Plasma calcium levels before and after injection of anterior lobe or posterior lobe extracts of the pituitary gland or physiological saline

Experiments	No. of assay rats	Infused solutions	Plasma calcium levels (mg%) Control values	Lowest values	*P* values
1	30	Anterior lobe extract	10.990 ± 0.084	9.013 ± 0.084	< 0.01 as compared with physiological saline infused control
2	30	Posterior lobe extract	10.956 ± 0.093	10.383 ± 0.093	> 0.05 as compared with physiological saline infused control
3	10	Physiological saline	10.260 ± 0.145	9.810 ± 0.145	

Statistical analysis: the difference between the means were tested by Student's *t*-test[6].

Results and discussion. There was no significant fall in plasma calcium levels in posterior lobe extract or saline injected groups. A significant fall in plasma calcium level was observed in anterior lobe extract injected group (Table). The fall in plasma calcium occurred within 10 min after injection in 12 cases, after 20 min in 7 and after 30 min in 11 cases. A total of 17 rats died, 6 of them had been injected with anterior lobe extract and 11 with posterior lobe extract. The cause of death is still under study. Preliminary results[7] show that the injection of the pituitary extract produces a prominent fall in blood pressure and shock. At autopsy, severe congestion in all tissues, being more pronounced in adrenal gland, was found. Neither hypothalamic and brain tissue extracts nor physiological saline produced a fall in blood pressure and subsequent death. The infusion of glucocorticoids before the administration of pituitary extract protects the animals from shock and death.

Zusammenfassung. Erste Untersuchungen der Autoren über einen blutkalziumsenkenden Faktor aus der Hypophyse wurden ergänzt. Dabei wurde festgestellt, dass dieser kalziumsenkende Faktor nur im Hypophysenvorderlappen vorhanden ist.

M. S. ZILELI, S. CAGLAR, G. URUNAY, T. GUNER, E. MUFTUOPLU and G. KANRA

Metabolic Division, Hacettepe Medical School, Ankara (Turkey), 1 July 1968.

[6] G. W. SNEDECOR, in *Statistical Methods*, 5th edn (Iowa State University Press, Iowa 1956).
[7] M. EKICI, G. KANRA, S. CAGLAR, G. URUNAY and M. S. ZILELI, unpublished data.

Separation of Two Components of Adenovirus Type 12 Induced T-Antigens with Sephadex G-50

Hamsters and mice carrying virus-free tumours induced by adenovirus type 12 develop complement-fixing humoral antibodies to tumour antigens (T-antigens)[1-5]. These T-antigens are characterized by (a) their virus specificity; that is, they are coded for by viral information incorporated in the genetic apparatus of the tumor cells[2,5-7]; (b) their antigenicity, distinct from viral antigens; their synthesis does not require replication of virus DNA[5-7].

The biological function of the T-antigens is not yet known. Another question is whether the T-antigens consist of 1 single or more antigenic components. SHIMOJO et al.[8] reported 2 antigens; 1 non-precipitable by ultracentrifugation, called TS-antigen, the other precipitable by ultracentrifugation, TP-antigen. GILEAD and GINSBERG[9,10] isolated and purified a heat-labile single type of T-antigen, obtained from KB cells infected with adenovirus type 12, with an average sedimentation coefficient of 2.40 S. HOLLINSHEAD et al.[11,12] described a method, using Sephadex G-100 chromatography, for isolation of a heat-stable and a heat-labile species of T-antigens, also obtained from KB cells infected with type 12 adenovirus. In this preliminary communication we report a method for separation of 2 components of antigens from adenovirus type 12 induced hamster tumours with Sephadex G-50 gel filtration.

