cellular activities; the other gives aid over three years to researches under Professor M. V. Visscher on the mechanism of osmosis in living systems. To the Johns Hopkins University a four-year appropriation was made for a group program on the chemical structure of biologically important compounds. To Oxford University funds were voted to build an extension to the research laboratory of organic chemistry under Sir Robert Robinson. The five appropriations in this group totaled $197,875.

A second group of appropriations emphasized the application of physics to biological problems. Funds were given to Washington University, St. Louis, to construct a cyclotron which will be used in biological and medical experimentation; and a three-year grant was made to Professor Lawrence's group in support of similar activities at the University of California. A three-year grant to the University of Chicago is assisting studies in molecular spectra, under Professor R. S. Mulliken. The Memorial Hospital of New York received a grant covering three years, for research in the spectroscopic aspects of anemia, under Dr. C. P. Rhoads. The four grants in this group totaled $149,000.

Two grants were made in the field of genetics. The University of Missouri, where there has been an important recent development in this subject, was assisted in building a research laboratory of genetics, and was given a five-year grant toward its research program. An appropriation covering five years was made to Brown University to aid the genetics researches of Professor P. B. Sawin. These two grants totaled $109,000.

A five-year grant to the biology group at Amherst College also involved support of genetics, as well as of experimental embryology and growth studies. Such assistance to groups or departments, in contrast to support of specific projects, has been an important part of the division's program. Thus during 1939 a ten-year grant was made in support of research in biology at Stanford University. Also involving assistance to a group activity was a grant in support of the Cold Spring Harbor symposia on quantitative biology. The appropriations in this classification totaled $242,500.

Emphasis on several interests of the Foundation was included in a grant of $224,000 in support of further activities of the Yale Laboratories of Primate Biology. A minor portion of this sum covered the cost of erecting and equipping a small new physiological laboratory at Orange Park, Florida, where there are already located extensive facilities for breeding and rearing chimpanzees for research purposes. The remainder of the grant will contribute, over a five-year period, to the support of a general program in which these animals, so close to man in many important regards, are to be utilized in the study of a wide range of physiological, psychobiological, neurological, nutritional, serological and biochemical problems.

In addition to these appropriations, funds were voted to the National Research Council in support of its general budget (61,956.54) and of its fellowship program ($180,000).

Once during the year an appropriation was made for a purpose somewhat removed from the program of the Foundation in the natural sciences under its policy of concentration. Political interference in Germany having threatened the integrity of the leading world journal for abstracting mathematical literature, a grant was given to the American Mathematical Society to aid in the founding of such a journal in the United States. The editorial offices of this new journal are at Brown University. A second grant was made for the establishment of a microfilm laboratory at Brown, through which an important microfilm service in mathematics has been set up in conjunction with the new journal. These two grants totaled $61,500.

SPECIAL ARTICLES

ASSOCIATION OF THE HETEROGENETIC ANTIGEN WITH A MATERIAL IN NORMAL AND TUMOR TISSUES SEDIMENTABLE AT HIGH SPEED[1]

THERE have been numerous investigations[2, 3, 4] on the nature of the substances in tissues of different species and in micro-organisms which have the ability to produce hemolysins in rabbits against sheep erythrocytes. The procedures applied to the characterization of these substances were usually drastic and yielded no precise information concerning the antigenic substances as they occur in tissues although they contributed greatly to the knowledge of its hapten, that is, the part of the molecule determining immunological reactivity.

Studies on the nature of agents producing leukosis and sarcoma of chickens have shown that they can be sedimented in the ultracentrifuge at 27,000 rpm for one hour and are associated with a normal substance which has approximately the same sedimentation rate.[5, 6, 7] Immunological studies failed to show dif-

[1] These studies have been supported by the Jane Coffin Childs Memorial Fund, the International Cancer Research Foundation and the Anna Fuller Fund.

[2] K. Landsteiner, "The Specificity of Serological Reactions," C. C Thomas, Springfield, Illinois, 1936.

[3] F. E. Brunius, Dissertation, Stockholm, 1936.

[4] H. Schmidt, "Die Heterogenetische Hammelblut Antikörper und Ihre Antigene," Leipzig, 1924.

[5] A. Claude, SCIENCE, 90: 213, 1939.

[6] K. G. Stern, *et al.*, SCIENCE, 89: 609–610, 1939.

ferences between the heavy substance obtained from chicken tumors and from normal chicken spleens[8] and suggested that the agent might be present in tumor extracts in concentrations too small to produce antibodies on injection. The possibility that the agent and the heavy material present in normal tissues are closely related can not be excluded and, indeed, Claude[9] assumes the agent is but a modified form of the normal heavy material.

