attributable to exposure to dimethylsulfoxide and/or the low temperatures, but the observations of Schwarzacher and Schnedl(8), and our results with the Down's syndrome, where there was no such exposure, have made these possibilities seem less tenable. Notwithstanding, if normal ploidy is a consideration, the use of frozen tissue fragments for tissue culture is precluded.

Summary. Tissue fragments frozen and stored at —90°C for 15 months were capable of initiating growth in culture. Chromosomal analysis revealed that such cells had a high incidence of tetraploidy and endoreduplication.

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Boracéia Virus. A New Virus Related to Anopheles B Virus.* (31526)

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During the studies of the epidemiology of arbovirus made in Casa Grande region of the State of São Paulo, a virus was isolated from mosquitoes. This virus has been named Boracéia after a mountain located near the site of isolation.

Methods. The methods used for identification have been described(1). Tissue culture studies were done with the BHK-21 cell line. The growth medium was a modified Eagle's with 20% calf serum, and the maintenance medium included 2% calf serum. Neutralization testing in BHK-21 cells was done by a constant serum—varying virus technique. Serum virus mixtures were incubated for one hour at 37°C prior inoculation.

Isolation and characterization. The virus, SP Ar 395, was isolated from a pool of 95 *Anopheles (Kerteszia) cruzii* collected in the Boracéia field station on March 30, 1962. Of 12 baby mice inoculated with the mosquito suspension, one sickened with evidence of central nervous system disturbance on the seventh day, when a suspension of brain was passed intracerebrally (i.c.) to another group of 2-day-old mice. The remaining 11 mice were dead by the ninth day. By the second passage, the average survival time was 5.0 days after i.c. inoculation and 9.0 days after intraperitoneal (i.p.) inoculation. Nearly half of the adults inoculated i.c. became paralyzed and died; the rest recovered. Adults inoculated i.p. showed no signs of disease.

In BHK-21 cells SP Ar 395 showed a

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TABLE I. Serological Comparison of SP Ar 395 and Anopheles B Viruses by Complement-Fixation (CF) and Neutralization (NT) Tests.

Serum		CF		NT	
		Anopheles B	SP Ar 395	Anopheles B	SP Ar 395
Anopheles B	3 i	128/512*	16/16	3.0†	1.0
SP Ar 395	2 i	16/512	64/256	—	—
SP Ar 395	3 i	16/64	128/256	1.5	3.5

i—No. of injections
* Reciprocal of serum titer/reciprocal of antigen titer.
† Results expressed as $\log_{10}$ neutralization index.
— = Not done.

cytopathic effect in 4 to 5 days after one blind passage. The cells became granulated, rounded, and fell from the tube wall. The titer was 4.5 log LD_{50}. By contrast, Anopheles B virus did not require adaptation to BHK-21 cells, producing cytopathic effect with mouse brain inoculum.

The virus was filterable through a Millipore® HA membrane with 450 mμ pore size. DCA inactivated SP Ar 395. The control titer was 6.6 log LD_{50} and the titer after exposure was less than 2.5 log LD_{50}. The virus was not reisolated from the original suspension, however, the fact that it is a new virus is evidence that the isolation was valid. Anopheles B virus, obtained from the Rockefeller Foundation Virus Laboratories, was first opened on April 16, 1964, two years after the isolation of SP Ar 395.

Identification. Attempts to obtain a hemagglutinin from infected baby mouse brain, liver, and serum were unsuccessful. An antigen from baby mouse brain which reacted by complement fixation (CF) test with homologous serum was tested with sera of arbovirus groups A, B, C, Bunyamwera, and Guama as well as several other grouped and ungrouped viruses. A cross-reaction was observed with Anopheles B immune mouse serum and not with the other sera. Confirmation of this observation was obtained by further CF and neutralization (Table I) testing in BHK-21 cells. Both CF and neutralization test results indicated SP Ar 395 was related to, but different from, Anopheles B.

