thus significantly higher. This is important not only in relation to increasing the oxygen capacity of the artificial oxygen carrier, but also in connection with the possibility of using various additional components during its administration, including polymers, in the attempt to develop a blood substitute of complex composition and polyfunctional action — an "artificial blood" [3].

A preliminary biological study of a solution of PHb-PP in experiments on noninbred albino mice and rats showed absence of toxicity in doses of 3 g/kg body weight, and an identical circulation time in the blood stream with that of PHb.

These investigations thus showed that the new model of an artificial oxygen carrier, developed by the writers, possesses basic functional properties similar to those of donors' blood.

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PEPTIDE MAPPING OF HUMAN ADENOVIRUS TYPE 6 AND SIMIAN ADENOVIRUS TYPE 7 HEXON AND CORE PROTEIN

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One of the most interesting objects in modern molecular biology and genetic engineering is the adenovirus, which is widespread in nature. By now about 80 serotypes of animal and human adenoviruses have been identified immunologically [5]. As criteria for isolation of subgroups of adenoviruses, their hemagglutinating properties, their oncogenicity, the homology of their nucleic acids as revealed by the hybridization method, and also the protein spectrum of the virions, determined electrophoretically, are usually used [6, 7, 11, 12].

In the investigation described below the method of tryptic mapping of proteins (fingerprinting) was used to determine similarity between human type 6 and Simian type 7 adenoviruses with respect to two major proteins — one external (hexon) and the other internal (core) [4].

EXPERIMENTAL METHOD

Adenovirus Ad6 was obtained in a culture of HeLa cells and SA7 virus in a culture of green guenon kidney cells [1, 2]. The viruses were extracted from infected cells by freezing and thawing, treated with freon-113, and purified by centrifugation in CsCl [10]. The

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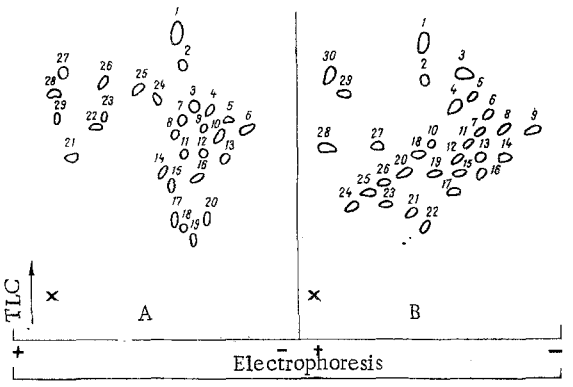

Fig. 1

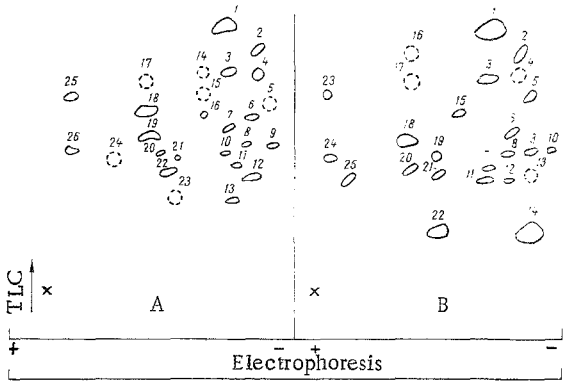

Fig. 2

Fig. 1. Tryptic mapping of hexons (polypeptide II). A) Hexon of Ad6 virus; B) hexon of SA7 virus. Cross indicates start.

Fig. 2. Tryptic mapping of core proteins (polypeptide V). A) Protein of Ad6 virus; B) protein of SA7 virus. Cross marks start.

virions were disintegrated by heating in dissociating buffer, the proteins were separated by electrophoresis by the method described in [8], and the strips were stained with 0.5% Coomassie. The proteins were iodinated by the method described previously [3]. Pieces of gel containing ^{125}I-labelled protein were dried, trypsin free from chymotrypsin (from Worthington, USA) in 0.5 ml 0.05 M NH_4HCO_3, pH 8.3, was added to the samples in doses of 25 mg each, and the samples were incubated for 16 h at 37°C. The gel was removed, the samples freeze-dried, 1 ml of deonized water was added to each, and they were again freeze-dried to remove traces of NH_4HCO_3. Oligopeptides were separated in a thin layer of cellulose (10 × 10 cm plates, from Merck, West Germany) in a two-dimensional combination of electrophoresis (500 V, 1 h) in a system of pyridine–acetic acid–water (25:1:225) and chromatography in a system of pyridine–n-butanol–acetic acid–water (10:15:3:12). Kodak (USA) film was used for autoradiography.

