markedly different on the two sides. For these two subjects, the EEG scoring apparatus was set to score alpha activity above a criterion of 7 μv at 9 hz. Both subjects showed more alpha activity on "easy" tasks than on "hard" tasks, more alpha activity with eye closed than open, and similar patterns of alpha activity on the two sides. When a burst of alpha activity was present on one side, a burst was generally also present on the other side. The amount of alpha activity simultaneously present on both sides far exceeded the probability predictions based on an assumption that the two sides were independent. Sample records from these one-eyed subjects are shown in Fig. 2. Although the amplitude of alpha activity tended to be slightly higher on the side with the normal eye, instances can be found where the reverse is true. A clinical EEG recorded preoperatively from subject N.A. appeared similar to those obtained by us postoperatively, including the tendency for alpha activity to have slightly higher amplitude on the left side. Clearly neither subject showed amplitude differences from the two sides as great as predicted by the ocular artifact hypotheses. If either the corneoretinal potential or the extraocular muscles were needed to produce alpha EEG, we should have found that the amplitude of alpha EEG recorded from the side with the remaining eye exceeded that on the other side by at least twofold. This would follow if the observation of more than a 2 : 1 ratio of alpha EEG amplitude found with an experimental procedure (unilateral light adaptation) is due to change in the corneoretinal of the ipsilateral eye, as Lippold interpreted (*5*). The same electrode placements (*9*) were used to record the data in Fig. 2. Data obtained from additional bipolar electrode placements at O_1–T_5 and O_2–T_6 (standard 10–20 system) yielded the same conclusions: no marked alpha asymmetry.

Kappa EEG activity recorded across the temples (F_7–F_8) was also obtained from both one-eyed subjects. Like normal subjects (*8*) both one-eyed subjects had more kappa EEG scores on "hard" tasks than on "easy" tasks, more kappa activity with eye open than closed (24 percent of normals), and independent occurrences of kappa and alpha activity (amount of simultaneous activity close to chance prediction).

The presence of normal alpha and kappa EEG activity in a bilaterally enucleated subject and the absence of marked left-right differences in two one-eyed subjects whose eyeballs and extraocular muscles were removed refute the explanations of alpha and kappa EEG activity in terms of ocular artifacts. These data, together with those previously reported, lead to the conclusion that alpha and kappa activity are not directly dependent on the corneoretinal potential of the eyeball, tremor of the extraocular muscles, eye position, accommodation, or eyelid flutter (*10*).

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8. R. M. Chapman, J. C. Armington, H. R. Bragdon, *ibid.* **14**, 858 (1962). In the present experiment bipolar recordings, using a Grass model 78 polygraph, were made from (i) the midline over the parietal and occipital regions (P_z and O_z) for alpha activity, (ii) from the left and right temporal areas (F_7 and F_8) for kappa activity, and (iii) from electrodes mounted near the absent right eye (1 cm from the lateral canthus and 1 cm below the middle of the lower lid) for periorbital electrical activity. Both ears were grounded. The half-amplitude frequency response of the recording system was 0.1 to 50 hz. The two EEG signals were then entered into a two-channel automatic scoring apparatus for quantitative determination of alpha and kappa EEG activity [W. J. Kropfl, R. M. Chapman, J. C. Armington, *Electroencephalogr. Clin. Neurophysiol.* **14**, 921 (1962)]. Each of the two identical channels contained a bandpass filter with a center frequency of 9 hz and a half-amplitude bandwidth from 7 to 12 hz, a rectifier, a short time-constant filter, and a trigger circuit that fired whenever the preset voltage level was exceeded (15 μv at 9 hz, except where noted otherwise). When EEG activity met these scorer criteria, markers were displayed on the EEG record and clutches of electric timers were activated. These timers summated the amounts of time that the parieto-occipital EEG contained alpha activity, the transtemporal EEG contained kappa activity, and the amount of time that alpha and kappa activity were present simultaneously. The subject was seated in a comfortable chair inside an electrically shielded, sound-damped, small room.
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10. After this report was submitted for publication, uniocular data supporting our conclusions were reported by M. Abbott and A. M. Dymond [*Lancet* **1970-II**, 933 (1970)].
11. We thank the subjects for their cooperation, John Swain for his technical assistance, Alison Lee for aid with the apparatus, and Marion Northern for help with the manuscript. This work was partially supported by PHS research grant EY 00490 from the National Eye Institute to the Eye Research Foundation which is an affiliate of the University of Maryland.

