spleen. Absorption tests indicate that this group reaction is entirely heterogenetic since all of the cross reactivity can be removed by absorbing the serum with sheep erythrocytes (Table III).

The possibility of obtaining specific reagents for each of the two identified components present in the heavy fractions of organs and in tumor extracts permitted an assay of these antigens in the crude extract and its fractions. The specific F serum was obtained by immunization of rabbits with the heavy material from chicken spleen heated to 100° C. for one hour; the species specific antibody was obtained by absorbing serum against unheated high speed deposits of chicken spleen, with sheep erythrocytes. The assay of the F antigen was complemented by specific inhibition tests made according to the technic of Brahn and Schiff.[14]

Table IV shows that the heterogenetic antigen present in tumor extracts is sedimented at high speed

TABLE IV

ASSOCIATION OF FORSSMAN AND TISSUE SPECIFIC ANTIGENS WITH THE HEAVY FRACTION OF TUMOR EXTRACT

Antigen	Total N concentration	Antigen dilutions	Complement fixing power of extract		
			Antiserum to high speed deposit from tumor, absorbed with sheep erythrocytes	Antiserum to high speed deposit from tumor, heated to 100° C. during 1 hr.	Inhibition of sheep cell hemolysis
	mg/ml		(Assay for Ts)	(Assay for F)	(Assay for F)
A	2.30	1/16	0	0	0
Crude extract		1/32	0	tr	ac
		1/64	0	ac	c
		1/128	st	c	
		1/256	ac		
B	1.90	1/8	0	c	ac
Supernatant after centrifugation at 27,000 rpm for 1 hour		1/16	tr	c	c
		1/32	st	c	
		1/64	c	c	
C	0.42	1/32	0	0	0
Fraction sedimented at 27,000 rpm for 1 hour		1/64	0	0	ac
		1/128	tr	ac	c
		1/256	ac	c	

In complement fixation tests serum Ts was used in dilution 1:75, serum F in dilution 1:200. In the inhibition reaction approximately 2 units of hemolysin were used (Serum F diluted 1:2500). Antigens and sera alone did not fix complement in the dilutions tested.

together with a species specific antigen (or antigens). Similar results were obtained with extracts from chicken spleen. Centrifugation at 27,000 rpm for one hour invariably concentrated both the Forssman and species specific antigens though small amounts of both often remained in the supernatant.

Summary. These studies show that there is in tissue extracts a material sedimentable at high speed with which are associated two distinct immunological specificities. One of these is heat labile and is species specific; the other is heat stabile and is identical with the heterogenetic (Forssman) antigen. Antisera containing specific antibodies for each have been prepared. Experiments are in progress to determine whether the heavy species specific substance and the F antigen are distinct chemical entities or whether the F hapten is part of the former.

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[14] B. Brahn and F. Schiff, *Klin. Woch.*, 8: 1523, 1929.

ELECTROPHORETIC ANALYSES OF HYPERIMMUNE SERA

TISELIUS and later other investigators have shown that the normal sera of many animals, including the horse, contain four components. One of these is the albumin; the other three, globulins, are commonly designated α, β and γ. Tiselius and Kabat[1] described an antipneumococcic horse serum in which the activity seemed to be associated, not with one of these globulins, but with a new component having a mobility between those of β and γ. In a previous note we described[2] results of an electrophoretic study of a number of antipneumococcic horse sera. All these hyperimmune sera showed only the three normal globulins, increase in antibody content being in each case reflected by an increase in the γ-component. We have also published[3] electrophoretic analyses of a number of tetanus antitoxic sera. There is reason to believe that their antitoxin is associated with a new (T) component rather than with the γ-globulin. This T-component has essentially the same mobility as the antipneumococcic component of Tiselius and Kabat $-u = ca - 2.2 \times 10^{-5}$ $cm^2\ sec^{-1}\ volts^{-1}$. A globulin of similar mobility has been seen in diphtheria antitoxic sera by Pappenheimer, Lundgren and Williams.[4]

This apparent association of antibody activity sometimes with one serum constituent and sometimes with another presents an interesting series of problems. In a previous paper[5] we suggested that since all our antipneumococcic sera were from horses long subject to hyperimmunization the antibody mobility might change as immunization proceeds. Since then we have examined serum drawn from a horse about six months after the start of hyperimmunization. Its electro-

[1] A. Tiselius and E. A. Kabat, SCIENCE, 87: 416, 1938; *Jour. Exp. Med.*, 69: 119, 1939.

