The rainfall was a little higher than average during the collection period at both locations. However, it was evenly distributed throughout the period, and there were few severe storms at critical cover periods. For these reasons, soil losses were lower than average. At La Crosse, something over 1 ton/acre was lost on the corn and oats plots and only 0.03 ton/acre on the clover plot. At Tifton, the soil losses were 0.3 ton/acre in plots planted to oats, 0.6 ton/acre in those planted to corn, and 0.7 ton/acre in those planted to peanuts.

The average fallout between runoff collections was 32 $\mu\mu$c of Sr^{90} per square foot at La Crosse and 20 $\mu\mu$c at Tifton. Usually about 1 percent of the fallout appeared in the runoff (Tables 1 and 2). The most notable exception was a runoff at La Crosse on 5 June, during which more than 8 $\mu\mu$c of Sr^{90} per square foot, or more than 25 percent of the fallout, was carried off the plots of corn and oats. Strontium-90 radioactivity is given as of the date of each sample collection.

Only a small percentage of the Sr^{90} that fell on these soils was removed in runoff. The percentage removed was greater on plots with greater amounts of soil loss. Thus, the transport of Sr^{90} would probably increase with more erosive soils, steeper slopes, and cultivation systems which leave bare soil exposed for long periods. However, these conditions are at a minimum in most agricultural areas, and the Sr^{90} content of such soils will not be reduced appreciably by erosion.

Soil samples taken from the plow layer showed 45 $\mu\mu$c of Sr^{90} per kilogram of soil at La Crosse on 11 April, and 16 $\mu\mu$c at Tifton on 11 March, 1957. The concentrations of Sr^{90} were about ten times as high as these in the soil carried by the runoff from most of these plots. However, soil in the runoff from the clover plot at La Crosse, which had the least amount of soil loss, showed 1300 $\mu\mu$c of Sr^{90} per kilogram. Thus it appears that a considerable concentration of Sr^{90} can occur in the limited areas where runoff sediments accumulate.

Table 2. Strontium-90 in fallout and runoff from corn, oats, and peanut plots at Tifton, Ga., in 1957.

Period	Strontium-90 ($\mu\mu$c/ft^2)			
	Fall-out	Runoff from plot		
		Corn	Oats	Peanuts
3/11–3/25	10	0.1	0.08	0.2
3/25–4/6	12	0.7	0.04	0.5
4/6–6/4	27	0.3	0.14	0.4
6/4–7/1	26	0.1	0.17	0.1
7/1–7/8	30	0.7	0.21	0.6
7/8–7/28	25	0.1	0.04	0.2
7/28–8/20	10	0.2	0.05	0.1
8/20–10/2	27	0.1	0.04	0.1
10/2–12/2	13	0.2	0.04	0.4
Total	180	2.5	0.81	2.6

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Separation of Serum Antibody Activities by Anion-Exchange Cellulose Chromatography

Abstract. Mumps, *Histoplasma capsulatum.* thyroglobulin, and typhoid H antibodies were found in fraction 1, which contained only gamma globulins with ultracentrifugal sedimentation coefficients of 6.6 Svedberg units (S). Typhoid O antibodies and rheumatoid factor were only in fraction 5, composed principally of gamma macroglobulins with ultracentrifugal sedimentation coefficients of 18 S. In contrast isohemagglutinins, Rh antibodies, and anti-human liver nucleoprotein activities were found in two chromatogram fractions (1 and 5) with different physicochemical properties.

The gamma globulins in normal human serum are composed of two major groups of proteins. Ninety to 95 percent of the total gamma globulins have ultracentrifugal sedimentation coefficients of 6.6 S and 5 to 10 percent are 18-S gamma macroglobulins. The 6.6-S gamma globulins are known also to be a heterogeneous group of protein molecules with differing electrophoretic mobility and hexose content (*1*). Anion-exchange cellulose chromatographic techniques have been used to separate the 18-S gamma macroglobulins from most of the 6.6-S gamma globulins and also to subdivide the 6.6-S gamma globulins into four arbitrary fractions with distinctive physicochemical properties (*1*).

In view of this physicochemical evidence of gamma globulin heterogeneity and of the capacity of anion-exchange cellulose chromatography to fractionate the gamma globulins, a study of the distribution of normal and abnormal physiologic activities among the chromatographic gamma globulin fractions was undertaken.

Sera containing antibodies to viral and bacterial antigens and antibody-like activity against human tissue or serum components were collected. Two or more sera were utilized for each activity tested except in the case of mumps, histoplasmosis, and rheumatoid arthritis sera (*2*).

Fractionation by zone (polyvinyl block) electrophoresis (*1*) of sera from each category except rheumatoid arthritis was performed. The activities in each instance were found among the gamma globulins. The gamma globulin fractions were then pooled, concentrated by ultrafiltration and chromatographed as described below.

Each whole serum (1.0 ml) and electrophoretically prepared gamma globulin (from 1.0 ml of sera) was chromatographed as described in detail elsewhere (*1*) on diethylaminoethyl (DEAE) cellulose (3) columns. Samples and columns were equilibrated with a starting buffer of 0.02*M* phosphate (*p*H 8) prior to sample application. Proteins were eluted from the column in 150 ml of effluent by a progressively increasing ionic strength gradient while *p*H 8 was maintained, and the protein distribution in the effluent fractions was determined by measurement of the optical density at 280 mμ. Subsequently the effluent was divided into 10 or more pools and, if necessary, the pools were concentrated to 1.0 ml by ultrafiltration and dialyzed thoroughly against *p*H 7.6 buffered saline prior to assay.

