TABLE I. Effect of Adjuvants on the Adaptation of IB45 Virus to Mice.

Adjuvant	No. of trials	No. of passages	Virus
None	5	3,3,4,4,6	Ab
H. influenza, living cells, A*	2	3,27	P
" heat-killed cells, A	5	6,7,8,8,10	P
" streptomycin treated, A	1	8	P
" glycine extract, A	3	6,10,15	P
" glycine extract dialyzed, A	2	8,23	P
" living cells added once	1	3	Ab
" filtrate of cells frozen and thawed 20 times, A	1	8	Ab
D. pneumoniae, type III, heat-killed cells, A	1	7	P
Group A beta hemolytic streptococci, heat-killed, A	1	7	P
V. comma, boiled 1 hr, A	1	4	P
Pneumococcus Group III polysaccharide, A	1	6	P
V. comma, filtrate of 10-day broth culture, A	1	6	P
3% chicken red blood cells, A	2	4,8	P
" " " " " heated, A	2	4,8	P
Laked 3% chicken red blood cells, A	1	9	Ab
Powdered pyrex glass, A	1	8	P
Soluble starch, A	1	8	P
Kaolin, A	1	8	P
Glycine, A	1	4	Ab
1% gum arabic, A	1	8	Ab

A* = Added each passage. Ab = Absent. P = Present.

In the series in which living cells of *H. influenzae* were added, and 27 passages in mice made, the adjuvant was omitted after 4, 8, 12 and 20 passages, and the virus remained present from 5 to 15 passages thereafter; further passages were not made. Occasional titrations in mice showed infectivity titers of at least 10^{-4} to 10^{-5}.

In the series in which a dialyzed glycine extract was the adjuvant, it was discontinued after the 20th passage, and the virus continued to propagate; the animals died and showed lesions to the same extent as before.

is no evidence that any one of the preparations possessed greater action than the others, although the addition of killed bacterial cells seemed to give somewhat more rapid adaptation of the virus in the mouse than cell extracts or non-cellular substances. An influenza B virus that was initially non-infective for mice became infective when certain adjuvants of bacterial or non-bacterial origin were added to each passage of virus. A procedure such as this may be useful in the isolation and adaptation of influenza viruses in mice.

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Protective Effect of Salt on Reduction of Viscosity of Sodium Thymonucleate Solutions by Heat. (18182)

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The high viscosity of aqueous solutions of sodium thymonucleate, which is characteristic of the polymerized, asymmetric molecular state of this material, is readily reduced by a variety of agents. Among these are added salts(1,2), heat(1), changes in pH(3), and ultraviolet(4) and X-irradiation(5-8). The

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1. Hammarsten, E., *Biochem. Z.*, 1924, v144, 383.

2. Greenstein, J. P., *J. Nat. Cancer Inst.*, 1940, v1, 77.

nature of the action of these agents on the nucleate is as yet obscure. The present communication is concerned with the effect of two of these agents, namely salts and heat, applied simultaneously.

Methods and material. Several preparations of sodium thymonucleate obtained by the modified Hammarsten procedure(5) were employed. The relative viscosity of aqueous solutions of these materials was measured in Bingham and Jackson type viscometers at a constant external pressure of 6 cm H_2O (cf 2). Salt mixtures with nucleate were prepared by adding known volumes of salt of designated concentration to equal volumes of nucleate solution. The heating experiments were performed by immersing solutions in a carefully controlled hot bath.

Experimental. Thymonucleate solutions in water show a slow but definite decrease in viscosity on standing. When, however, such solutions are treated with sodium chloride to 0.5 M concentration, there is an immediate fall in viscosity to a value which is maintained quite constant over many days. This phenomenon suggested that added salt might protect the nucleate against the spontaneous

TABLE I. Protective Action of Salt on Heated Solutions of Sodium Thymonucleate.*

Final conc. of NaCl, M	Relative viscosity†	
	Before heating	After heating
Control (no salt)	15.6	3.5
2	8.9	8.9
1	8.8	8.7
0.5	7.9	7.8
0.1	6.5	5.4
0.01	7.4	2.7
0.001	12.2	3.4

* Final concentration of nucleate 0.5%.
† 6 cm H_2O external pressure.

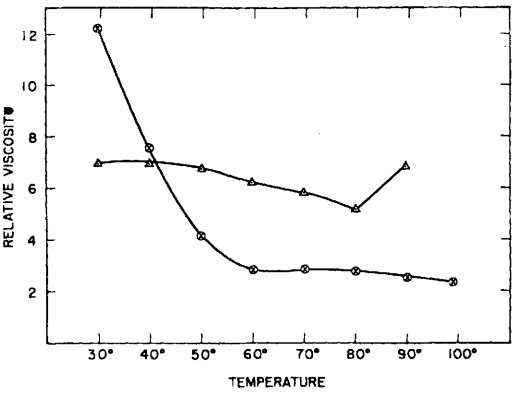

FIG. 1.

Effect of various temperatures on the reduction of relative viscosity, at 30° and 6 cm H_2O pressure, of sodium thymonucleate in the presence and absence of added sodium chloride. ○ 0.5% nucleate in water, △ in 1 M NaCl solution.

