durch die Anstreichgeschwindigkeit, die im Mittel 7 mm/s beträgt, das Stridulieren mit einer oder beiden Pars stridens sowie durch die Wahl feingerippter proximaler bzw. grobgerippter distaler Teile der Pars stridens verändert. Der Lautanteil des Abdomino-elytralen Instrumentes hat einen regelmäßigeren Amplitudenverlauf. Er nimmt in Silbe A und B erst zu, dann ab. Die Impulsfrequenz liegt zwischen 5 und 8 kHz. Das Spektrum des Protestlautes, der von beiden Instrumenten erzeugt wird, liegt im Bereich von 100 Hz bis 20 kHz. Letzteres ist die obere Grenze der bisher von mir gemachten Lautaufnahmen. AUTRUM [3] stellte für *Geotrupes sp.* einen Ultraschallanteil bis 40 kHz fest.

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Gross Chemical Composition of Strain Flury HEP Rabies Virus

H. D. SCHLUMBERGER*, L. G. SCHNEIDER**, H.-P. KULAS*, and H. DIRINGER*

* Max-Planck-Institut für Virusforschung, Tübingen and ** Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen

(Z. Naturforsch. 28c, 103—104 [1973]; received August 16/November 3, 1972)

Rabies virus protein, RNA, lipid, carbohydrate

The Flury HEP strain of *rabies* virus, grown in BHK 21/C 13-cells was purified by adsorption and elution from aluminium phosphate gel followed by a cycle of high and low speed centrifugation as previously described [1]. The specific infectivity (LD_{50}/mg dry mass) of the purified virus was 1000 to 4000 times higher than of the infectious tissue culture fluid. The crude virus preparation reacted in the complement fixation assay with 8 units of anti-BHK 21 rabbit serum up to dilutions of 1 : 4000 to 1 : 8000. On the other hand the purified and 200 to 300 fold concentrated virus preparations reacted in the same test up to dilutions of 1 : 4 to 1 : 8 indicating that the purified virus contained $2 \cdot 10^5 - 3 \cdot 10^5$ less host antigens than the crude virus preparation. Thus the antigenic impurities possibly present in the purified virus preparations seem to be negligable and the same most likely applies for non-immunogenic impurities. Although the virus was grown in a medium containing bovine serum albumin (BSA) anti BSA sera did not react in a complement fixation with purified virus.

Preparations of purified virus contained per mg dry mass in average 10^{10} LD_{50} determined by intracerebral inoculation of suckling mice, 10^4 complement fixing units (CFU) and 5×10^4 hemagglutinating units (HAU). The results are in good agreement with the specific activites of the same virus purified by other methods [2].

The main chemical constituents of the virus were determined for four different batches. Purified virus was dialyzed exhaustively against destilled water at 4 °C, lyophilized, and dried at 20 °C over P_2O_5 under reduced pressure for 48 hours. The water uptake of the dried material during the weighing procedure was recorded and the weight was corrected accordingly.

Lipids were extracted with chloroform/methanol/water (40/20/3) at 20 °C for 3 min, using 6 ml of the solvent per 40 mg of dry mass. The residue was again extracted with half the volume of a mixture, containing 20 parts of chloroform, 40 parts of methanol and 3 parts of water.

In average 25 % (24,6–28 %) of the dry mass was solubilized by this treatment. The weight of the insoluble residue represented in average 74,3 % (72–76 %) of the original dry mass.

The protein content of the virus, as determined by quantitative analysis of the amino acid composition of the insoluble residue, ranged from 63,4 to 70,0 %

Table I. Lipid, protein, carbohydrate and RNA content of strain Flury HEP rabies virus.

constituent	% of dry weight
Lipids:	
(Chloroform/methanol soluble fraction)	25.8 ± 1.5 *
phosphorus	0.41 ± 0.01
chloroform/methanol insoluble fraction	74.3 ± 1.9
phosphorus	0.38 ± 0.06
Protein:	67.0 ± 3.6
Protein-bound carbohydrates:	
hexoses	0.7
fucose	0.1
sialic acids	0.8
galactosamine	0.3
glucosamine	1.0
	2.9
RNA	3.9 ± 0.6
total mass of the insoluble residue:	73.8 ± 3.6
total mass of the virus:	99.6 ± 2.5

* Mean ± standard deviation.