The distribution of the gamma globulins on chromatography of whole serum is illustrated in Fig. 1 (upper left corner). The distribution of electrophoretically prepared gamma globulin when chromatographed separately is the same. The physicochemical and immunochemical characteristics of gamma globulin fractions 1 to 5 have been described (*1*). Fraction 1 is the first protein peak and is obtained between 5 and 15 percent of the effluent volume. Fraction 2 is obtained between 15 and 25 percent, fraction 3 includes 25 to 45 percent, fraction 4 ranges from 45 to 58 percent, and fraction 5 from 58 to 80 percent.

The distribution of antibody activities after gamma globulin or whole serum chromatography is illustrated in Fig. 1. Antibodies to mumps virus and to *Histoplasma capsulatum* were found in fraction 1 which is composed of 6.6-S gamma globulins. Antibodies to thyroglobulin in the first three sera tested (*4, 5*) were also found in this fraction.

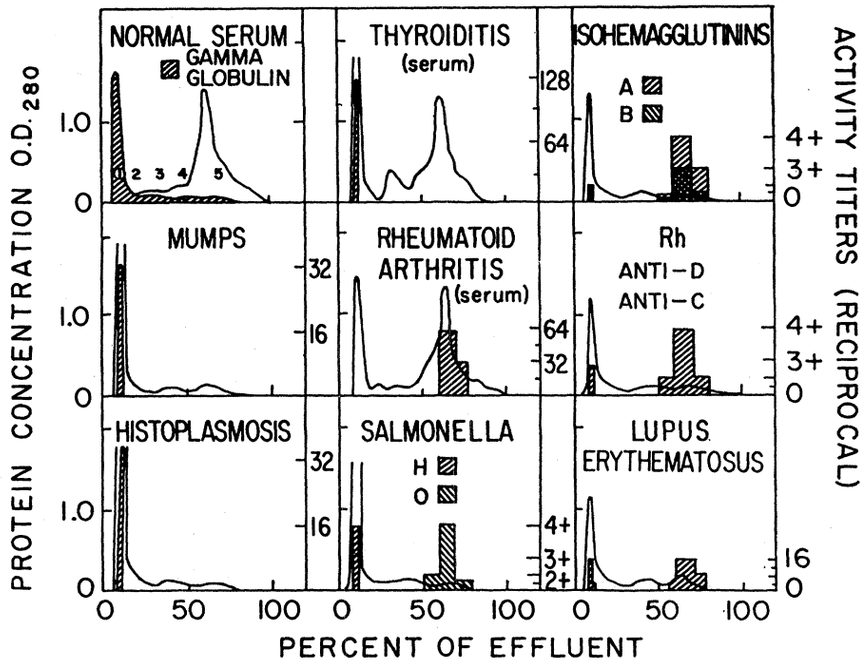

Fig. 1. Distribution of antibody activities after anion-exchange cellulose chromatographic fractionation of whole serum or of electrophoretically prepared gamma globulins. In the top left corner the gamma globulin distribution (shaded area) is illustrated in a normal chromatogram of whole serum.

The rheumatoid factor was found only in gamma globulin fraction 5, consistent with the location of the 18-S gamma macroglobulins in chromatogram fraction 5 and the gamma macroglobulin characteristics of the rheumatoid factor (*6*). Antibodies to the *Salmonella* (typhoid) O and H antigens were separated chromatographically. The antibodies to the H antigen were found in fraction 1 and the O antibodies were found in fraction 5, indicating different physicochemical properties for these two antibodies.

Fractions 2, 3, and 4 were not demonstrated to contain the major component of any physiologic activity as tested. These preliminary observations on fractions 2, 3 and 4, which together comprise about 20 percent of the total gamma globulin, do not reveal the functional role of these gamma globulins.

Several activities were found in both fractions 1 and 5. The anti-A and anti-B isohemagglutinins, the Rh antibodies, and the anti-liver-nucleoprotein extract activity, when present in the serum of patients with lupus erythematosus, were found in gamma globulin fractions 1 and 5. The finding of the same activity in both fraction 1 and fraction 5 indicated that the antibody activity was associated with distinctly different gamma globulin molecules. The activities in fraction 1 could be assumed to be with 6.6-S gamma globulins, the only protein found in that fraction (*1*), and the activities in fraction 5 were likely to be 18-S gamma macroglobulins, which compose 90 percent of this gamma globulin fraction.

Ultracentrifugal studies have confirmed the physicochemical differences between the antibody activities in these two fractions. Isohemagglutinins (*7*) and anti-human-liver-nucleoprotein extract activity (*5*) in fraction 1 were found to sediment in the manner of 6.6-S gamma globulins and in fraction 5 to sediment as 18-S gamma macroglobulins.

The findings of two different gamma globulin fractions with apparently similar reactivity, however, does not necessarily indicate that the two types of proteins possess antibody activity at the same antigenic site. The materials used in the assay systems deserve close scrutiny. The red cell surface which determines isohemagglutinin and Rh factor reactions is well known to be antigenically complex, and the nucleoprotein extract used in testing for lupus erythematosus very likely contained many components. The question thus raised, whether gamma globulins, differing physicochemically enough to be separable from one another by chromatographic procedures, can still react with identical antigen sites will have to await further study.

Anion exchange cellulose chromatography should prove to be a useful tool in immunologic investigations. A study of the chromatographic distribution of many of the activities in lupus erythematosus sera demonstrated that some factors capable of reactions with cell nuclear material could be chromatographically distinguished from factor(s) responsible for lupus erythematosus cell formation (*8*). The ability to separate two different gamma globulin molecules with apparently similar antibody activity will allow further study of these antibodies as well as of conditions favoring formation of the physicochemically distinct 6.6-S and 18-S antibodies (*9*).

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