The fraction sedimentable in the ultracentrifuge from normal and tumor tissues has been shown to consist of lipoid and nucleoprotein.[10] Because of its complex nature, it was desirable to use the sensitive immunological properties as a guide to further chemical differentiation. Both species and organ specificities of these substances were studied.

Tissues were ground in the cold with sand and saline and clarified by preliminary centrifugation at 8000 rpm. The heavy fraction was obtained by centrifugation at 27,000 rpm for one hour. The supernatant was decanted and the sediment washed and suspended in saline.[11] Antisera were prepared in rabbits by injections of sediment resuspended in saline.

It was noted in the course of these studies that immune sera directed against high speed deposits from normal chicken spleen contained two different antibodies. One of these agglutinated and hemolyzed sheep cells in high dilutions and may be regarded to be a heterogenetic or Forssman (F)[12] antibody; the other (Ts) is species specific and reacts with high speed deposits of tissue extracts even after their immune sera have been deprived of the F antibodies by absorption with sheep erythrocytes. Heating to 100° C. for one hour destroyed reactivity of the species specific antigen but had no effect upon the heterogenetic component (Table I). An antiserum prepared by immunizing rabbits with the heavy fraction of chicken spleen heated to 100° C. produced only heterogenetic antibody (Table II).

TABLE I

DEMONSTRATION OF HETEROGENIC AND SPECIES SPECIFIC ANTIBODIES IN ANTISERUM TO A MATERIAL FROM CHICKEN SPLEEN SEDIMENTABLE AT 27,000 RPM

	Serum dilutions 50	150	450	1350	4050	12150
I. Serum (#26) unabsorbed						
Complement fixation with homologous heavy material						
(a) unheated	–	0	tr	c	c	–
(b) heated to 100° C. during 1 hour	–	0	0	c	c	–
Hemolysis of sheep erythrocytes	–	c	c	c	sl	0
II. Serum (#26) absorbed with sheep erythrocytes						
Complement fixation with homologous heavy material						
(a) unheated	–	0	c	c	–	–
(b) heated to 100° C. during 1 hour	ac	c	c	c	–	–
Hemolysis of sheep erythrocytes	0	0	0	–	–	–

0 = no hemolysis, tr = trace, sl = slight, st = strong, ac = almost, c = complete hemolysis.

TABLE II

ANALYSIS OF IMMUNE SERUM AGAINST HEAVY MATERIAL OF CHICKEN SPLEEN HEATED TO 100° C. DURING 1 HOUR

	Serum dilutions 50	100	200	400	800	1600
I. Serum (#45) unabsorbed						
Complement fixation with heavy fraction						
(a) unheated	–	0	0	0	tr	–
(b) heated to 100° C. during 1 hour	–	0	0	mod	c	–
Hemolysis of sheep erythrocytes	–	–	–	c	c	st
II. Serum (#45) absorbed with sheep erythrocytes						
Complement fixation with heavy fraction						
(a) unheated	st	c	c	–	–	–
(b) heated to 100° C. during 1 hour	mod	c	–	–	–	–
Hemolysis of sheep erythrocytes	0	0	0	–	–	–

Immune sera prepared with the high speed sedimentable fractions of a chicken tumor (Strain 13[13]) likewise contained the two antibodies (Ts and F). Alcoholic extracts of chicken spleen reacted only with the F antibody; they were not antigenic themselves but produced F antibodies when injected into rabbits mixed with pig serum.

These rabbit immune sera against the high-speed sedimentable fraction of chicken spleen and chicken tumor reacted strongly in complement fixation tests with similar deposits from mouse and guinea pig spleen but did not react with a similar fraction from rabbit

TABLE III

REMOVAL OF CROSS REACTING ANTIBODIES FROM ANTISERUM TO HEAVY MATERIAL FROM CHICKEN SPLEEN BY ABSORPTION WITH SHEEP ERYTHROCYTES

Antiserum (#26) to high speed sediment from chicken spleen	Serum dilutions	High speed sediment from spleen: Chicken	Mouse	Guinea pig	Hemolysis of sheep erythrocytes
Unabsorbed	1/150	0	0	0	c
	1/450	0	0	0	c
	1/1350	c	ac	c	c
	1/4050	c	c	c	sl
	1/12150	–	–	–	0
Absorbed with sheep erythrocytes	1/50	–	c	c	0
	1/150	0	c	c	0
	1/450	c	c	c	0

[7] E. A. Kabat and J. Furth, *Jour. Exp. Med.*, 71: 55, 1940.
[8] *Ibid.*
[9] A. Claude, *op. cit.*
[10] *Ibid.*
[11] E. A. Kabat and J. Furth, *op. cit.*
[12] Abbreviations: F = Forssman, T = tissue antigen.
[13] E. L. Stubbs and J. Furth, *Jour. Exp. Med.*, 61: 593, 1935.

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.