THE POSSIBILITY OF USING FREON DEPROTENIZATION AND ULTRACENTRIFUGATION FOR ROUS VIRUS PURIFICATION

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In 1956 a new method was proposed for isolating and purifying animal and plant viruses [4]. The method was based on the deproteinization of a suspension containing the virus by means of fluorocarbon (Freon - 112).

These experiments were repeated [2, 3], and the method of fluorocarbon (Arcton-63) purification was successfully used for isolating and electron microscope studying of the Rous sarcoma virus. A detailed description of fluorocarbon purification of this virus is given in another work [9]. In this investigation, carried out with the B 77 chicken fibromyxosarcoma virus, the author was able, after sixfold treatment of the tumor extract with flurocarbon (Ledon-113), to appreciably purify it from nonviral protein and with subsequent ultracentrifugation to obtain a residue of highly active virus. Even more effective was a sixfold extraction with Freon of a suspension of the virus preliminarily precipitated on the ultracentrifuge.

The successful development of methods of purifying Rous virus from tissue proteins is of primary importance for investigations involving a number of problems: the interrelationship of this virus with the tumor cell, the antigenic and immunogenic properties of Rous virus, the genetic and biochemical investigations of viral RNA, and others. The method of differential centrifugation [1, 6, 8] previously used for purifying this virus proved to be insufficiently effective for the aforementioned purposes, since the viral preparation obtained in this case contained an appreciable admixture of tissue tumor protein. The combined method of purification with the use of fluorocarbon (Freon-113) and ultracentrifugation has definite advantages in this respect. At the same time the aforementioned works, where fluorocarbon was used for Rous virus purification, contain certain contradictions. Thus, according to some data [5] ultracentrifugation of the Freon-purified Rous virus at 144,000 g led to the formation of a small dense precipitate having a weak oncogenic activity. The supernate, on the other hand, contained the bulk of the active virus. In another study [9] opposite results were obtained: it was possible to precipitate the bulk of the virus by ultracentrifugation at 80,000 g from an extract preliminarily purified by Freon.

In connection with the contradictory data in the literature and in view of the great importance of the problem of purifying oncogenic viruses, we considered it expedient to obtain our own experimental data on this problem.

METHOD

The present study was carried out with Rous sarcoma (Carr strain) passaged on 4-week-old White Russian chicks by means of a cell suspension. The tumor, freed from hemorrhagic and necrotic areas, was extracted with 0.02 M phosphate-citrate buffer (pH 7.2) with sucrose (0.25 M solution). The buffer was used in a quantity equal to 1/5-1/10 of tissue weight. A solution of hyalouronidase in an end concentration of 1% was added to the extract. The tissue was ground in a blenderat 16,000 rpm for 8-10 min or at 9000 rpm for 15-20 min. After homogenization the extract was centrifuged at 5000 rpm for 15 min. All these and subsequent manipulations were done at 0-2°. To the supernate of the extract (SE) we added fluorocarbon (Freon-113) in a volume equal to half the

Purification of Virus by the Method of Ultracentrifugation After Its Twofold Purification with Freon

No. of Experiment	SE after 2-fold Freon treatment		Precipitate after ultracentrifugation		SE after ultracentrifugation	
	Virus titer	Protein (in mg/ml)	Virus titer	Protein (in mg/ml)	Virus titer	Protein (in mg/ml)
1	1:10 000	3,12	1:500	0,76	1:10	2,58
2	1:25 000		1:1 000		1:10	
3	1:5 000	3,75	1:1 000	0,72	—	2,52
4	1:1 000	1,2	1:10	0,1	—	1,06
5	1:10 000	2,92	1:100	0,31	1:10	2,47
6	1:1 000	2,0	1:100	0,75	—	1,62
7	1:10 000	2,12	1:500	0,5	—	1,87
8	1:50 000	5,68	1:5 000	0,67	1:10	5,06
9	1:100	2,5	—	0,37	—	2,34
10	1:1 000		1:10		—	

Comment: The preparation is biologically inactive; the unfilled lines indicate that the protein was not determined.

volume of the SE. The mixture of SE and Freon was thoroughly mixed in a blender at 16,000 rpm for 3-5 min and then centrifuged at 3000 rpm for 5 min. The supernate of the extract formed here (SE I) contained the virus, and the precipitate consisted of a compound of the tumor protein and Freon. Treatment with Freon was done 5-6 times until the addition of Freon caused the formation of the precipitate, and a thin white film formed at the boundary of the interaction of the SE and Freon.