Materials and methods. Preparation of the tumour-antigen-extracts: tumours were produced by s.c. inoculation of adenovirus type 12 (strain Huie) preparations (infectivity titre in KB cells $10^{2,6}$ $TCID_{50}$/0.2 ml) in newborn hamsters. Tumour transplants were carried out by s.c. inoculation of a single cell suspension of adenovirus type 12 induced tumours into suckling hamsters. Preparing

[1] R. J. HUEBNER, W. P. ROWE and W. T. LANE, Proc. natn. Acad. Sci., U.S. *48*, 2051 (1962).
[2] R. J. HUEBNER, W. P. ROWE, H. C. TURNER and W. T. LANE, Proc. natn. Acad. Sci., U.S. *50*, 379 (1963).
[3] Y. YABE, L. SAMPER, E. BRYAN, G. TAYLOR and J. J. TRENTIN, Science *143*, 46 (1964).
[4] R. A. FUGMANN and M. M. SIGEL, J. Virol. *1*, 678 (1967).
[5] R. J. HUEBNER, in *Perspectives in Virology* (Academic Press, New York 1967), vol. 5, p. 147.
[6] Z. GILEAD and H. S. GINSBERG, J. Bact. *92*, 1853 (1966).
[7] Z. GILEAD and H. S. GINSBERG, J. Bact. *90*, 120 (1965).
[8] H. SHIMOJO, H. YAMAMOTO, E. YOSHIKAWA and T. YAMASHITA, Jap. J. med. Sci. Biol. *19*, 9 (1966).
[9] Z. GILEAD and H. S. GINSBERG, J. Virol. *2*, 7 (1968).
[10] Z. GILEAD and H. S. GINSBERG, J. Virol. *2*, 15 (1968).
[11] A. C. HOLLINSHEAD and R. J. HUEBNER, Nature *210*, 1381 (1966).
[12] A. C. HOLLINSHEAD, T. C. ALFORD, S. OROSZLAN, H. C. TURNER and R. J. HUEBNER, Proc. natn. Acad. Sci., U.S. *59*, 385 (1968).

the antigen extract necrotic and hemorrhagic parts of the tumours were removed by dissection, the residue washed free of debris and blood with PO_4-buffer, pH 7.2/0.06 *M*, put into 20% suspension with the same buffer, homogenized with a tissue grinder and clarified by centrifugation (3000 rpm for 15–20 min). The supernatant fluid was used as crude tumour antigen extract.

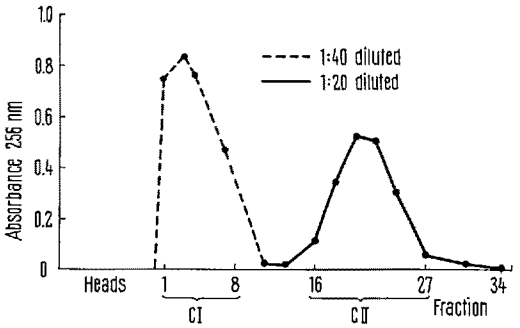
Gel-filtration: Sephadex G-50 was swelled in PO_4-buffer overnight at room temperature. A gel volume of 84 ml was packed into a Sephadex K 15/90 tube, according to conventional methods, and washed with PO_4-buffer pH 7.2/0.06 *M*. 20 ml of the crude tumour antigen extract were layered on top of the column (approximately 25% of the total bed volume). Elutions were done with 150 ml PO_4-buffer at room temperature and a flow rate of 0.8 ml/cm² per min. Fractions of 3 ml were collected, diluted 1:20 or 1:40 and tested in a Beckmann DB Spectrophotometer for their absorption at 256 nm. That is the same absorption as for the original crude extract. Homogeneous fractions were pooled and partially concentrated.

Concentration procedures: the method of KOHN[13] was used with polyethylene glycol 20 *M*. A concentration 6- to 10-fold was achieved.

Complement fixation test (CFT): the crude tumour antigen extracts and the pooled and partially concentrated fractions were titrated in the CF-test with conventional methods with narrow hamster tumour sera.

Heat treatment: the pooled and partially concentrated fractions were tested for susceptibility to heat in a water-bath at 56 °C for 30 min. After the taking out of the water-bath, the samples were immediately cooled at 4 °C.

