

myocardial damage in hyperthyroid patients. Indeed, it has been shown that CK-BB in the serum of about 50% of the patients suffering from thyrotoxicosis, declined following medical treatment<sup>21</sup>. Thus there is a potential for diagnostic confusion when CK isoenzyme profiles are used.

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## Distinction of influenza viruses of different host cell origin

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**Summary.** Influenza A viruses grown in different animal or human cells retain their antigenic make-up as tested by the usual immunological assays. With the aid of a *Sambucus nigra* (L.) extract containing its lectins the viruses can be distinguished after one single passage in a different cell type by a change in their hemagglutinating properties. Binding of such lectins to influenza viruses may be a means for a more subtle classification, relating to the host cell origin of the virus.

**Key words.** *Sambucus nigra* (L.); influenza virus; lectin.

Influenza A viruses grown in different cell types retain their antigenic make-up. The hemagglutinin and the neuraminidase, the two main surface antigens, remain of the same type. Antigenic shifts or drifts which occur in vivo can only be mimicked in vitro by growing the virus in antibody-containing media<sup>1</sup>. Subtle differences which are due to different cellular origins, such as changes in the sugar composition of the hemagglutinin may, however, not be detected by routine immunological methods because of the weak immunogenicity of sugars. By means of a *Sambucus nigra* (L.) extract containing several lectins<sup>2</sup> it is demonstrated that the hemagglutinin of an influenza A virus may vary, depending on the cellular origin.

### Materials and methods

***Sambucus nigra* (L.) extracts.** The ripe fruits were collected in northern Switzerland during the second half of September. Ten grams of fruit were frozen in 50-ml Fal-

con tubes (Falcon Plastics, Oxnard, CA) at -20 °C. After thawing, the specimens were mashed in the tubes, with a glass homogenizer, and Eagle's minimum essential medium (MEM) was added to a volume of 20 ml. The samples were frozen at -80 °C. The thawing was then done on a vortex at maximum speed in order that the lumps of ice would homogenize the fruits further. After centrifugation at 2250 × g for 30 min at 4 °C the supernatants were stored at -80 °C in aliquots of 1 ml until used.

**Virus.** Avian influenza virus (AIV; A/Turkey/England/63, Hav1 Nav3, Langham strain) had been adapted to primary cultures and cell lines of chicken, mouse, and human origin as described earlier in detail<sup>3-7</sup>.

**Hemagglutinin determinations.** The hemagglutinin titrations were effected using WHO standard procedures<sup>6</sup>.

**Hemagglutination reduction assay.** AIV grown in different cells were mixed with an equal volume of different dilutions of *S. nigra* extract or dilution medium (MEM

plus 2% fetal bovine serum) alone. After 14 h of incubation at 20°C, hemagglutination determinations were done.

### Results and discussion

The table shows the results for the reduction of hemagglutination of AIV of different cellular origins by *S. nigra* extract. Two groups of viruses can be distinguished: Group 1: The hemagglutinating property is abolished by *S. nigra* extract. Group 2 viruses: The hemagglutination titer is not changed by incubation with *S. nigra* extract. Viruses from group 1 change to group 2 after one single passage in chicken cells. Group 1 viruses also originating from chicken cells<sup>8</sup> change their hemagglutinating properties after a passage in the cells mentioned under group 1 in the table. The figure shows a representative experiment demonstrating this hemagglutination reduction by *S. nigra* with the AIV grown in a human breast cancer cell line (BT20).

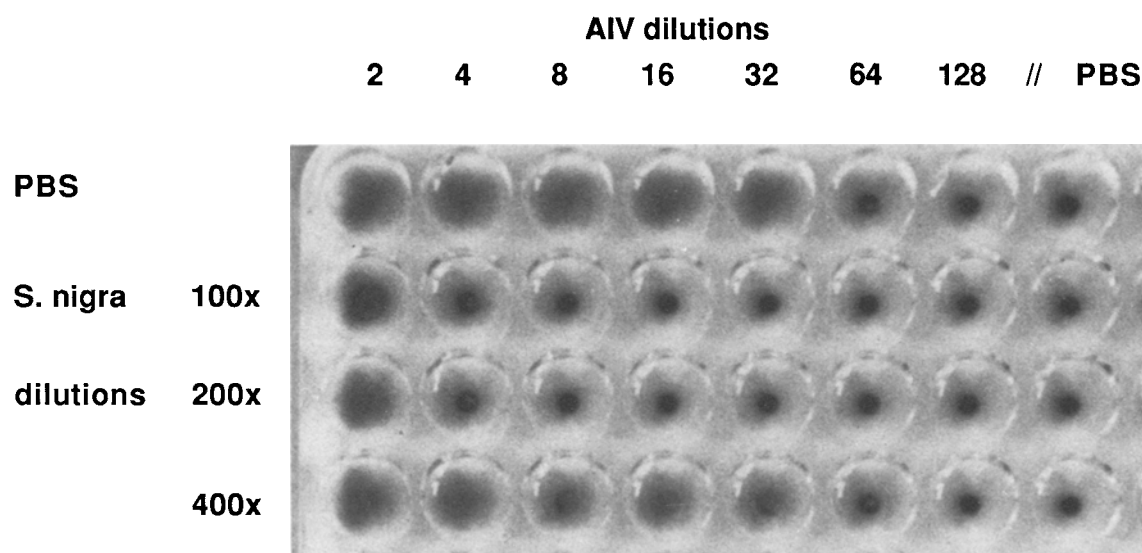
Host cell-mediated variations of influenza viruses were shown using a panel of monoclonal antibodies<sup>9</sup>. In some instances these variations were due to a single amino acid substitution in the hemagglutinin. However, in other instances the amino acid sequences were identical, although these viruses exhibited antigenic differences when examined with anti-hemagglutinin monoclonal antibodies. Therefore, single amino acid changes in the hemagglutinin molecule may not be the sole cause of antigenic changes in host cell-mediated variation. The observation described here, that influenza viruses can be distinguished after one single passage in a different host cell (AML and AML plus one egg passage; BT20 and BT20 plus one egg passage) by *S. nigra* lectins points to variations of the sugar composition of the hemagglutinin. Glycosylation of influenza hemagglutinin is very pro-

