be tripled by a new plant. Calendered "Vinylite" sheets have applications as rubber and leather successors, particularly in articles of attire. The development of collapsible tubes formed of vinyl sheet material was reported. "Saran," a vinylidene-chloride thermoplastic resin, is employed in the manufacture of synthetic rattan. The production of melamine resins ("Melamac"), especially for laminating and surface coatings, was announced.

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SPECIAL ARTICLES

COMPLEMENT-FIXATION IN ENCEPHALITIS AND RABIES VIRUS INFECTIONS

THE use of brain extracts as an antigen in the complement-fixation test presents considerable difficulties on account of the anti-complementary and non-specific effects involved.[1] Mainly for this reason the test has remained unsatisfactory as a specific method for diagnosing central nervous system virus infections, especially when no other source of antigen than the infected brain is available.

A technique has now been devised, however, by which the disturbing variables have been largely removed or controlled. The main features of the procedure consist in (*a*) preparing the antigens from a heavy brain emulsion by repeated freezing and thawing and centrifugation in the angle head centrifuge; (*b*) inactivating the sera at the proper temperature according to the animal species.

Diluted guinea pig serum constitutes the complement. In each test it is titrated in duplicate in the presence of the antigens, and also in saline. One set of tubes is incubated for one half hour at 37° C., the other at icebox temperature 18 hours before addition of the hemolytic system. The former set at 37° C. determines the amount of complement to use in the later specific test and the latter set at icebox temperature, run with the specific test, indicates the validity of the result by showing the actual amount of free complement present in the system at the time the sensitized blood cells are added. This double titration discloses that some preparations, especially crude brain emulsions, when merely centrifuged in the horizontal centrifuge, show no anti-complementary effect following incubation at 37° C. but do have a strong inhibitory effect when kept in the cold for 18 hours. Thus they are unsuitable as antigens. Antigens prepared as described above, however, usually exhibit no anti-complementary effect until after at least 3 months' aging. The titre of the complement in the presence of antigen is the same as in saline at 37° C. and often even better. Two full units of complement in saline are used in the final specific reaction—an amount equivalent to 2, sometimes 2½ units in the presence of antigen at 37° C. The result of the titration of complement at icebox temperature usually parallels that at 37° C.; besides it gives more exact information on the inhibitory effect of the antigen than the usual antigen control.

TABLE I

EFFECT OF HEATING AT DIFFERENT TEMPERATURES FOR PERIODS OF 20 MINUTES ON THE SPECIFIC AND NON-SPECIFIC COMPLEMENT-FIXING POWER OF SEVERAL NORMAL AND IMMUNE SERA

Sera	56°		60°		65°	
	Homologous antigen	Heterologous antigen or normal brain	Homologous antigen	Heterologous antigen or normal brain	Homologous antigen	Heterologous antigen or normal brain
Rabies immune, Rabbit No. 1	*1/128	1/16	1/128	1/16	1/128	0
" " " No. 2	1/32	1/32	–	–	1/32	0
Eastern equine encephalomyelitis, Rabbit No. 3	1/96	1/24	–	–	1/48	0
Normal, Rabbit No. 4	–	1/16	–	1/16	–	0
Rabies immune, Guinea pig No. 1	1/12	0	1/6	–	1/3	–
" " " No. 2	1/96	0	–	–	1/24	–
Rabies immune, Mouse (pooled sera)	1/24	1/6	1/12	0	–	–

* 1/128 = Highest dilution at which serum gave a 2+ or better reaction.
0 = No reaction in any of the tubes, the first dilution being usually 1/3 or 1/4.
– = Not tested.

Infected mouse or dog brains have constituted the antigens. A suspension of infected brain is made up in ten times its weight with diluent consisting of 0.85 per cent. saline containing 2 per cent. inactivated normal guinea pig serum. This suspension remains in the icebox 14 to 20 hours and is then spun in a horizontal centrifuge at 2,500 r.p.m. for 30 minutes. The supernatant is removed, frozen and thawed five times in a dry ice-alcohol mixture, and spun in a Swedish angle centrifuge for 1 hour. The supernatant is again removed and, after the addition of 1/10,000 merthiolate, is stored in the icebox.

Sheep red blood cells plus anti-sheep hemolysin constitute the hemolytic system. The hemolysin is

[1] B. F. Howitt, *Jour. Immunol.*, 33: 235, 1937.

titrated at long intervals. 3 M.H.D. of hemolysin and 3 per cent. suspension of packed red cells in saline are employed.

