extent. In these experiments, the results of which are presented in Table III, there was a slight decrease in plasma inorganic phosphate, the greatest decrease occurring at the 30-59-minute period and amounting to 13% of the initial value.

It is not possible from the data obtained to derive any conclusions concerning the exact cause of these changes occurring in the plasma phosphorus concentration of restrained dogs. The principal factors appear to be those of posture and of emotional disturbance. This paper does not attempt to establish the relative contribution of each of these factors to the phosphorus decrease; its purpose is to report the observation of this phenomenon so as to bring it to the attention of those investigators whose experiments may be affected by its occurrence.

Summary. 1. A decrease in the plasma inorganic phosphate concentration amounting to 30-60% of the initial concentration was observed in dogs restrained in the supine position. This change was also evident in the total acid soluble and the total phosphorus fractions, while the lipid phosphorus fraction remained constant during an entire experiment.

2. The decrease in inorganic phosphate was accompanied by an increase in plasma glucose.

3. Dogs restrained on their backs exhibited a greater decrease than those restrained in a stock.

16149

Effect of Salicylate and Tripelennamine Hydrochloride (Pyribenzamine) on the Arthus Reaction and on Bacterial Allergic Reactions.*

EDWARD E. FISCHEL. (Introduced by R. F. Loeb.)

From the Departments of Medicine and Bacteriology, Columbia University College of Physicians and Surgeons, and the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, New York City

The tissue damage that results from the union of certain antigens with their corresponding antibodies may be prevented by non-specific agents.[1,2] In the case of the positive tuberculin reaction, cachexia and fever appear to inhibit the expected reactions.[2] As yet, however, little attention has been directed to the inhibition of necrotizing allergic reactions by therapeutic agents. The more transient reactions of classical anaphylaxis are found to be prevented effectively by the Mosnier compounds which have striking anti-histamine properties.[3,4] Urticarial reactions to specific allergens or to histamine can be inhibited by many of these synthetic compounds, of which the most widely used in this country are diphenhydramine hydrochloride (Benadryl) and tripelennamine hydrochloride (Pyribenzamine). Clinically, hayfever and analogous reactions have been benefitted by these agents.[4] These substances, however, have not been demonstrated to be beneficial in the "bacterial" type of allergic response, or in the local Arthus reaction, although these allergies may be important in the pathogenesis of many diseases. Reubi[5] reported that 2-(N-phenyl-N-benzyl-aminoethyl)-imidazoline (antistine) prevented the occurrence of the kidney damage that usually results when duck anti-kidney serum is in-

* This work was done during the tenure of a Life Insurance Medical Research Fellowship.

1 Mitchell, A. G., Wherry, W. B., Eddy, B., and Stevenson, F. E., *A. J. Dis. Child.*, 1928, **36**, 720.

2 Gay, F. P., and Associates, *Agents of Disease and Host Resistance,* Springfield, Charles C. Thomas, 1935.

3 Feinberg, S. M., *J. A. M. A.*, 1946, **132**, 703.

4 Friedlander, S., *Am. J. Med.*, 1946, **1**, 174.

5 Reubi, F., *Helvetia Medica Acta,* 1946, **13**, Supp. XVIII.

jected into rabbits. Attempts to use similar compounds such as diphenhydramine or tripelennamine to inhibit the tuberculin reaction in animals were unsuccessful.[6] Likewise the use of these drugs in two cases of serum sickness and 3 of rheumatic fever has been disappointing in our experience.

Among the agents said to inhibit the more severe allergic tissue reactions, salicylates have been frequently mentioned. Derick, *et al.*[7] used aspirin to prevent the appearance of some of the manifestations of serum sickness. Several workers have presented data which led them to believe that salicylates inhibited antibody formation.[7-10] Coburn demonstrated that concentrations of sodium salicylate larger than those usually obtained clinically inhibited the union of antigen and antibody *in vitro*,[11] and on this basis used salicylates prophylactically to prevent recurrences of rheumatic fever following streptococcus infections.[12]

It appeared of interest to study whether, in the presence of known quantities of antibody and antigen,[16] salicylate or tripelennamine could inhibit the Arthus reaction induced passively in rabbits. The "bacterial" type of allergic reaction in humans actively sensitive to tuberculin or to streptococcal nucleoprotein was also studied.

