Nuclear Protrusions in the Polymorphonucleated Leucocytes of the Anthropoid Apes[1]

In a preliminary note, we studied the frequencies of the so-called drumstick in the polymorphonucleated leucocytes of different species of female monkeys[2].

Here we refer to a more extensive analysis of the frequency of the appendices of the type A and C of the KOSENOW classification[3] associated with the polymorphonucleated leucocytes of the anthropoid apes. The present data are not comparable with one of the previously published lists[4] because they refer only to the polymorphonucleated leucocytes.

We analysed the blood smears of 6 female and 3 male orangs, 7 female and 3 male gorillas, 1 female and 1 male chimpanzees. For each animal we studied 1000 polymorphonucleated leucocytes. The data are reported in the Table.

From the above results we can first observe the higher frequencies of this type of protrusion in the anthropoid apes in comparison with those of man. Secondly we can differentiate the 3 genera of anthropoid apes according to the frequency of these appendices. In the gorilla a mean of 14.16% with a higher frequency of 18.7% and a lower of 10.4% was reckoned. In the orang a mean of 7.38% with a higher frequency of 10.6% and a lower of 4.8% was reckoned. In both the chimpanzees studied a frequency of 4% was reckoned. The frequencies of these appendices in the different sexes of the species studied are not appreciably different.

Moreover, as shown in the microphotographs presented in the Figure, some polymorphonucleated leucocytes of the different species studied show that more than one of these protrusions are present in one nucleus and that, in some cases, they are very similar in size and morphology. The present data do not allow us to interpret these structures as due only to the allocyclic condition of the *X*-chromosome, as was done in the case of a similar structure, the so-called drumstick, in man.

Research is in progress to establish whether these structures are eventually related to the inactivation of some other chromosomse[5].

Frequencies of appendices observed in the polimorphonucleated leucocytes of the anthropoid apes

Species, name and sex		% of appendices observed in respect to the polymorphonucleated leucocytes
Pongo pygmaeus		
Jowata	(♀)	10.6
Jada	(♀)	4.8
Kitchee	(♀)	6.3
Sya	(♀)	8.1
Tupa	(♀)	6
Sungei	(♀)	6.4
Dyak	(♂)	10.1
Kampong	(♂)	6
Bukit	(♂)	8.2
Gorilla gorilla		
Fini	(♀)	14.6
Shamba	(♀)	14.3
Choomda	(♀)	11.5
Oko	(♀)	14.5
Katooma	(♀)	16.2
Banga	(♀)	15.4
Anka	(♀)	14
Rawn	(♂)	10.4
Badam	(♂)	18.7
Ozoun	(♂)	12
Pan troglodytes		
TO_1	(♀)	4
TO_2	(♂)	4

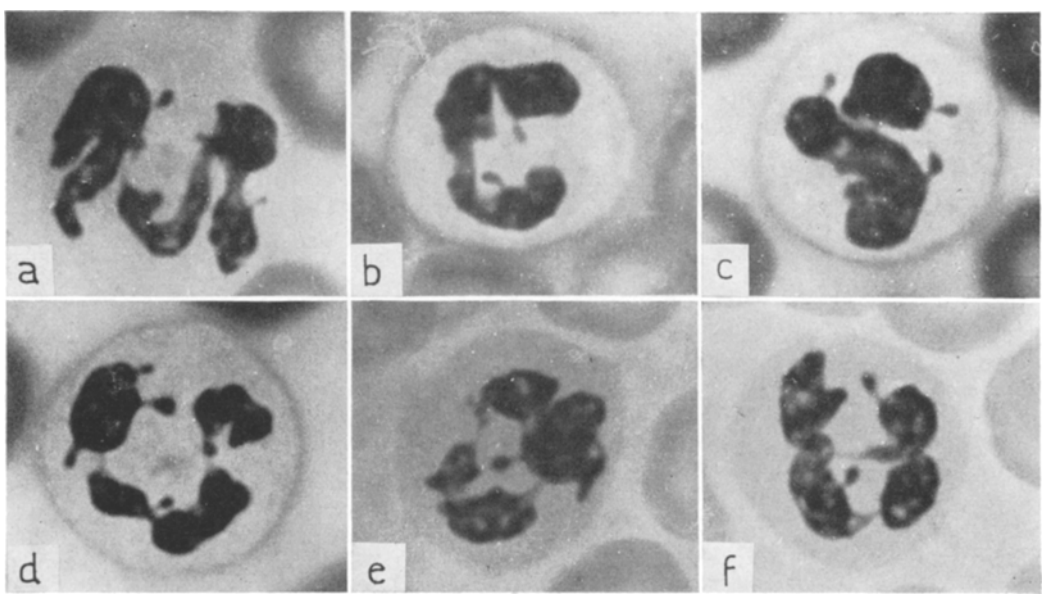
[1] Supported in part by a grant from the Italian 'Consiglio Nazionale delle Ricerche', Grant No. 7854/H.

[2] B. CHIARELLI and L. BARBERIS, Caryologia *17*, 567 (1964).

[3] W. KOSENOW, Trinagolo *2*, 309 (1966).

[4] B. CHIARELLI and L. BARBERIS, Experientia *20*, 679 (1964).