Hyperimmune sera prepared with homologous infected brain tissue have been tested. In contrast to guinea pig sera, rabbit sera usually and mouse sera often give non-specific reactions with unrelated antigens or with normal brain. This effect has been thought to be due to thermolabile substances (Takenomata; Mackie and Finkelstein[2]) and has been removed largely in our tests by establishing temperatures of inactivation for guinea pig serum of 56°, mouse serum, 60°, rabbit serum, 65°. All sera are heated for 20 minutes. This procedure eliminates non-specific as well as anti-complementary reactions without materially disturbing the specific effect (Table I).

TABLE II

COMPLEMENT-FIXATION TESTS WITH MOUSE BRAIN ANTIGENS AND MOUSE IMMUNE SERA

Antigens	Sera inactivated for 20 minutes at 60° C.									
	St. Louis No. 3	Japanese B No. 2604	Japanese B No. 17	Japanese B No. 12	Lymphocytic choriomeningitis	Eastern equine encephalomyelitis	Louping-ill	Rabies (fixed)	Rabies (street)	Saline
St. Louis No. 3	*1/32	0	0	0	0	0	0	0	0	0
Japanese B No. 2604	0	1/32	1/32	1/64	0	0	0	0	0	0
" " No. 17	0	1/32	1/64	1/128	0	0	0	0	0	0
" " No. 12	0	1/32	1/128	1/128	0	0	0	0	0	0
Lymphocytic choriomeningitis	0	0	0	0	1/128	0	0	0	0	0
Eastern equine encephalomyelitis	0	0	0	0	0	1/64	0	0	0	0
Louping-ill	0	0	0	0	0	0	1/64	0	0	0
Rabies (fixed)	0	0	0	0	0	0	0	1/8	1/16	0
" (street)	0	0	0	0	0	0	0	1/8	1/32	0
Saline	0	0	0	0	0	0	0	0	0	–

* 1/32 = Highest dilution at which serum gave a 2+ or better reaction.
0 = No reaction in any of the tubes, the first dilution being usually 1/3 or 1/4.
– = Not tested.

The specific reaction is carried out with 0.25 cc of undiluted antigen, plus two full units of complement in 0.5 cc volume, and 0.25 cc of serum. The serum is used in twofold dilutions commencing with 1 to 3 or 1 to 4. These reagents are placed in the icebox 18 hours and then left at room temperature for one half hour. The hemolytic system is then added, consisting of 0.25 cc of the 3 per cent. suspension of sheep cells plus 0.25 cc of hemolysin containing 3 M.H.D. The total volume per tube is then 1.5 cc. The tubes are incubated at 37° C. for one half hour. The degree of hemolysis resulting in each tube is expressed from 0, indicating complete hemolysis, to 4, indicating no hemolysis.

Specific complement-fixation has been obtained with the viruses of St. Louis encephalitis, Japanese B encephalitis, Eastern equine encephalomyelitis, lymphocytic choriomeningitis, louping-ill and rabies (fixed and street). Table II shows the type of result obtained.

Sera have been shown to react in dilutions as high as 1 to 192 and antigens 1 to 128. Rabies antigen is destroyed at 70° C. in 30 minutes. It is affected but little by ultraviolet light irradiation sufficient to render the preparation avirulent. Centrifugation in the high-speed vacuum centrifuge or filtration through Berkefeld N candles, though reducing the virulence considerably (10,000 to 100,000 times), does not alter the antigenicity materially.

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[2] N. Takenomata, *Zeitschr. Immunitätsforsch*, 41: 508, 1924; T. J. Mackie and M. H. Finkelstein, *Jour. Hyg.*, 28: 172, 1928–29.

ALCOHOLIC AND NON-ALCOHOLIC KETOSTEROIDS AND THE ZIMMERMAN COLOR REACTION[1]

IN the course of isolating steroids from ether extracts of acid-hydrolized urines from cancerous and non-cancerous persons we noticed that the non-alcoholic ketonic fraction of the neutral material contributed considerably to the total 17-ketosteroid titer as determined by the Zimmerman[2] reaction. Since androsterone, a 17-keto hydroxy steroid, is chiefly responsible for the androgenic activity of urinary extracts, this observation may partially explain the divergence existing between the relatively high 17-ketosteroid colorimetric titer and the biological activity.

Evidence has recently been obtained that the non-alcoholic ketonic fraction contains steroid compounds. Burrows *et al.*[3] obtained $\Delta^{3:5}$ androstadiene-17-one

[1] Aided by grants from the Dazian Foundation for Medical Research and the National Research Council Committee for Problems of Sex. Works Progress Administration Project No. 65–1–14–2949.

[2] W. Zimmerman, *Ztschr. Physiol. Chem.*, 233: 257, 1935.