Summary. Comparison of absorption and urinary recovery of chloramphenicol and 2 of its derivatives, Thiocymetin and U-15,442, has been carried out following administration of single oral doses of 500 mg each in 6 healthy human volunteers. Similar peak concentrations of these drugs were noted in serum but they were achieved later after the dose of Thiocymetin. At 25 hours slight antibiotic activity was still discernible with the 2 derivatives, but not with chloramphenicol. In view of the superior antimicrobial activity of chloramphenicol the 2 derivatives are considered to be inferior to the parent compound in producing antimicrobial activity in serum. In contrast, much higher urinary recoveries were achieved with the 2 derivatives than with chloramphenicol. In the case of Thiocymetin urinary recovery was so great as to more than offset its inferior antimicrobial activity; this was not the case with U-15,442. The high recovery of active Thiomycetin in urine suggests that it is inactivated more slowly or by a different mechanism than is chloramphenicol.

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A Simple Chromatographic Method for Preparation of Gamma Globulin.* (25476)

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Many serological procedures might be improved by use of gamma globulin rather than whole serum as the source of antibody. For example, non-specific inhibitors of viruses might be removed in some cases where antiviral sera are being studied. Furthermore, in work with fluorescent antibody, non-specific staining might be reduced if gamma globulin fractions were used instead of serum. Most methods for preparation of gamma globulin are not convenient for use by the usual virology or immunology laboratory. The high capacity of the cellulose ion exchange adsorbents(1), and the relatively mild conditions of salt and pH required for adsorption and elution have suggested their use in such preparation(2). A slight modification of the chromatographic procedure described by Sober and Peterson(3) is presented here which allows preparation of gamma globulin in a stepwise procedure, free from macroglobulins, high molecular weight serum protein with gamma globulin mobility, and non gamma globulin serum proteins.

Methods. Ion exchange adsorbents. The anion exchanger, diethylamino-ethylcellulose (DEAE), (40-100 mesh, 0.96 meq/g) was prepared by the method of Peterson and Sober(1), or purchased. It was washed with 0.5 N NaOH 3 times to remove color and to regenerate the column, then suspended in water and washed to neutrality. The slurry was adjusted to pH 6.3 by adding 0.2 M NaH_2PO_4, and washed several times with the starting buffer (0.0175 M phosphate (Na^+), pH 6.3). A large batch of the adsorbent may be so prepared and stored in the refrigerator.

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Before use it is resuspended gently, and the slowly sedimenting "fines" discarded as they unduly interfere with the flow rate of the column. The cation exchanger, carboxymethylcellulose (CM) (100-230 mesh, 0.74 meq/g) was synthesized as described(1). It was prepared for use by washing 3 times with 0.5 M NaCl—0.5 M NaOH, then with water till neutral, then with 0.0175 M PO_4 pH 6.3, till the pH of the wash was 6.3. *Antibody assay.* Adenovirus antibodies were assayed according to procedures given by Rowe *et al.*(4). ECHO virus antibodies as well as antibody to the tissue on which the viruses were grown were assayed according to Halonen(5). Antibodies to diphtheria and tetanus toxoids were determined by hemagglutination technics, using serial dilution of serum against erythrocytes sensitized by the method of Landy *et al.*(6), using as a standard a modified antitoxin of known unitage. Neutralizing antibodies to Russian Spring-Summer Encephalitis were estimated by the method of Rivers *et al.*(7). *Separation procedure.* (1) The serum to be fractionated is dialyzed overnight *vs.* 100-200 volumes of 0.0175 M PO_4 buffer (Na^+), pH 6.3 in the cold. The slight precipitate formed may be removed by centrifugation or may be retained and put on the column. (2) For 2 ml of the dialyzed serum, a column of DEAE-cellulose is prepared of about 1 cm diameter and 5 cm height, using pressure, not to exceed about 10 lb/sq in, preferably in the form of nitrogen. The column should not be allowed to dry out during the preparation. The column is then washed with about 50 ml more of the starting buffer. (3) With the liquid level of the starting PO_4 buffer just at the top of the adsorbent, the serum is carefully added and pushed into the column with gentle gas pressure. The gamma globulin, the only serum protein that is not adsorbed under the indicated conditions, is washed through with more of the pH 6.3 buffer. For 2 ml of serum about 6 ml of buffer will remove most of the gamma globulin, but about 20 ml is needed for complete recovery. The macroglobulin remains on the column with the other non-gamma globulin serum proteins and may be removed from the column by washing with 2 M NaCl in 0.4 M Na-phosphate, pH 5.2.