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16 October 1970

Acute Lymphocytic Leukemia in Owl Monkeys Inoculated with Herpesvirus saimiri

Abstract. *Our study demonstrates for the first time that* Herpesvirus saimiri *can induce acute lymphocytic leukemia in owl monkeys* (Aotus trivirgatus) *and that malignant lymphoma can be induced in this species of nonhuman primates by the inoculation of the virus by various routes (intravenous, subcutaneous, and intradermal).*

Herpesvirus saimiri, a viral agent indigenous to the squirrel monkey (*1*), induces malignant lymphoma after intramuscular inoculation in cotton-top marmosets (*Saguinus oedipus*) and owl monkeys (*Aotus trivirgatus*) (*2, 3*). Mortality in marmosets (*Saguinus* sp.) was 100 percent within 18 to 48 days, associated with marked invasion and organ replacement by elements resembling reticulum cells. Some animals showed terminal leukocytosis, lymphocytosis, and the presence of immature lymphocytes, lymphoblasts, and reticulum cells in the peripheral blood. Of ten owl monkeys inoculated with *Herpesvirus saimiri*, all developed malignant lymphoma within 29 days. Peripheral blood was not studied, but the histopathological features were similar to those in marmosets (*3*).

The studies reported here were conducted to determine whether peripheral blood changes characteristic of leukemia develop in owl monkeys; whether routes of inoculation other than intramuscular are effective in inducing malignant lymphoma; and, if not, whether this antigenic exposure would confer immunity to intramuscular inoculations.

A group of 12 owl monkeys were inoculated each with approximately 316,000 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of *H.*

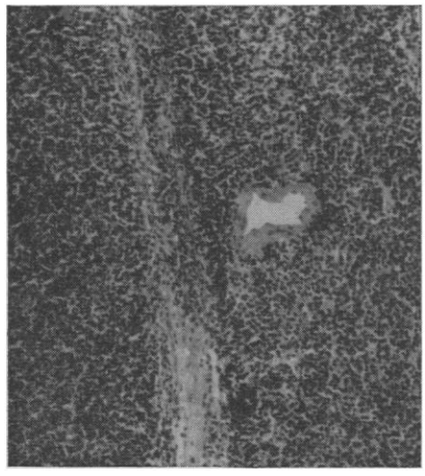

Fig. 1. Lymph node from owl monkey No. 622-69. The lymph node had been replaced by a solid sheet of lymphocytic cells which invaded through the capsule into perinodal tissues. Hematoxylin and eosin; × 60.

saimiri, strain S-295C. The virus was propagated and titered in continuous cultures of owl monkey kidney cells as previously described (*4*). The animals were inoculated as follows: subcutaneously (Nos. 621-69, 622-69, and 630-69), intradermally (Nos. 633-69, 636-69, and 639-69), intraperitoneally (Nos. 626-69, 631-69, and 638-69), and intravenously (Nos. 629-69, 632-69, and 635-69). Serums collected before inoculation and on day 21 after inoculation did not reveal neutralizing antibodies to *H. saimiri*. When possible, blood was also collected weekly for complete blood counts and differential counts.

Animals No. 622-69 (inoculated subcutaneously), Nos. 636-69 and 639-69 (intradermally), Nos. 631-69 and 638-69 (intraperitoneally), and No. 635-69 (intravenously) were each reinoculated by the intramuscular route with 316,000 $TCID_{50}$ of *H. saimiri* in the 23rd week. Surviving animals were killed 36 weeks after inoculation.

