Second is the finding that plasma obtained from whole blood previously treated with ragweed antigen confers a diminished capacity upon normal cells to release histamine. The larger the quantity of ragweed used for prior treatment, the less histamine release occurred on subsequent sensitization of normal cells with such plasmas (Table II). This observation strongly suggests that in the initial treatment of sensitive whole blood with ragweed antigen that sensitizing antibody is actually used up in some fashion and hence there is less remaining for subsequent sensitization of the normal cells. Thus, less histamine release occurs on later addition of ragweed to treated plasma-normal cell mixtures. Diminution in transferable sensitizing antibody activity could occur in at least 3 different ways: 1) humoral antibody complexing with antigen, thus preventing reaction of cellular antibody and antigen and subsequent histamine release; 2) binding of antibody to cells during the course of histamine release reaction; 3) formation of a product in the release reaction which inhibits histamine release in a fresh reaction system.

Had the interaction of sensitizing antibody, antigen and cells in the sensitive blood caused generation of any stable anaphylatoxin-like substance, then addition of plasma from such a system to normal blood might be expected to cause a release of histamine without further addition of ragweed antigen. The last column of Table II shows that such plasmas added to normal blood without antigen (saline control) do not bring about a significant release of histamine. It appears, therefore, that this type of allergic reaction is not accompanied by anaphylatoxin generation.

Summary. *In vitro* sensitization of non-allergic human leukocytes by plasma from ragweed sensitive subjects was carried out. In 10 attempts with 6 sensitive plasmas and 7 normal bloods, only 3 pairs of individuals provided systems which demonstrated passive sensitization as determined by histamine release in presence of ragweed antigen. This finding is not related to major blood group incompatibility, and is unexplained. Diluted sensitive plasma brings about less histamine release. Plasma obtained from sensitive whole blood previously treated with ragweed antigen confers a diminished capacity upon normal cells to release histamine. There was no evidence that such plasmas possessed anaphylatoxin-like activity.

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Method of Concentration of Viral Diagnostic Reagents Using Hydrophilic Agents. (25795)

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A number of methods other than procedures primarily dependent upon high speed centrifugation and lyophilization have been employed for concentration of viral and rickettsial suspensions. Several chemical methods have been described(1,2,3,4,5). Kohn(6) outlined a procedure for concentration of urine using polyvinylpyrrolidone (PVP)* and polyethylene glycol (Carbowax 20-M)† as hy-

* K and K Laboratories, Jamaica, N. Y.

† Carbide and Carbon Chemicals Co., Union Carbide and Carbon Corp., N. Y.

Use of trade names does not imply endorsement by Public Health Service.

drophilic agents. It occurred to us that with certain modifications Kohn's general method would be of possible value in the production of viral diagnostic reagents.