The biological activity of the fractions obtained after Freon treatment (SE I-SE IV) was tested by intracutaneous injection of the test materials into two fresh chickens. At the same time the SE of the initial extract was titered on these same chickens. The protein in the test samples was determined by the micro-Kjeldahl method.

RESULTS

It was estimated in the experiments that a 5–6-fold treatment of the extract with Freon causes about a 30% drop in the protein content, the biological activity of the virus in this case dropping about 10-100 times. To elucidate the question as to whether such a drop of activity during Freon treatment is associated with mechanical precipitation of the virus together with protein, we set up experiments in which the precipitate (Freon + protein) was resuspended in the starting volume of buffer, thoroughly mixed in a blender for 5-10 min, and centrifuged at 3000 rpm. The supernate was titered on chickens.

The results obtained confirmed the assumption that a certain portion of the virus is mechanically precipitated with the protein upon treating the extract with Freon. Here we observed a direct relationship between the mass of precipitate and its content of virus: the largest precipitate formed after the first addition of Freon contained virus in a 1:100-1:10 titer. The volume of the precipitate after the second treatment of the extract with Freon was appreciably less, and the precipitate either did not contain the virus at all or contained it in a small quantity. Taking these data into account, we carried out all subsequent experiments in the following manner: the precipitate of protein with Freon was suspended each time in 20-30 ml of buffer, mixed in a blender, and centrifuged. The supernate was added respectively to SE I-IV and only after this was the next extraction with Freon done. With this setup of the experiments the first two extractions with Freon as a rule did not cause a drop in the activity of the virus in comparison with the starting SE. However, with 5-6-fold treatment with Freon the activity of the virus dropped by a factor of 10-100. These data indicate that in addition to mechanical loss of the virus its partial inactivation occurred during the long treatment with Freon.

Taking this fact into account, and also that the bulk of the protein is precipitated by Freon in the first two extractions, in subsequent experiments we purified the virus in the following manner: the supernate of the tumor extract was treated twice with Freon and then the virus was precipitated in the ultracentrifuge at 39,000 rpm for 1½ h at 0-2°. The precipitate, which formed as a thin film, was resuspended in 5-10 ml of buffer or Earl's

solution (pH 7.2) and thoroughly ground in a manual glass mixer. The volume of the suspension was brought up to the initial volume, after which the resuspended precipitate and supernate, which were taken in descending dilutions, were injected intracutaneously into 2 chickens. In each experiment titration of the initial extract and the SE after twofold treatment with Freon and of the precipitate and SE after ultracentrifugation was done on the same pair of chickens.

The results of these experiments are given in the table. They indicate that with the proper centrifugation conditions it is possible to precipitate from the extract the bulk of the virus (experiments No. 2, 3, 6, 8). It is necessary, however, to note that in a number of experiments (No. 1, 4, 5, 7, 9, 10) the virus titer in the precipitate was 20-100 times lower than in the extract before ultracentrifugation. The supernate after ultracentrifugation was either completely without virus or it contained it in an insignificant quantity. At the same time the bulk of the tumor protein (about 83% on the average) remained in the supernate. For further purification of the viral precipitate from other proteins, we set up experiments in which the precipitate obtained after ultracentrifugation of the Freon-purified extract was again subjected to 2–3 fold treatment with Freon by the method described above. The preparations obtained were titered on chickens and we investigated the protein content (by the micro-Kjeldahl method or test with acetic acid).

The obtained results indicate that the above-described combined method (purification of the extract with Freon before and after ultracentrifugation) makes it possible to obtain highly purified preparations which do not contain slight traces of tissue protein.

However, we were not able to obtain by this method a highly active viral suspension: in two experiments the preparations were inactive in general, in two the titer of virus reached 1 : 10, and only in two experiments was it 1 : 100. These results are in contradiction with the data cited above [9], according to which highly active viral preparations could be obtained after sixfold treatment with Freon of a suspension of virus precipitated at 80,000 g. At the same time investigators who have purified infectious viruses by means of fluorocarbons have noted in a number of cases inactivation of these viruses during purification. For example, the titer of Aujeszky virus after threefold treatment of the infectious culture liquid with Ledon-113 dropped from 10^8 to 10^2 TID_{50}/ml [7].

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