Comments. Anopheles B virus was originally isolated in Eastern Colombia by Roca-Garcia(2) from a pool of *Anopheles (K.) boliviensis*. It is proposed that, because of the serological cross-reaction between Anopheles B and Boracéia viruses, a new arbovirus antigenic group, the Anopheles B group, be established. Casals and Whitman(3) stated that to establish a new antigenic group, it is necessary to show cross-reactions among members of the new group, and absence of cross-reactions between members of the new group and viruses of previously described groups. Anopheles B has been studied extensively and considered as an ungrouped virus and no cross-reactions have been found in the present study between Boracéia and arboviruses other than Anopheles B, indicating that requirements to establish a new group are satisfied.

Another point of interest is that both Anopheles B and Boracéia were isolated from anopheline mosquitoes of the same subgenus, *Kerteszia*. This finding emphasizes the importance of further study on the role of anophelines in arbovirus transmission in the Americas.

There is evidence that Boracéia virus neutralizing antibody exists in human residents of the Casa Grande region. This finding will be reported later.

Summary. Boracéia virus, a new arbovirus isolated from *Anopheles (K.) cruzii*, is described. Serological cross-reactions between Boracéia and Anopheles B virus were demonstrated. It is proposed that these viruses form a new arbovirus antigenic group, the Anopheles B group.

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Absorption of Vitamin B_{12} in a Rectal Suppository. (31527)

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Physiological absorption of vitamin B_{12} requires intrinsic factor (IF). In conditions where IF activity is lacking in the gastrointestinal tract, a therapeutically significant absorption of B_{12} occurs only when given in large quantities. Such absorption seems to be independent of IF, and constitutes the basis of the oral B_{12} therapy in massive dosages for pernicious anemia and agastric B_{12} deficiency. Rectal absorption of large doses of B_{12} in solution has been noted by Ungley(1) and Brigeau *et al*(2), but the results have been inconsistent. The mechanism for the non-IF-mediated absorption has not yet been elucidated, although a physical phenomenon like diffusion has been suggested, and some observations are rather against such a mechanism(3).

In this study, radioactive B_{12} in 2 extremely different doses was given to human subjects *per rectum* in suppositories and absorption was measured in comparison with oral administration of the same dosage. The demonstrated rectal absorption was further corroborated in animals.

Methods. Hospital patients with minimal disease not involving the intestine or the kidney were used. Shortly after a morning bowel movement, a suppository containing Co^{57}-cyanocobalamin ($Co^{57}B_{12}$)* was placed in the rectum to be retained for at least 4 hours. Two hours after the rectal administration, 1 mg of unlabeled B_{12} was injected subcutaneously for flushing and 24 hours' urine collection was made as in the Schilling test(4). In some subjects, feces were also collected for 3 days for measurement of the unabsorbed portion. For comparison, the regular Schilling test was carried out in a separate group using the same doses of $Co^{57}B_{12}$ in aqueous solutions *per os* and the same flushing procedure.

The suppository, weighing 2 g, consisted of the base of isococoa butter and a small amount of polyoxyethylene lauryl ether as the emulsifier. Aqueous $Co^{57}B_{12}$ with a specific activity of about 14 μc per μg was mixed with either lactose powder or crystalline B_{12}, dried *in vacuo*, pulverized, redried, and thoroughly pulverized $Co^{57}B_{12}$ powder was suspended in the suppository base melted at 50°C. Two extreme doses of B_{12}, 10 mμg and 2,000 μg, were used, but the radioactivity was so adjusted that each suppository contained 20,000-100,000 cpm as measured in a well type gamma scintillation counter.

The radioactivity in each suppository was measured in the same geometry before use, and the variation in count was found to be within 10% of the average. One suppository with about the average count was dissolved in 100 ml of warm 80% ethanol and quantitatively transferred to measuring tubes and bottles as the standards. Corrections were made from the differences in count of the used suppositories from the original standard suppository. Urine was condensed, feces were homogenized with water and radioactivities in aliquots were determined.

For the animal study, adult rabbits were used. A suppository, half in size and containing 1,000 μg $Co^{57}B_{12}$ was placed in the rectum, the anus was ligated for the prevention of ejection, and the animal was sacrificed 15 hours later. The contents of the colon after homogenization, as well as the

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