[2] D. H. Moore, J. van der Scheer and R. W. G. Wyckoff, SCIENCE, 90: 357, 1939.

[3] J. van der Scheer and R. W. G. Wyckoff, *Proc. Soc. Exper. Biol. and Med.*, 43: 427, 1940.

[4] A. M. Pappenheimer, H. P. Lundgren and J. W. Williams, *Jour. Exper. Med.*, 71: 247, 1940.

[5] D. H. Moore, J. van der Scheer and R. W. G. Wyckoff, *Am. Jour. Immunol.*, 38: 221, 1940.

phoretic pattern did not differ essentially from that of serum from a horse that had been producing pneumococcic antiserum for ten years. We have also found no significant difference on electrophoresis between tetanus antitoxic sera drawn from horses four and 30 months after beginning hyperimmunization.

Recently we have completed the electrophoretic study of the sera of horses hyperimmunized with a number of antigens of bacterial origin. Some have shown with more or less prominence the T-component absent in normal sera; others have shown no T but, like our pneumococcic antisera, an enhanced γ-glubulin peak. Antitoxic sera against Cl. welchii, Cl. sordelli and Cl. oedematiens, as well as against C. diphtheriae and Cl. tetani show large T components. Scarlet fever, botulinus, staphylococcus, histolyticus and vibrion septic antitoxic sera have also contained some T-globulin. In certain of these sera there has been more T than γ, in others it has been present in small amounts only. The amount of γ in these sera has been greater than in normal sera. Several antisera against the meningococcus have resembled our antipneumoncoccic sera both in the extraordinarily large amounts of γ and in the absence of detectable amounts of T. Sera against the organisms of hemolytic septicemia and swine erysipelas have also been very rich in γ. The latter serum has shown a small amount of a component having approximately the mobility of T. A serum against the Shiga and Flexner strains of dysentery bacteria has contained both T and a moderately enhanced γ. These various sera have been taken, some from freshly immunized horses, others from old serum producers.

All these results make it clear that horses respond to some antigens by an increase in the already existing γ-globulins, to others by the production of a new T component. In the experiments here reported all antitoxic sera have contained T while the most impressive increases in γ have been seen in anti-carbohydrate sera. More work will, however, be needed before we can specify those qualities of an antigen which determine the production of a T or a γ antibody.

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ON THE URINARY EXCRETION OF "FREE" SULFAPYRIDINE

CERTAIN theories relative to the mode of action of the sulfanilamide series of drugs involve oxidation-reduction mechanisms. We have isolated a monohydroxyl derivative of sulfapyridine from dog urine following the administration of the drug. The compound melts at 180°–181° (corr.) and depresses the melting point of sulfapyridine. The ultra-violet absorption data are shown in Fig. 1. The compound gives a

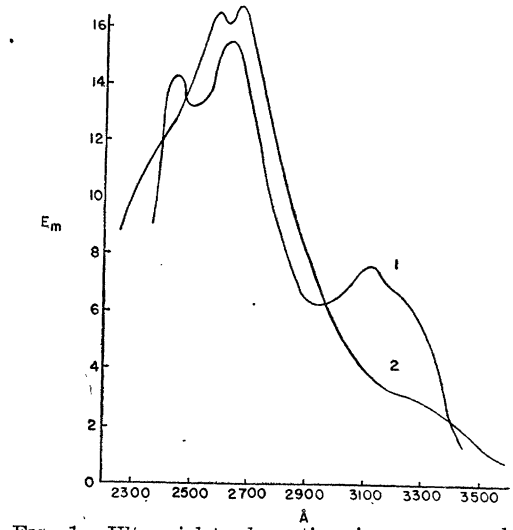

FIG. 1. Ultra-violet absorption in aqueous solution. 1. Sulfapyridine. 2. Hydroxy-sulfapyridine.

positive diazo and ferric chloride reaction.

Calculated for $C_{11}H_{11}O_3N_3S$:
C = 49.81; H = 4.15; N = 15.84.
Found:
C = 50.33; H = 4.26; N = 15.96.

A glucuronate of this substance has been isolated and characterized as its silver salt.

Calculated for $C_{11}H_9O_2N_3SAg \cdot OC_6H_8O_6Ag \cdot 3H_2O$:
C = 28.77; H = 3.24; N = 5.92: Ag = 30.46; H_2O = 7.61.
Found:
C = 28.98; H = 3.38; N = 5.73; Ag = 30.16; H_2O = 6.85.

Since the hydroxy-sulfapyridine glucuronate is very water-soluble, this product is of interest in relation to the problem of the formation of renal calculi.

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