TABLE I. List of Antisera Fractionated and Tested.

Immunizing agent	Species of serum	Test method	% antibody recovered in: Gamma globulin	% antibody recovered in: Remainder
Adenovirus, Type 2	Rabbit	Comp. fix.	50	0
Idem	"	Neut.	100	0
ECHO 6	Guinea pig	Comp. fix.	80	0
Monkey kidney t.c.	"	*Idem*	80	0
ECHO 6	Rabbit	"	80	0
" 14	"	"	40	0
Tetanus toxoid	Human (2)	HA	100	0
Diphtheria toxoid	"	"	50	0
Russian S-S enceph.*	"	Neut.	300	0

* Russian Spring-Summer Encephalitis.

Results. The systems to which the procedure has been successfully applied are given in Table I. It should be realized that reproducibility of some of the above testing systems is no better than plus or minus 2-fold. Therefore the results of Table I indicate that the described fractionation procedure separates a non-adsorbed chromatographic fraction, which contains only gamma globulin and which accounts for all the antibody. All the gamma globulin fractions were examined qualitatively in the Spinco paper electrophoresis apparatus, or in the Aminco-Stern boundary electrophoresis apparatus in veronal buffer at pH 8.6 and some were analyzed quantitatively. All samples proved to be gamma globulin either without detectable contamination or contaminated only with a small amount of β-globulin. The "adsorbed-fraction," that was eluted from the column by the higher salt concentration, showed the other serum components with the small amount of gamma globulin that could be attributed to the macroglobulin component.

In addition to antisera mentioned in Table I, 3 samples of horse serum that showed anti-type I poliovirus activity were fractionated by the present method. All the anti-poliovirus activity was found in the non-adsorbed gam-

ma globulin fraction. For several reasons this material has not been considered as an antibody(8).

It should be noted that the present method dilutes the gamma globulin 4-10 fold. If this dilution should be undesirable, the gamma-globulin eluate may be applied directly to a carboxymethylcellulose (CM) column, equilibrated with pH 6.3, 0.0175 M PO_4 buffer onto which the gamma gobulin will be adsorbed(2, 3). It may be then eluted in a more concentrated form by 2 M NaCl in 0.05 M Na-phosphate pH 6.9. Any other concentration method may, of course, be used.

Summary. A relatively simple method for separation of gamma globulin from serum has been presented. The procedure has been applied to a number of antisera, and has been shown with them at least to give reasonably good localization of antibody in the gamma globulin fraction.

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Effect of Pluronic F68 on Growth of Fibroblasts in Suspension on Rotary Shaker. (25477)

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Recent investigations have established the feasibility of propagating certain strains of mammalian cells in suspension, and a number of technics have been described. These include the tumble tube of Owens *et al.*(1), the rotary shaker of Earle *et al.*(2), the roller tube of Graham and Siminovitch(3), the suspended stirrer (spinner culture) of Cherry and Hull(4), the fermentor of McLimans *et al.*(5), and a variety of modifications of the foregoing(6,7,8,9,10). Use of conventional rotary shakers to provide agitation eliminates the need for special equipment and the method is particularly suitable for experiments involving many cultures of relatively large volume. Formation of a non-cellular precipitate(11) under certain conditions, however, is a major drawback of the shaker method. In media containing serum, the precipitate is presumably protein in nature(11,12) and its formation is related to strain of cells and environmental conditions employed(11). Precipitation of constituents of the medium not only results in erratic growth of cells but imposes severe limitations on their use in studies of metabolism. This report describes the use of Pluronic F68 in shake cultures as a means of essentially eliminating the precipitate formed in a medium containing horse serum.

Materials and methods. The Pluronics† are blockpolymer surfactants prepared by adding ethylene oxide to both ends of a polyoxypropylene polymer. Pluronic F68, hereafter referred to as F68, is a solid with a molecular weight of approximately 8750. About 80% of the molecule, by weight, consists of hydophilic polyoxyethylene groups and the remainder of hydrophobic polyoxypropylene groups. Stock cultures of strain U12-29 fi-

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