TABLE II. Intraperitoneal Mouse Pathogenicity with Mucin of Staphylococci in Relation to Their *In Vitro* Reactions.

In vitro test	Reaction	Total strains	Mouse pathogenicity with mucin: Pathogenic No.	Pathogenic % total	Non-pathogenic No.	Non-pathogenic % total
Coagulase	Positive	49	41	83.7	8	16.3
	Negative	11	1	9.1	10	90.9
Pigmentation	Positive	39	34	87.2	5	12.8
	Negative	21	8	38.1	13	61.9
Hemolysin	Positive	42	31	73.8	11	26.2
	Negative	18	11	61.1	7	38.9
Mannite fermentation	Positive	52	41	78.9	11	21.1
	Negative	8	1	12.5	7	87.5

genicity with mucin, the animal virulence test reported upon is proposed as a supplementary method for directly assessing this property in staphylococci since it represents the determination of the capacity of a strain to induce death, which is the acme of virulence.

Summary. Significant enhancement of mouse pathogenicity was observed following intraperitoneal injection of staphylococci with gastric mucin. By means of the adjuvant, the mouse was converted from a host resistant to staphylococcus infection by this route into one readily susceptible with 37 strains. High to fair degree of correlation, depending upon the test, was found between the ***in vitro*** presumptive biochemical tests and intraperitoneal mouse pathogenicity with mucin but some discrepancies were noted between both. The procedure under consideration is proposed as a supplementary method for the direct determination of virulence of staphylococci.

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Comparison of Continuous and Strip Paper Electrophoresis Technics for Study of Serum Glycoproteins.* (22658)

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Paper strip electrophoretic technics are now widely used for fractionation studies of serum proteins. Application of the Hotchkiss-McManus technics for tissue polysaccharides to paper strips by Koiw and Gronwall(1) allows studies of carbohydrate components of the serum proteins. Technics for continuous paper electrophoresis apparatus using vertical curtains have been described by Grassmann and Hannig(2) and by Durrum(3). The use of such equipment allows the collection of serum fractions in quantities which make possible chemical analysis for the glycoprotein and protein components. The work described here was undertaken to compare results obtained by continuous electrophoresis technics with the less tedious paper strip methods.

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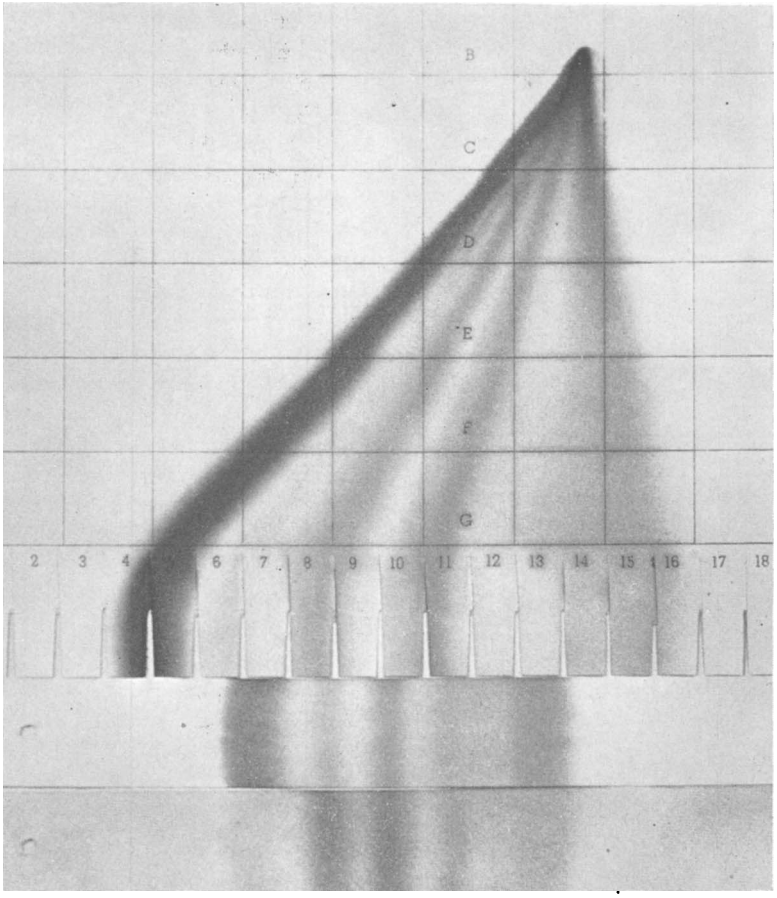