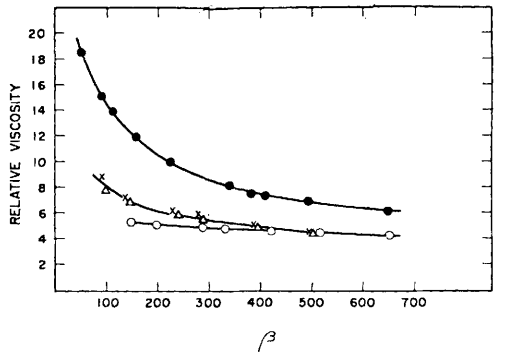

FIG. 2.

Relation of related viscosity at 30° of heated and unheated solutions of sodium thymonucleate in the presence and absence of added sodium chloride as a function of the velocity gradient β (cf. 2). ● 0.2% nucleate in water, unheated; ○ same solution after heating at 70° for 20 min.; ×, △ 0.2% nucleate in 1 M NaCl solution before and after heating at 70° for 20 min., respectively.

diminution in its viscous property and perhaps even against induced falls in viscosity by such agents as heat.

Stock solutions of 1.0% nucleate were diluted by equal volumes of either water or NaCl solutions of various concentrations. The mixtures were then heated at 70° for 20 minutes, cooled rapidly to 30°, and their relative viscosities measured at this temperature. At concentrations of 0.5 M NaCl or higher, heating had apparently no effect on the viscosity of the nucleate solutions. The data are given in Table I.

Similar results to those in Table I were

3. Vilbrandt, C. F., and Tennent, H. G., *J. Am. Chem. Soc.*, 1943, v65, 1806.

4. Hollaender, A., Greenstein, J. P., and Jenrette, W. V., *J. Nat. Cancer Inst.*, 1941, v2, 23.

5. Sparrow, A. H., and Rosenfeld, F. M., *Science*, 1946, v104, 245.

6. Taylor, B., Greenstein, J. P., and Hollaender, A., *Arch. Biochem.*, 1948, v16, 19.

7. Butler, G. C., *Canad. J. Res.* (B), 1949, v27, 972.

8. Butler, J. A. V., Gilbert, L. A., and Smith, K. A., *Nature*, 1950, v165, 714.

TABLE II. Reversibility of the Protective Action of Sodium Chloride.

Mixture†	Relative viscosity*			
	Before heating	After heating	After dialysis	After heating dialyzed sol.
Nucleate in H_2O	27.3	—	6.4	3.4
"	27.3	5	3.8‡	3.3
Nucleate in salt	12.8	—	10.2	3.9
"	12.8	12	10.2‡	3.6

* Viscosities measured at 30° and 6 cm H_2O external pressure.

† Final concentrations of nucleate and sodium chloride 0.5% and 1.0 M, respectively.

‡ Refers to the viscosity of the heated solutions after dialysis.

obtained with potassium chloride and magnesium chloride.

Fig. 1 describes the effect of heating at various temperatures on the viscosity of aqueous and of salt-containing mixtures with nucleate. Final concentrations of nucleate and of sodium chloride were 0.5 and 1 M, respectively. The period of heating was 20 minutes. At temperatures over 85°, mixtures of nucleate and salt sometimes yielded a small amount of white precipitate.

The structural character of the viscosity of the nucleate heated in the presence of salt is revealed in Fig. 2.

The reversibility of the salt protection was demonstrated by dialysis. Aqueous and salt-containing solutions of nucleate were heated, dialyzed, made up with water to equal concentrations of nucleate as measured spectrophotometrically in the ultraviolet, and heated again. Several such experiments revealed without exception that after loss of salt by dialysis the nucleate solution suffered a loss in viscosity on heating. A sample experiment is described in Table II. An interesting finding is that not only does salt protect against the effect of heat, but also apparently against the equally marked effect of dialysis on reducing the viscosity of the nucleate solution.

In another series of experiments, water and sodium chloride solutions (2M) were added to equal volumes of heated 1.0% nucleate solutions in water. Only a marked decrease in viscosity resulted after addition of the salt solution. The salt must be present while heating the nucleate solution in order to prevent the viscosity drop.

Summary. The viscosity of aqueous solutions of sodium thymonucleate is reduced both by heating and by addition of sodium chloride and other salts. If, however, the nucleate solution is heated in the presence of a sufficiently high concentration of salt, there is no further decrease in viscosity beyond that induced by the salt. The protective effect of the salt is reversible, for if the salt is removed by dialysis from the heated nucleate-salt mixture, the residual aqueous solution of nucleate again suffers a marked reduction in viscosity on heating.

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Human Plasma as a Source of Prothrombin in the Ac-Globulin Assay.* (18183)

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The approach to the problems of blood coagulation from the point of view of studying single factors received a major stimulus with the development of the 2-stage prothrombin assay by Warner, Brinkhous and Smith (1). This provided for the measure of pro-

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1. Warner, E. D., Brinkhous, K .M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.