EXPERIMENTAL RESULTS

Two electrophoretically pure proteins from Ad6 and SA7 viruses were chosen for mapping: the hexon, i.e., the principal surface protein of the capsids (polypeptide II), with a molecular weight of 134,000 and 112,000, respectively, and polypeptide V, a core protein covering about half of the virus DNA (mol. wt. 49,000 and 51,000) [4].

There are as yet no data in the literature of the amino acid composition of the proteins chosen for testing. However, it is known that the hexon of human type 2 adenovirus, belonging to the same serologic subgroup C of human adenoviruses as AD6, contains altogether 85-90 arginine and lysine residues, accessible to the action of trypsin, and about 60 tyrosine residues, capable of binding ^{125}I [9]. The number of spots on the autoradiograph, allowing for the irregularity of distribution of amino acid residues usual in such cases, ought therefore to be 30-40. A significant increase in this number would mean incompleteness of tryptic hydrolysis. In fact, 29 spots were observed for the Ad6 hexon and 30 for the SA7 hexon (Fig. 1), i.e., the subsequent interpretation of the results was quite correct.

When the peptide maps are analyzed attention must be paid not only to absolute agreement between the positions of the spots, which is easily verified by fractionating the combined tryptic digest of test proteins. Much information is given by the general pattern of the fingerprints. This is due to the high sensitivity of the peptide mapping method, higher, for example, than that of hybridization of the corresponding template nucleic acids. Changes in individual triplets cannot decisively affect hybridization of sufficiently long polynucleotides. Meanwhile, replacement of a single amino acid in an oligopeptide can shift the spot on the peptide map to a greater or lesser degree.

Comparison of tryptic digests of the Ad6 and SA7 hexons (Fig. 1) revealed several characteristic groups of spots, first, fragments 1 and 2, which coincided for both hexons. The group of spots 3–16 was similarly arranged, although only two of them coincided (oligopeptides 4 and 6 for Ad6, 6 and 9 for SA7). Fragments with low electrophoretic mobility (21, 27-29 for Ad6 and 28–30 for SA7) were characteristically arranged. Spots 21 of Ad6 and 28 of SA7

overlapped. On the other hand, oligopeptides lying in the center and lower part of the map differed clearly in their mobility and, consequently, in their primary structure. It can thus be concluded that hexons of viruses Ad6 and SA7 contain both coincident and noncoincident regions, but nevertheless the principle of organization of their primary structure is of the same common character.

The general similarity was even more marked when the investigation moved on from the external to the core proteins (Fig. 2). In the latter case three groups of spots with high, average, and low electrophoretic mobility can be distinguished on the tryptic map, within each of which a similar distribution of fragments with respect to R_f values could be observed. For the core protein of Ad6 virus the groups were formed by oligopeptides 1-13, 17-23, and 24-26, whereas for SA7 they were formed by 1-3, 16-22, and 23-25, respectively. Under these circumstances about half of the spots overlapped partly or completely. The most significant difference between these two proteins was that two intense spots (14 and 22) with R_f values of 0.23 and 0.24 were present on the SA7 map.

It can thus be concluded that proteins of adenoviruses Ad6 and SA7 possess definite similarity, which increases during passage from the hexon to the main core protein. With regard to the technique of peptide mapping, since it is much more sensitive and delicate than gel electrophoresis of whole proteins, and is inferior from this point of view only to direct sequencing of biopolymers, it is of considerable interest for the classification of adenoviruses on the basis of their complement of structural proteins. This method can also reveal more precisely the antigenic determinants of the different adenoviruses and study their interconnection, evolution, and possible origin.

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