[5] Acknowledgment: The authors are indebted to the Yerkes Primate Centre for providing the blood smears of the orangs and gorilla; and to the Turin Zoo for those of the chimpanzees. – The diligent cooperation of Mrs. PAOLA PIZZETTI is also acknowledged.



Microphotographs of the nuclear protrusions of different individuals of anthropoid apes: (a) female orang outan (Jawata); (b) and (c) female gorilla (Oko); (d) female gorilla (Shamba); (e) and (f) male gorilla (Colobar). × 1000.

Riassunto. È stata condotta una indagine sul numero delle protrusioni nucleari del tipo drumstick nei leucociti polimorfonucleati di diversi esemplari di Antropomorfe (*Gorilla, Pan, Pongo*). Queste protrusioni sono state ris contrate in entrambi i sessi, talvolta anche più di una (fino a 6) per nucleo ed in proporzione diversa nelle diverse speci.

B. Chiarelli and M. Sarti

Centro di Primatologia, Istituto di Antropologia, Università di Torino (Italy), May 22, 1966.

Quantitative Determination of Adenovirus Antigens by Means of a Fluorescent Precipitin Test[1]

A quantitative fluorescent precipitin test for determination of submicrogram and microgram amounts of antigens was described recently[2]. The present communication shows that this test can be successfully applied to studies on adenovirus antigens.

Prototype seed viruses, obtained from the American Type Culture Collection, were propagated in monolayer cultures of HeLa cells. Infectivity titrations were performed in KB cells.

The globulin fraction, obtained from reference equine antisera[3] (prepared against KB cell-grown adenoviruses) by precipitation with half-saturated ammonium sulfate, was dissolved in the original volume of a buffer solution, 0.025 *M* with respect to both sodium carbonate and sodium bicarbonate, and was dialyzed overnight against 10 volumes of the same buffer containing 0.1 mg/ml of fluorescein isothiocyanate (FITC). The unbound FITC was then removed by exhaustive dialysis against phosphate-buffered saline (0.007 *M* phosphate, 0.14 *M* sodium chloride, pH 7.2). An equal volume of control antigen (non-infected HeLa cells and fluids with medium and newborn calf serum treated under conditions identical to those given for infected cells and fluids) was added to the FITC-conjugated preparations of antibodies. The mixtures were then incubated for 30 min at 37 °C, left overnight at 4 °C and stored at − 20 °C.

The fluorescent precipitin test has been described in detail elsewhere[2]. Aliquots of 0.2–1.0 ml of antigen and 0.2 ml of FITC-conjugated antibody preparation (constant amounts of each antigen and antibody throughout the experiments) were used for each test.

The results summarized in the first 2 columns of the Table show the sensitivity of the test. The ratio of $TCID_{50}$ value to fluorescence reading ranged from $1.2 \cdot 10^2$ to $2.9 \cdot 10^3$ with the exception of adenovirus 5, which had a ratio of $1.3 \cdot 10^4$. These differences probably reflect different levels of antigen production by cells infected with different adenoviruses. The reactions of antigen preparations with homologous antisera in general greatly exceeded the reactions obtained with heterologous antisera. To obtain comparable data on the reciprocal cross reactions between antigens present in the individual virus preparations, the values of R[4] were calculated and are also presented in the Table. These values correspond to the geometrical averages of readings obtained from heterologous reactions divided by the geometrical aver-

[1] This investigation was supported in part by contract PH43-63-80 from the National Institutes of Health.

[2] A. R. Neurath, Z. Naturforsch. *20b*, 974 (1965).

[3] Kindly supplied by Dr. J. B. Lucas from the Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia.

[4] I. Archetti and F. L. Horsfall jr., J. exp. Med. *92*, 441 (1950).

Fluorescent immunoprecipitation reactions between adenovirus antigens and antisera

Antigen			R values[a]										
Type	Log titer[b]	Fluorescence[c]	Antiserum type										
			1	2	3	4	5	6	7a	8	9	10	13
1	4.9	120	1.00	0.21	0.21	0.12	0.21	0.06	0.10	0.29	0.14	0.43	0
2	5.4	95	0.11	1.00	0	0	0	0	0	0	0	0	0
3	4.0	16	0.21	0	1.00	0	0.31	0.10	0.90	0.19	0	0	0
4	5.1	66	0.12	0	0	1.00	0	0	0	0.39	0	0	0
5	6.3	150	0.21	0	0.31	0	1.00	0.08	0.13	0	0	0	0
6	5.5	120	0.06	0	0.10	0	0.08	1.00	0.13	0	0	0	0
7a	4.7	32	0.10	0	0.90	0	0.13	0.13	1.00	0.40	0	0	0
8	4.1	45	0.29	0	0.19	0.39	0	0	0.40	1.00	0	0.30	0.25
9	3.6	20	0.14	0	0	0	0	0	0	0	1.00	0.18	0
10	3.7	28	0.43	0	0	0	0	0	0	0.30	0.18	1.00	0
13	3.5	26	0	0	0	0	0	0	0	0.25	0	0	1.00

[a] R = reciprocal cross reaction[8]; the method of calculation is shown in the text. [b] Log_{10} $TCID_{50}$ in KB cells (absolute amount of virus in test). [c] Fluorescence readings on the Turner fluorometer after reactions with homologous sera.