Results. After gel filtration the photospectrometric examination of the fractions at 256 nm indicated 2 distinct peaks, called CI and CII, wherein T-antigens have always been found. CI, eluated in the fractions 1–8, consist of 24 ml and correspond to antigen samples, layered on top of column, in amount and appearance. CII, with essentially less absorption as CI, eluated in the fractions 16–27, consist of about 36 ml and has aqueous appearance.



Sephadex G-50 gel-filtration of crude tumour antigen extracts. 20 ml of antigen sample was applied on the 84-ml gel bed in a K 15/90 column. Eluation was done with PO_4-buffer, 3 ml fractions were collected, 1:40 or 1:20 diluted and their absorption at 256 nm tested photospectrometrically.

Determination of the thermal stability of the components CI and concentrated CII

	Not heated	Heated 56 °C/30 min	Thermal stability
CI	1:32	< 1: 4	labile
Concentrated CII	1:32	1:32	stable

Samples were heated for 30 min at 56 °C and the CF-titres of the residual antigens were tested.

CI and CII, tested for complement fixing reactivity, indicated for CI a positive, for CII a negative result. However, when CII was 6- to 10-fold concentrated, the complement fixation test was positive. The controls were: the 6-fold concentrated heads, the pooled and 6- to 10-fold concentrated fractions, 9–15, 28–34; the CF-tests were always negative. The concentrated CII, but not CI, was revealed to be heat-stable, when aliquots were tested for their complement fixing reactivity after heat treatment in a water-bath at 56 °C for 30 min.

Intensive mixing of the crude tumour antigen extracts with air-oxygen in a Vir-Tis homogenizer inactivated the CII-antigen components. After Sephadex G-50 gel filtration the complement fixing test of the concentrated CII components is negative, but not of the CI components. Therefore CII is oxygen-sensitive, whereas CI remains oxygen-insensitive.

Discussion. The crude tumour antigen extracts, obtained from tumour transplants (third passage) were separated with Sephadex G-50 gel filtration in 2 antigens, both reactive in CF-test with sera of A_{12} tumour-bearing hamsters. They differ in their susceptibility to heat, in their oxygen-sensitiveness and apparently in their molecular weight. Based on the properties of the Sephadex G-50[14], we can assume that the heat-labile and oxygen-stable components CI have molecules larger than the largest pores of the swollen Sephadex G-50, that they cannot penetrate the gel particles and therefore pass through the bed in the liquid phase outside the particles. CI are thus eluated first and have a molecular weight over 30,000 (exclusion limit for proteins for Sephadex G-50 is about 30,000).

The heat-stable but oxygen-sensitive components CII, consisting of smaller molecules, penetrate the gel particles to an extent depending on their size and shape and are eluated from Sephadex bed later than the larger CI molecules. The molecular weight of CII is therefore less than 30,000. Without having exactly determined the molecular weight of CI and CII, we can say that these results correspond well with the results of HOLLINSHEAD et al.[11]. These authors have isolated by gel chromatography from adenovirus type 12 infected KB cells a heat-stable antigen component with a molecular weight of about 20,000 and a heat-labile component with a molecular weight of about 80,000.

Further investigation is needed to isolate and purify the 2 antigen components and to estimate their molecular weights more precisely.

Zusammenfassung. Es wird aus Hamstertumoren, die durch Adenovirus Typ 12 induziert wurden, ein Tumorantigenextrakt hergestellt. Dieser kann mit Sephadex G-50 Gelfiltration in 2 mittels Komplementbindung nachweisbare T-Antigen-Komponenten aufgetrennt werden.

P. ERB, M. GASSER and H. LOEFFLER

Institut für Mikrobiologie und Hygiene der Universität, 4000 Basel (Switzerland), 17 September 1968.

[13] J. KOHN, Nature *183*, 1055 (1959).
[14] H. DETERMANN, *Gelchromatographie* (Springer Verlag, Berlin 1967).