Prior to reinoculation, six animals (Nos. 621-69, 626-69, 629-69, 630-69, 632-69, and 633-69) (Table 1) died between 11 and 14 weeks after initial virus inoculation. Two (Nos. 621-69 and 632-69) exhibited histopathological findings of malignant lymphoma, including marked lymphocytic replacement or invasion of lymph nodes, spleen, liver, kidney, and heart (Fig. 1). In contrast to previous findings in marmosets and owl monkeys, the cell type was lymphocytic rather than reticular. In three of the animals that died (Nos. 629-69, 630-69, and 633-69) there was hyperplasia of lymphocytes in lymph nodes, and hepatic sinusoids contained an excessive number of lymphocytes. However, invasion of the capsules of lymph nodes, seen in animals with malignant lymphoma, and infiltration of other organs and tissues were not found. In one animal (No. 626-69) with no lesion resembling malignant lymphoma or lymphocytic hyperplasia, the cause of death was not determined.

Leukemia with a total white blood cell count in excess of 90,000 per cubic millimeter, consisting primarily of lymphocytes (Fig. 3), developed in No. 621-69 7 days before death (Fig. 2). There were numerous large lymphocytes, prolymphocytes, and lymphoblasts in each sample.

Of the six animals reinoculated in the 23rd week, four (Nos. 622-69, 636-69, 638-69, and 639-69) died, having the gross and histopathological features of lymphocytic malignant lymphoma. One (No. 622-69) developed lymphocytic leukemia 1 week prior to reinoculation and two (Nos. 638-69 and 639-69) developed a leukemia identical with that in No. 621-69, 3 weeks and 1 week after reinoculation, respectively. Two animals (Nos. 631-69 and 635-69) killed at 36 weeks (13 weeks after reinoculation) showed no lesions.

This study clearly confirms induction of malignant lymphoma in owl mon-

Table 1. *Herpesvirus saimiri* disease produced in owl monkeys (*Aotus trivirgatus*) by various routes of inoculation.

No. and sex of animal	Route of inoculation	Survival* time (weeks)	Histopathologic and hematologic findings
621-69 (M)	Subcutaneous	11	Malignant lymphoma with leukemia
622-69 (M)	Subcutaneous	24†	Malignant lymphoma with leukemia
630-69 (M)	Subcutaneous	14	Lymphocytic hyperplasia
633-69 (M)	Intradermal	12	Lymphocytic hyperplasia
636-69 (F)	Intradermal	24†	Malignant lymphoma
639-69 (F)	Intradermal	36†	Malignant lymphoma with leukemia
626-69 (M)	Intraperitoneal	9	No significant lesions
631-69 (M)	Intraperitoneal	36†	No significant lesions
638-69 (F)	Intraperitoneal	29†	Malignant lymphoma with leukemia
629-69 (M)	Intravenous	6	Lymphocytic hyperplasia
632-69 (M)	Intravenous	14	Malignant lymphoma
635-69 (M)	Intravenous	36†	No significant lesions

* All animals died with the exception of Nos. 631-69 (M) and 635-69 (M), which were killed at the termination of the experiment. † Reinoculated intramuscularly in 23rd week.