Hemagglutination reduction of AIV by *S. nigra* (L.) extract (dilution 1:50)

Cellular origin of AIV	HA <sup>a</sup>	
	Extract	Control
Group 1		
Acute myelogenous leukemia (human AML, pr.cult.) <sup>4</sup>	neg	16
Human astrocytoma IV (primary culture)	neg	32
BT 20 (human breast cancer cell line) <sup>5</sup>	neg	16
HeLa <sup>3</sup>	neg	8
Flow 2000 (diploid human lung fibroblasts)	neg	16
EL-4 (mouse lymphoma)	neg	4
Group 2		
BEN (human lung cancer cell line) <sup>7</sup>	16	16
Human breast cancer (primary culture) <sup>6</sup>	32	32
BT 20 plus one egg passage (chicken)	16	16
Acute myelogenous leukemia plus one egg passage	16	16
Chicken embryo fibroblasts <sup>3</sup>	32	32

<sup>a</sup> HA: Hemagglutination titer; neg, negative, i.e. no hemagglutinin detected.

nounced, since carbohydrates are attached on most potential glycosylation sites<sup>10</sup>. Extensive analysis of the oligosaccharides of the hemagglutinin of the WSN strain of influenza showed that the oligosaccharide components varied with the host cell type<sup>11</sup>. The experiments presented here point to a difference in the galactose content of the hemagglutinin, since AIV was found to be neutralized by the D-galactose specific lectin of *S. nigra*<sup>2</sup>. With the aid of *S. nigra* extracts a rapid distinction of certain influenza viruses from different host cells can be made without the painstaking analysis by monoclonal antibodies or oligosaccharide analysis. Binding of lectins to influenza viruses may be a means for a more subtle classification relating to the host cell origin. Similar classifications could be devised for other enveloped viruses like the HIV viruses, which opens the possibility of tracing HIV viruses with a view to a more precise elucidation of the origin of individual viruses.



Agglutination of chicken erythrocytes by AIV (grown in the human breast cancer cell line BT 20) preincubated with increasing dilutions of *Sambucus nigra* (L.) extract. Black dot on the ground of the well shows

a negative hemagglutination. Note the decrease in the AIV hemagglutination titer from 32 (control) to 2 up to a *S. nigra* dilution of 200×. All dilutions were prepared with phosphate buffered saline (PBS).

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## Announcements

The European Molecular Biology Organization (EMBO) will organize the following Workshops and Courses in 1989. Those interested should contact the organizers directly at the addresses given for each individual event.

### EMBO Workshops 1989

Subject	Organizer(s)	Address for inquiries	Date, Place
The <i>scid</i> mouse mutant: cellular/molecular characterization and lymphoid reconstitution	• W. Schuler M. Bosma R. Phillips	Institute for Immunology Grenzacherstrasse 487 4005 Basel Switzerland	20–22 February CH-Basel
Genetics of nervous system development	J. Campos-Ortega	Institut für Entwicklungsphysiologie Universität zu Köln Gyrhofstrasse 17 5000 Köln 41 Federal Republic of Germany	3–7 April D-Simonswald
Molecular biology of retroviral viruses and retroviral elements	• T. Hohn J. Fütterer H. Schaller	Friedrich-Miescher-Institut P.O. Box 2543 4002 Basel Switzerland	4–7 April CH-Flumserberg
Chromosome 21: Impact of the new genome technology in human genetics	• N. Sacchi P. Durand G. Romeo G. Bernardi	International School of Pediatrics Istituto G. Gaslini Genoa Italy	18–20 May I-Genoa
Illegitimate recombination	S. D. Ehrlich	Laboratoire de Génétique Microbienne Institut de Biotechnologie INRA-Domaine de Vilvert 78350 Jouy en Josas France	28–31 May F-Paris
The molecular biology of plant virus pathogenicity	• J. Davies M. Mayo	Department of Virus Research AFRC Institute of Plant Science Research Colney Lane Norwich NR4 7UH England	16–19 July GB-Kent
Molecular and cell biology of gap junctions	K. Willecke • P. Meda	Institute of Histology University, C.M.U. 1, rue Michel Servet 1211 Geneva 4 Switzerland	18–23 July D-Issee
Molecular biology of filamentous fungi	• J. Knowles H. Arst, H. van den Broek, C. van den Hondel, C. Scazzocchio, U. Stahl	Biotechnical Laboratory, VTT Technical Research Centre Tietotie 2 02150 Espoo Finland	2–7 August SF-Espoo
Comparative structure and function of membranes in chloroplasts and cyanobacteria (blue-green algae)	• G. Peschek P. Böger R. Douce G. Papageorgiou	Biophysical Chemistry Group Institute of Physical Chemistry Währinger Strasse 42 1090 Wien Austria	4–8 September GR-Sounion