FIG. 1. Typical continuous and strip paper electrophoretic patterns. (Albumin is at the left in all patterns.) Top: Continuous electrophoretic curtain stained for proteins with bromophenol blue. Middle: Strip pattern stained with bromophenol blue. Bottom: Similar strip of same serum after development of periodic acid Schiff reaction for glycoproteins.

Methods. A Karler-Misco Continuous electrophoretic unit† was used with a constant voltage supply.‡ The plate supporting the paper curtain was found to make too much contact with the paper and was modified by making a plastic plate with fewer contact points. A slow stream of air was bubbled through the anode chamber to prevent plating out of buffer ions. A .045 barbital buffer (pH 8.6) was used for all studies. The apparatus was allowed to equilibrate for about 8 hours until the current became stable at 12 milliamperes. Electrophoresis was carried out at 350 volts for 24-48 hours. A paper wick (S and S No. 470) was used to feed the serum sample to the paper curtain.

At the end of each run the curtain was removed and the pattern developed with bromophenol blue in the same manner as used for strip electrophoresis. The collecting tubes, previously calibrated at 3 ml, were labeled and the volume in each adjusted to 3 ml. Aliquots were taken from each tube for estimations of protein by the biuret reaction and bound carbohydrate by the method of Shetlar, Foster, and Everett(4). Samples were also taken from each tube for paper strip electrophoresis. From these studies the protein constituents of each tube were identified. If more than one fraction was noted on the paper strip, it was scanned in the Spinco Analy-

† Microchemical Specialties Co., Berkeley, Calif.

‡ Model 1400 Research Specialties Co., Berkeley, Calif.

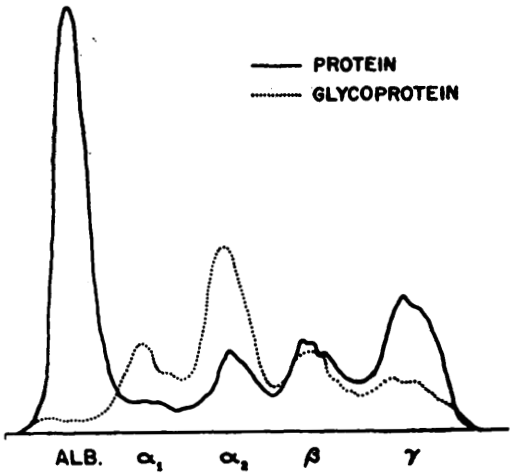

FIG. 2. Typical densitometer tracings of strip electrophoresis patterns for proteins and glycoproteins. Patient with active rheumatoid arthritis. Glycoprotein, 201 mg/100 ml; protein, 8.05 g/100 ml.

trol and the relative amounts of each fraction estimated.

Paper strip electrophoresis. Strip electrophoresis was carried out essentially by the method of Block, Durrum and Zweig(5) using Spinco hanging strip electrophoresis cells. 3MM filter paper was used for all work. Electrophoresis was conducted at constant current (5 milliamperes for 8 strips) using barbital buffer, .075M, pH 8.6. Duplicate strips, 10 λ for protein and 30 λ for glycoprotein studies, were made of each sample. For estimation of protein the strips were developed with bromophenol blue essentially as described by Block *et al.*(5). For glycoprotein studies, the strips were subjected to the periodic acid Schiff reaction as applied by Koiw and Gronwall(1) except that the reducing solution was prepared as described by Roboz *et al.*(6). The protein strips were quantitated with the Spinco Analytrol using blue filters; the glycoprotein strips were similarly evaluated with the same instrument using Klett 52 (green) filters. The total area under the protein curve was equated to the serum protein as determined by the biuret reaction, and the area under the glycoprotein curve was similarly made equal to the total serum glycoprotein obtained by the tryptophan method(4).