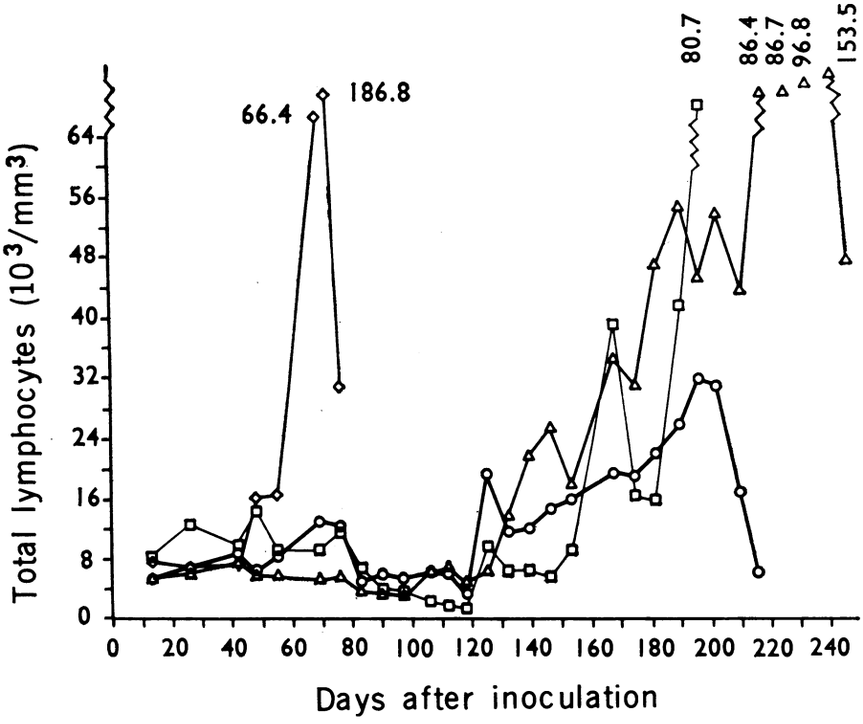

Fig. 2. Absolute lymphocyte count in four owl monkeys that developed leukemia. ◇—◇, Animal No. 621-69; □—□, No. 622-69; ○—○, No. 638-69; and △—△, No. 639-69.

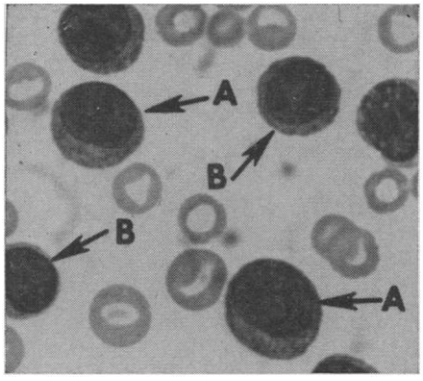

Fig. 3. Lymphoblasts (*A*) and prolymphocytes (*B*) in peripheral blood of a leukemic owl monkey. Wrights stain; × 500.

keys by *H. saimiri* and demonstrates, for the first time, development of lymphocytic leukemia in this species in association with malignant lymphoma. Further, malignant lymphoma was induced by virus given subcutaneously and intravenously, as two animals (Nos. 621-69 and 632-69) died prior to reinoculation. The intradermal route was also probably effective; animal No. 636-69 died with malignant lymphoma 1 week after the intramuscular reinoculation, and it seemed unlikely that the disease developed in 1 week. It appeared unlikely, also, that immunity developed in the six animals surviving the first inoculation, as four died of malignant lymphoma after reinoculation. Two (Nos. 622-69 and 636-69) no doubt had the disease prior to reinoculation, because one was then leukemic, and both died 1 week after reinoculation. The other two did not die of malignant lymphoma until 6 weeks (No. 638-69) and 13 weeks (No. 639-69) after reinoculation.

The incidence of malignant lymphoma in this study was 50 percent as compared with 100 percent of all owl monkeys inoculated with *H. saimiri* in earlier studies (*2–4*). The course of the disease extended to 36 weeks in the present study, whereas in previous studies no animal survived longer than 4 weeks, even when the viral inoculum was 100 times more dilute. It is difficult (if not impossible) to explain the lower incidence and longer course despite the use of an undiluted viral inoculum. Variation in virus virulence must be considered, but there has been no in vitro evidence of it thus far. A more likely explanation is variation in the host, since, with few exceptions, New World monkeys available for research are poorly defined. Genetic variability, age, indigenous viruses, parasitic diseases, and other factors might influence the pathogenicity of *H. saimiri.*

Six animals, four dying prior to reinoculation and two killed 13 weeks after the second injection of *H. saimiri,* did not have lesions indicative of malignant lymphoma. Lymphocytic hyperplasia of lymph nodes and increase in lymphocytes in hepatic sinusoids were observed in three animals (Nos. 630-69, 633-69, and 629-69), but a possible relation of these changes to virus inoculation can only be speculative. The cause of death of the four animals was not ascertained. If related to *H. saimiri,* the result would suggest that the virus can induce a disease other than malignant lymphoma. However, lack of evidence of disease suggests that infection was not established. Development of leukemia in owl monkeys inoculated with *H. saimiri* and extension of the course of disease to 70 days are of particular significance and importance as an indication of possible usefulness of the lymphoma as a model for studying chemotherapeutic procedures. Further, this leukemia induced by a herpesvirus in nonhuman primates may provide a convenient virus-host system for study of similar conditions of man.

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5. We thank Douglas Jackman, Jill Cadwallader, Martha Williamson, Martha Elliot, John Martino, and Marjorie Vallee for their technical assistance. Supported by PHS grant No. RR 00168-09.

13 October 1970; revised 16 November 1970 ■

Immunoglobulin M and Secretory Immunoglobulin A: Presence of a Common Polypeptide Chain Different from Light Chains

Abstract. *Unique polypeptide chains have been isolated from* S-*sulfonated light-chain fractions of human serum immunoglobulin M and colostral immunoglobulin A. Their electrophoretic mobilities, molecular weights, peptide maps, amino acid compositions, and antigenic determinants are very similar or perhaps identical but differ from those of light chains and secretory piece.*

External secretions of man contain immunoglobulin A (IgA) which, in contrast to serum IgA, has a higher molecular weight and is associated with another polypeptide chain. The polypeptide, termed the secretory piece (SP), has also been found free in secretions. These considerations have been reviewed by Tomasi and Bienenstock (*1*).

After disruption of disulfide bonds, the secretory IgA (S-IgA) molecule dissociates into heavy (H) and light (L)

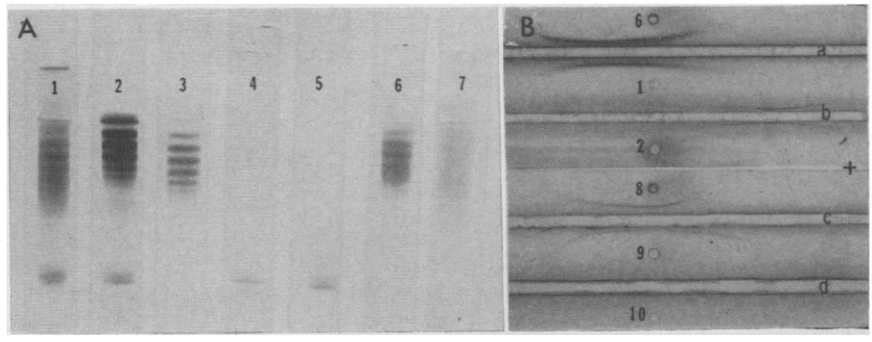

Fig. 1. Disc-electrophoretic (A) and immunoelectrophoretic (B) patterns of S-IgA and IgM subunits. (1) L-chain fraction of S-IgA; (2) L-chain fraction of IgM; (3) L-chain fraction of IgM with J chain removed; (4) J chain from S-IgA; (5) J chain from IgM; (6) L-chain fraction of IgG; (7) L-chain fraction of serum IgA (7*S*); (8) whole colostrum diluted 1 : 2; (9) SP-enriched fraction of colostrum; (10) L-chain fraction of S-Iga with J chain removed. Antiserums: (a) to L chain; (b) to J chain; (c) to SP.