

sive edema and ascites appeared, showing that NaCl is a factor in producing edema where the colloid osmotic pressure reduction leads to "Oedembereitschaft" or Edema Tendency.

The exact mechanism whereby the NaCl leads to increased edema formation we are leaving for a future publication. Suffice it to say that these experiments prove that the statement that salt is held back through renal insufficiency in nephroses and nephritis with edema is probably false.

We also noted in our work that the blood corpuscles should not be returned to the vascular system by intravenous injections because this slight injury to the veins tends to cause increase in venous pressure and local edema when "Oedembereitschaft" is present. The experiment is clean cut only when the corpuscles are returned to the right ventricle.

*Conclusions.* Lowering plasma proteins to below 3% and osmotic pressure of plasma colloids to below 10 mm. produces a tendency to edema formation. The edema is very slight and does not increase rapidly until NaCl is given, when the urinary output falls suddenly and massive edema appears.

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### Colloidal Gold Test for Serum Antibodies in Poliomyelitis.\*

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In a previous communication<sup>1</sup> a method was described for the detection and titration of immune bodies in poliomyelitis, with reference to the applicability of the test in the study of susceptible persons, carriers, therapeutic value of serums from normal adults, and in relation to prognosis and convalescence.

A total of 363 serums has been studied to date. These included 100 normal adults and 100 normal children (age groups 6 weeks to 19 years), 58 convalescent serums (monkey, adult human, and children), 76 normal animals (monkey, horse, goat, and sheep),

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<sup>1</sup> Ebersson, F., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 405.

and 29 immune animal serums (monkey, horse, goat and sheep).

The results have agreed with known facts. The specificity of the test has been demonstrated also by observations made during all stages of poliomyelitis in the human and in the monkey.

The progressive development of immune bodies could be shown to occur during convalescence, particularly in those cases where rapid and complete recovery ultimately took place. These antibodies could not be found in the blood serum of patients or animals with a poor outlook as to recovery. When the disease went on to a protracted convalescence with poor or slow restoration of muscular activity, these immune bodies were found to be weak or negligible in quantity.

The serums from infants and very young children did not show any protective properties whatsoever and the same was true for 34% of adults, thus agreeing with the known facts of susceptibility to poliomyelitis and the neutralizing power against the virus of serums from certain normal adult persons. The following results were obtained in 200 serums that were tested for poliomyelitic antibodies: 100 *adults*, ranging in age from 25 to 50 years, gave a *positive* test for immune bodies in 64% of the cases (30%, strongly positive; 20%, moderately strong; and 14%, weakly positive). One hundred *children*, ranging in age from 6 weeks to 19 years, gave a *negative* test in 77% of the cases. Of 50 in the *age group from under 1 year to 8 years*, 90% gave *negative* tests for immune bodies, 6% a weakly positive, and 4% a moderately strong test; among 50 in the *age group of 9 to 19 years*, 64% were *negative* and 36% were positive for immune bodies (12%, strongly positive; 16%, moderately strong; and 8%, weakly positive). (Table I.)

The nature of the precipitation phenomenon exhibited by certain serums subjected to the colloidal gold test was studied more minutely by means of *in vivo* neutralization experiments in the monkey. It was especially desirable to evaluate the protective property of serum obtained from normal adults. For this purpose 2 serums were selected from such a group, one serum giving a negative and the other a definite positive result with the colloidal gold test. In the experiments, a highly potent virus obtained through the courtesy of Dr. Simon Flexner was used. The virus filtrate in 0.5 cc. and 0.62 cc. amounts from a 10% and 20% emulsion was mixed with 0.5 cc. amounts of serum (previously inactivated one-half hour at 56°C), incubated at 37°C for one hour, allowed to stand over night

TABLE I.  
Incidence of Positive and Negative Tests for Antibodies in Different Age Groups.  
Adults (Age 25-50)

Number	Negative	Positive			Total Positive
		Weak	Moderately Strong	Strong	
100	36	14	20	30	64

  

Age	Number	Negative		Positive						Total Positive	%	
		Number	%	Weak	%	Moderately Strong	%	Strong	%			
												Total Positive
Under 1 yr.	4	4	100.00	0	0	0	0	0	0	0	0	0
1-4 years	19	19	100.00	0	0	0	0	0	0	0	0	0
5-8 years	27	22	81.5	3	11.1	2	7.4	0	0	5	18.5	18.5
Total	50	45	90.0	3	6.0	2	4.0	0	0	5	10.0	10.0
9-14 years	35	25	71.4	2	5.7	5	14.3	3	8.6	10	28.6	28.6
15-19 years	15	7	46.6	2	13.3	3	20.0	3	20.0	8	53.3	53.3
Total	50	32	64.0	4	8.0	8	16.0	6	12.0	18	36.0	36.0
Total	100	77	77.0	7	7.0	10	10.0	6	6.0	23	23.0	23.0

in the icebox ( $4^{\circ}$ - $6^{\circ}$ C), and the entire contents injected intracerebrally under ether anesthesia and careful surgical technic. Six monkeys were employed; 2 received normal, unpreserved human serum giving a negative test (7777777777), 2 others a human serum with a positive test (1111224477), and the other 2 received the virus alone. The control animals became paralyzed on the sixth to the eighth day (one died on the seventh day), the animals that

received the strictly normal serum came down on the seventh to the ninth day with typical poliomyelitis, and the 2 animals receiving the positive test serum remained free of all symptoms. In these instances the serum was effective against approximately 50-60 M.L.D. as calculated on the usual activity of the virus, and the specificity of the *in vitro* colloidal gold test was thus demonstrated. These experiments have a direct bearing upon the possibility of employing such "neutralized" or "protected" mixtures of virus and serum as determined by our rapid test for the purpose of active immunization. Such studies, now in progress on a larger scale, aim to use an appreciable excess of immune serum in combination with virus in such amounts that the colloidal gold test will still give a positive reaction with the "protected" mixture.

The specificity of the test was further shown by experiments in which serums were studied in the following manner: (1) Different concentrations of virus in fixed quantities were combined with varying dilutions of serum. (2) Different dilutions of a 5% or 10% virus in fixed amounts were combined with fixed quantities of serum. The protocols which will appear in the complete report may be summarized as follows: The precipitation of the colloidal gold preparation by immune serums did not occur in the presence of poliomyelitis virus when the virus was present in excess. Similarly, in the presence of an excess of antibodies due to incomplete absorption on the part of a given amount of virus, the serum gave a positive precipitation test. With encephalitis and herpes viruses no absorption occurred and immune serums gave typical positive precipitation reactions.

The application of the colloidal gold test in the study of poliomyelitis has been shown to have a direct bearing and practical value in the problems concerning: (1) Selection of donors' serum for therapeutic use in poliomyelitis. (2) Study of susceptibility to this disease among the general population. (3) Evaluation of therapeutic potency of serums from human and animal sources. (4) Prognosis during the course of poliomyelitis as related to the progressive development or complete absence of serum antibodies. (5) *In vitro* selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization.