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.

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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.

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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.

ages of readings from homologous reactions. An R value of 1 corresponds to the homologous reaction.

$$\left(R = \sqrt{\frac{\text{reading: serum A vs antigen B}}{\text{reading: serum A vs antigen A}}} \times \sqrt{\frac{\text{reading: serum B vs antigen A}}{\text{reading: serum B vs antigen B}}}\right)$$

One-sided cross reactions (R = 0) were observed with antisera of types 2 (antigens 5 and 7), 4 (antigens 3, 5, 6, 7 and 10), 10 (antigen 5) and 13 (antigen 5). The readings corresponding to these reactions did not exceed 10% of the readings obtained with homologous antigen, except those of antiserum type 2 with antigen type 7 (32%); antiserum type 4 with antigen types 3 (31%), 7 (18%) and 10 (121%); and antiserum type 10 with antigen type 7 (19%).

An absolute precision of about ± 3 fluorometer scale readings can be achieved, the relative error thus depending on the absolute value of fluorometer readings.

Since both type-specific and group-specific antigens give immunoprecipitates in the Ouchterlony test[5–8], and adenovirus virions are agglutinated by type-specific antibodies[9], the fluorescent precipitin test undoubtedly measures all these antigens when present in a crude preparation. The observed cross reactions thus include not only real crossings between type-specific adenovirus antigens but also must reflect the presence of some group-specific sites in the preparations tested. It is noteworthy that the greatest reciprocal cross reaction in our experiments was observed between types 3 and 7a adenovirus antigens, two types in which the serological relationship has been well established[10–15]. Recent data suggest that besides the type-specific antigen of adenoviruses, the group-specific antigen also contains type-specific sites probably different from those of type-specific antigen[16–18]. The preponderance of type-specific binding sites in each of the crude antigen preparations tested might be responsible for the relative type specificity of the fluorescent precipitin test. It should be emphasized that the values of R presented in the Table indicate relative type specificity in the fluorescent precipitin test in spite of the quantitative differences in the preparations.

The greatest advantages of the present method are the high sensitivity combined with high precision (about ± 5% under the usual conditions) and the possibility of directly measuring the interrelationships between antigens and antibodies.

Zusammenfassung. Es wird die Anwendung eines quantitativen Fluoreszenz-Präzipitin-Tests für Adenovirus-Antigene beschrieben. Die Methode wurde für 11 verschiedene Adenoviren angewendet und hat sich als relativ typenspezifisch erwiesen.

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ACTH-Stimulierung der Corticosteroidbiosynthese durch Freisetzung «gebundener» 21-Desoxypregnene

Nach HAYNES und BERTHET[1,2] beruht die Stimulierung der Corticosteroidbiosynthese durch ACTH auf einer vermehrten Bereitstellung des für die Steroid-Hydroxylierung benötigten[3,4] NADPH. KORITZ und PÉRON[5] postulierten einen zusätzlichen ACTH-Effekt, der auf der Freisetzung «gebundener» Endogenpräkursoren der Corticosteroide beruht.

Eigene Untersuchungen über die in vitro Biosynthese von 4-C^{14}-Corticosteroiden aus 4-C^{14}-Cholesterin und 4-C^{14}-Progesteron geben Anlass zu der Annahme, dass ACTH den Abbau von 21-Desoxypregnenen ermöglicht, welche nach ihrer Bildung aus Cholesterin in intaktem, adrenalem Rindengewebe zunächst in einer Form vorliegen, in der sie der Corticosteroidsynthese ohne Konditionierung durch ACTH nicht zugänglich sind.

Schnitte (0,5–1 mm^3 Würfelchen) der äusseren Rindenzonen frischer Rindernebennieren wurden in Krebs-Ringer-Bikarbonat-Glukose-(KRBG)-Lösung unter ständigem Begasen mit 95% O_2 + 5% CO_2 bei 37°C in einer Schüttelapparatur inkubiert. Nach Vorinkubationen von 30 min und – nach Erneuerung des Mediums – von weiteren 15 min Dauer wurde je Ansatz 1 g Gewebe in 10 ml frischer KRBG-Lösung mit 1,6 μCi (150 μg) 4-C^{14}-Cholesterin bzw. 4-C^{14}-Progesteron 120 min lang inkubiert. Pro Ansatz wurden zu Beginn der Hauptinkubation 8 IE ACTH («Cortrophine» Organon) zugesetzt. Der Prozent-

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