Materials and methods. Antigens. The following complement-fixing (CF) antigens were prepared for concentration experiments: mumps viral and mumps soluble(7), influenza A/PR8/34, influenza A2/Jap/305/57, influenza B/GL/1739/54, and influenza B/Maryland/1/59(8), lymphocytic choriomeningitis (LCM)(9), and poliovirus types 1 (Mahoney strain), 2 (Statler strain), and 3 (McMullen strain)(10). Before concentration the poliovirus CF antigens were treated with fluorocarbon (Genetron 113‡ was substituted for the Freon 112-*n*-heptane mixture described by Hamparian *et al.*(11)) to remove possible anticomplementary activity. The other antigens were concentrated with no prior treatment because they were not found to be anticomplementary. These antigens were selected because they represented 3 sources of host material: egg (mumps and influenza antigens), tissue culture (poliovirus antigens), and animal tissue (LCM antigens). *Concentration procedure.* To maintain optimal aseptic technic and facility of manipulation of large volumes, the following modification of Kohn's technic was utilized. An appropriate length (depending on volume of antigen used) of cellulose dialyzer tubing was fastened to a small funnel by cotton string and knots tied in the distal end of the tubing. The unit was sterilized in the autoclave. Antigen was added aseptically to the tubing, the funnel was removed, and the upper portion of the tubing was tied securely with cotton string. The tubing was then placed in a suitable container (battery jar, cylinder, beaker, etc.) and the top of the tubing was secured with adhesive tape to the top of the wall of the container in such manner that the fluid dialyzing out of the antigen within the tube would not ultimately reach the top portion of the membrane. The lower portion of the tubing was coiled so that it lay flat in bottom of the container. Carbowax (polyethylene glycol compound 20-M), in a ratio of 1 g to 9 ml of the antigen within the dialyzer tubing, was sprinkled over the entire outer surface of tubing. The assembly was kept at +4 to +8° usually overnight and in some cases for a longer period. Concentration is effected in shorter time periods when larger amounts of the agent are added, especially if the antigen is distributed in more than one dialyzer tube. Ratio of hydrophilic agent to volume of antigen to be concentrated is not critical. It has been possible to achieve a 10-fold volume concentration of several antigens within a 24-hour period. The product was finally removed from the dialyzer tubes in an aseptic manner using a bulbous beveled point (Horsley) needle and suitable syringe. *Serological methods.* Hemagglutination (HA) tests were performed in tubes using chicken erythrocytes and the ensuing cell pattern was read after a period of 1 hour at room temperature. The CF test employed in this study was that described by Sigel *et al.*(12) for all antigens other than poliovirus. The CF test procedure outlined by Schmidt and Lennette(13) was followed for testing poliovirus CF antigens. Titers were recorded as the reciprocal of the highest dilution giving the endpoint of titration.

Results. An increase in serologic activity was effected by concentration with PVP or Carbowax using both CF and HA tests. This concentration with increased serologic activity was demonstrated using all 3 categories of host material for antigen production. Volume concentration was effected from 2.8 to 22.4-fold of the original material. When the increases in HA and CF titers were compared for different lots of each type of antigen concentrated, there seemed to be no consistent relationship with the exception of 2 lots of influenza B/GL/1739 antigens. Here, 4-fold increases in titer were observed in both lots of antigen using 2 serologic technics with a 10-fold decrease in volume. In the case of the 2 lots of mumps soluble antigen, the original preparations showed negative HA tests in the tubes of undiluted antigen but elicited an HA pattern in high titers in both concentrates. The results are summarized in Table I.

Discussion. Methods describing concentra-

‡ Genetron trifluorotrichloroethane Purple label 113. Allied Chemical and Dye Corp., N. Y.

TABLE I. CF and HA Antigen Titers before and after Concentration.

Antigen	Vol in ml,* orig./conc.	Fold conc.	HA titers Conc./orig.†	HA titers Fold increase	CF titers Conc./orig.†	CF titers Fold increase
Mumps						
Viral: Exp. 1	590/210	2.8	64/32	2	8/2	4
2	1,060/101	10.5	256/32	8	8/<2	>4
3	730/73	10.0	512/64	8	32/2	16
4	640/64	"	1,024/64	16	16/4	4
Soluble: Exp. 1	386/37	10.4	32/undil.	>32	8/4	2
2	125/11	11.4	256/ "	>256	32/8	4
Influenza						
A/PR8/34: Exp. 1	600/80	7.5	512/128	4	16/2	8
2	365/20	18.2	16,384/512	32	64/4	16
A2/Jap/305/57:						
Exp. 1	515/23	22.4	4,096/512	8	128/8	"
2	570/57	10.0	2,048/512	4	"	"
B/GL/1739/54: Exp. 1	128/12	10.7	512/128	4	16/4	4
2	82/8	10.2	1,024/256	4	32/8	4
B/Maryland/59:						
Exp. 1	500/56	8.9	" / "	4	4/2	2
2	710/70	10.1	4,096/512	8	16/4	4
3	670/63	10.6	" / "	8	"	4
LCM						
Exp. 1	130/13	10.0			256/16	16
2	150/15	"			512/32	"
Poliovirus						
Type 1: Exp. 1	300/50	6.0			48/4	12
2: 1	"	6.0			24/2	"
2	470/80	5.9			10/2	5
3	600/50	12.0			"	5
4	750/80	9.4			12/4	3
3: 1	300/50	6.0			24/4	6
2	970/120	8.1			6/2	3
3	700/50	14.0			10/4	2.5
4	825/60	13.7			6/3	2