Results. Typical electrophoretic patterns by both technics are shown in Fig. 1, and typical analytrol curves for protein and glycoprotein are presented in Fig. 2. The difference in distribution of protein and bound carbohydrate is illustrated in both figures.

Average glycoprotein contents of serum fractions of patients with cancer and arthritis and for normal and pregnant subjects are summarized in Table I. The corresponding protein values are tabulated in Table II. Even with the small number studied, differences are apparent between the 4 groups.

In order to obtain some measure of the agreement between the two methods, the correlation coefficients for the 2 methods for the glycoprotein and protein of each fraction were calculated. Correlation coefficients for the glycoprotein moieties were: Albumin, .74; α_1, .81; α_2, .71; β, .78; and γ-globulin, .90; for the protein components they were: Albumin, .84; α_1, .75; α_2, .79; β, .55; and γ-globulin, .93. With the exception of β-globulin protein all of these correlation coefficients are significantly different from O at the 1% level of probability.

Discussion. Two different electrophoretic methods have been compared in this study. Not only do the electrophoretic technics vary, but the reactions used to quantitate the results are also different. For the continuous technic the protein of each fraction was estimated by the biuret reaction, while proteins by the strip method were estimated by densitometer readings made directly on the bromophenol blue dyed strip. Many studies have been made of the bromophenol blue staining technic as applied to paper strip electrophoresis(5) and a multiplicity of factors which affect the quantitation of protein fractions by this method have been noted. At present, it appears the best approach to use of this technic for study of serum fractions is an arbitrary one with standardized dyeing and scanning procedures. The continuous paper electrophoresis technic would appear to be more direct in its approach; however, this method is more difficult and is consequently unlikely to gain favor for extensive studies. The agreement of the continuous with the strip method

TABLE I. Distribution of Glycoprotein in Different Conditions.

Condition	No.		Bound carbohydrate* associated with					Total†	Sero-mucoid‡
			Albumin	α_1	α_2	β	γ		
Cancer	5	Strip	18	48	72	32	18	188	41
		Continuous	20	49	67	31	20		
Rheumatoid arthritis	2	Strip	15	38	80	35	30	198	27
		Continuous	18	30	79	35	38		
Late pregnancy	2	Strip	12	22	49	44	25	152	17
		Continuous	11	25	54	39	22		
Normal	3	Strip	12	18	36	33	24	122	15
		Continuous	13	16	35	31	24		

* mg/100 ml of serum.
† Bound hexose polysaccharide by tryptophan method(4).
‡ Determined by tryptophan method after isolation by method of Weimer *et al.*(7).

TABLE II. Comparison of Protein Distribution.

Condition	No.		Protein* associated with				
			Albumin	α_1	α_2	β	γ
Cancer	5	Strip	39.6	10.0	16.7	14.9	18.8
		Continuous	39.9	8.9	16.6	13.9	20.7
Rheumatoid arthritis	2	Strip	43.4	5.0	11.6	14.2	25.8
		Continuous	42.9	6.0	12.5	11.4	27.2
Late pregnancy	2	Strip	46.8	7.4	12.6	18.4	14.8
		Continuous	43.6	7.6	16.1	15.9	16.8
Normal	3	Strip	53.6	5.8	9.1	13.6	17.9
		Continuous	58.1	3.9	8.0	10.8	19.2

* Expressed as a % of total serum protein.

tends to strengthen the use of the latter method for routine work.

The agreement between glycoproteins by the two technics is rather surprising considering the great difference in technics and quantitation. The periodic acid Schiff reaction has not been evaluated as a quantitative test for serum glycoproteins, and the tryptophan method of Shetlar *et al.*(4) has not been thoroughly studied for purified serum fractions. However, the agreement noted in this paper tends to strengthen both of these technics as tools for investigations of glycoproteins.

Summary. A continuous paper electrophoretic technic was compared with a paper strip electrophoretic technic as a tool for the study of serum protein and glycoprotein fractions. Protein and glycoprotein for the continuous electrophoretic study were quantitated by analyses of the fractions by the biuret and tryptophan reactions. The glycoproteins by the strip technic were quantitated by the periodic acid Schiff reaction and the protein strips by the bromophenol blue staining. In both cases the strips were evaluated with an automatic photoelectric scanner. Good agreement between the two methods for both protein and glycoprotein was noted.

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