Requests for reprints should be send to H. DIRINGER, MPI für Virusforschung, *D-7400 Tübingen*, Spemannstr. 35.

(average 67,0 %) (Table I). To correct for amino acid decay during hydrolysis, samples of 0.6 to 0.8 mg of insoluble residue were hydrolyzed in 6 N HCl at 110 °C in sealed tubes under a nitrogen atmosphere for 24, 48, 72, 96 and 120 hours, respectively. After hydrolysis the samples were dried and analyzed in a Beckman amino acid analyzer. The results of the individual amino acid determinations were corrected by graphic extrapolation. The mass of protein was calculated from the corrected data (Table I).

The ribonucleic acid (RNA) content of the virus was calculated from the phosphorus content of the insoluble residue determined by the method of BARTLETT [3] (Table II). Possible phosphorylation of viral proteins was not considered in these calculations, and thus the data obtained (in average 3.9 % RNA (3.2 to 4.6 %) per mg dry mass), represent maximal values.

Table II. Amino acid composition of strain Flury HEP *rabies* virus.

Amino acid *	% of dry weight
Lys	5.0 ± 0.6
His	2.1 ± 0.2
Arg	4.3 ± 0.6
Asp	6.8 ± 0.7
Thr	3.9 ± 0.2
Ser	3.5 ± 0.15
Glu	8.8 ± 0.5
Pro	2.8 ± 0.2
Gly	2.5 ± 0.05
Ala	2.8 ± 0.3
Cys	1.4 ± 0.2
Val	4.1 ± 0.15
Met	1.8 ± 0.3
Ile	4.1 ± 0.4
Leu	6.5 ± 0.3
Tyr	2.9 ± 0.1
Phe	3.6 ± 0.1
	67 ± 3.6

* Tryptophan was not determined.

Carbohydrate analysis was carried out only with two virus batches. Neutral sugars present in the chloroform/methanol-insoluble residue were hydrolyzed and isolated according to WALBORG *et al.* [4]. Hexoses were determined by the orcinol-sulfuric acid reaction of WINZLER [5] employing a standard of galactose:mannose = 1 : 1. The data are corrected for the presence of ribose and fucose interfering with the determination of hexoses. Ribose was determined by the orcinol method [6]. Fucose was assayed by the cystein-sulfuric acid reagent according to DISCHE [7]. Sialic acids were determined by the method of WARREN [8], after hydrolysis with 0.1 N sulfuric acid for 1 hour at 80 °C. Standards of *N*-acetyl-neuraminic acid were treated similarly.

Hexosamines were analyzed directly in a Beckman amino acid analyzer after hydrolysis for 2,4 and 6 hours, respectively at 110 °C in 5 ml of 2 N HCl of each 5 mg of insoluble residue in sealed tubes under a nitrogen atmosphere. The molar ratio of galactosamine and glucosamine was found to be 1:3. The total carbohydrate content of the chloroform/methanol-insoluble residue was estimated to be 2.9 % (Table II). The balance of protein, carbohydrate and RNA in in good agreement with the weight determinations of the insoluble residue. The sum of the viral constituents adds up to about 100 % of the original viral mass.

The authors like to express their gratitude to Professor Dr. F. A. ANDERER and to Professor Dr. M. MUSSGAY for valuable discussions and the support of this work. We are indebted to Dr. M. A. KOCH and Dr. F. SOKOL for revising the manuscript. The skillful assistence of Miss D. TROMM and Mr. M. GENČIĆ is gratefully acknowledged.

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