* Numerator = Original vol. Denominator = Concentrated vol.
† Numerator = Titer after concentration. Denominator = Titer before concentration.

tion of viruses and viral antigens employing high speed centrifugation which have been conveniently utilized where volumes are small are usually impractical when large volume production methods are used. Methanol precipitation methods, although generally successful for concentration of mumps viral, influenza, and poliovirus CF antigens in this laboratory, have disadvantages of 1) excessive manipulation in the process, 2) observation of thermal restrictions, and 3) loss in final yield of serologic activity in some cases. Furthermore, optimal methanol concentration and pH of the eluting menstruum have to be ascertained. The method described here requires no special equipment. It can be easily applied to antigens eliciting various ranges of titers. Presumably, no chemical contamination takes place other than that which is present in the hydrophilic agent used. However, it should be realized that a concentration of all non-dialyzable components in the antigen also takes place. Indeed, the appearance of cloudy material was noted in some of the egg antigen concentrates. This was absent in the original fluids. However, none of the antigens tested with the CF technic were found to be anti-complementary after concentration. It has been noted during these experiments that another method of virus concentration has been described recently(14) using the principle of partition of viral agents in dextran-methylcellulose-water and dextran-Carbowax 6000-water systems.

Summary. A simple and economical method is described for concentration of viral

diagnostic antigens. Evidence of serologic increase in titer has been presented with influenza, mumps, lymphocytic choriomeningitis, and poliovirus antigens.

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Carbohydrate Constituents of Human Red Cell Stroma.*† (25796)

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Information concerning the carbohydrates in red cell stroma is scanty. Most of the work in this field has been done on specific compounds prepared from stroma. This paper is concerned with analysis of monosaccharides, hexosamines and sialic acid in human red blood cell stroma. In addition, studies were conducted to determine the effect of storage of blood in the cold on carbohydrate constituents.

Methods. Bloods obtained from 4 healthy young men were collected in acid-citrate-dextrose and aliquots were stored in the cold (4°C) under sterile conditions in stoppered bottles. Zero day samples and bloods stored for 15, 30 and 45 days were treated by the short-wash procedure for preparing stroma from dog erythrocytes described by Tishkoff *et al.*(1). This material was lyophilized and kept under vacuum over P_2O_5. Total hexose content was determined by the anthrone method(2). It was found essential to age the anthrone reagent for at least 24 hours to obtain constant results. Dried stroma (10 mg) was hydrolyzed in 3 ml 2N H_2SO_4 in a tube stoppered with a screw-cap lined with rubber. The tube was slowly rotated for one-half hour in a water bath maintained at 96°. Insoluble material was removed by centrifugation and clear supernatant was used for analysis. Galactose in 2N H_2SO_4 was used as a standard and values were expressed as galactose. To separate the lipid-carbohydrate complexes, dried stroma (25 mg) was extracted for one hour with occasional shaking with 10 ml of a chloroform-methanol mixture (2:1)(3) at room temperature. The stroma residue was re-extracted 3 times with one ml portions of the chloroform-methanol mixture; extracts were pooled and evaporated to dryness in a stream of nitrogen. The residual stroma and the extracted material were hydrolysed with 2N H_2SO_4 and analysed for hexoses. To obtain clear hydrolysates, it was necessary to remove the solution carefully so as to avoid the insoluble material which floated on the surface. Hexosamine was determined by the Boas method(4). Stroma (20 mg) was hydrolyzed for 5 hours in 2 ml 2N H_2SO_4 in a screw-cap tube rotated in a water bath at 96°. The hydrolysate was diluted to 8 ml and cen-

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