The Arthus Reaction. The desirability of inducing allergic reactions with known amounts of a single pure antigen and its corresponding antibody is obvious from the data of Kabat.[13] He and his coworkers[14,16] have presented the techniques for producing anaphylaxis and the Arthus reaction with measured amounts of antigen and antibody. The latter technique was employed in this study.

Albino rabbits (about 2200 g) were injected intracutaneously in single or multiple sites with dilutions of rabbit anti-chicken egg albumin serum containing amounts of antibody nitrogen known to give reactions of minimal and maximal severity.[16] The serum was prepared and analyzed by Dr. E. A. Kabat and kindly given for this study. Controls were injected in equal number simultaneously but were not given drug therapy. After a half hour, known amounts of a solution of 4 times recrystallized egg albumin were injected directly into the sites. The reactions were read at 6, 12, 24 and 48 hours, and those of the untreated animals were compared with groups receiving salicylate or tripelennamine. In some instances biopsies were taken of the involved areas for histological comparison. The intensity of the reactions was graded as previously described:[16] 0 = no reaction; ± = transitory erythema lasting 5 to 8 hours, and absent at 24 hours; + = erythema lasting 24 hours and measuring up to 1.5 cm in greatest diameter at that time; ++ erythema 1.5-2.5 cm at 24 hours persisting for one to 2 days, with slight edema and occasional slight brownish discoloration after 24 hours; +++ 2-3.5 cm erythema with moderate edema and slight brownish discoloration persisting 2 to 3 days; ++++ more than 2.5 cm of erythema, marked edema and moderate or marked brownish discoloration with changes lasting 3 to 5 days. Rabbits of the treated groups were given the medication every 4 hours on the day before, the day of, and occasionally on the day following the induction of the Arthus reaction, although by this time the reactions had reached their height and readings were conclusive. Sodium salicylate was given intravenously in doses of 2 to 5 ml of a 10%

[6] Birkeland, M., and Kornfeld, L., *J. Bact.*, 1947, **54**, 82.

[7] Derick, C. L., Hitchcock, C. H., and Swift, H. F., *J. Clin. Invest.*, 1928, **5**, 427.

[8] Swift, H. F., *J. Exp. Med.*, 1922, **36**, 735.

[9] Homburger, F., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 101.

[10] Jager, B. V., and Nickerson, M., *Am. J. Med.*, 1947, **3**, 408.

[11] Coburn, A. F., and Kapp, E. M., *J. Exp. Med.*, 1943, **77**, 173.

[12] Coburn, A. F., and Moore, L. V., *J. Pediat.*, 1942, **21**, 180.

[13] Kabat, E. A., *Am. J. Med.*, 1947, **3**, 535.

[14] Kabat, E. A., and Landow, H., *J. Immunol.*, 1942, **44**, 69.

[15] Kabat, E. A., and Boldt, M. H., *J. Immunol.*, 1944, **48**, 181.

[16] Fischel, E. E., and Kabat, E. A., *J. Immunol.*, 1947, **55**, 337.

TABLE I.
Effect of Salicylate and Tripelannamine on Severity of the Arthus Reaction Induced Passively with Known Amounts of Anti-egg Albumin and Crystalline Egg Albumin. Number of Reactions of Different Severity Related to Total Reactions in Each Group.

Antibody nitrogen (mg)	Treatment	Severity of reaction with 0.13 mg crystalline egg albumin nitrogen				
		++++	+++	++	+	0
.53	Control	3/3				
	Salicylate	2/3	1/3			
.22	Control	3/16	9/16	4/16		
	Salicylate	1/8	5/8	2/8		
	Tripelennamine		4/10	6/10		
.10	Control			1/2	1/2	
	Tripelennamine				2/3	1/3
.05	Control			4/7	3/7	
	Salicylate			4/7	2/7	1/7
	Tripelennamine			4/5	1/5	
.025	Control				2/2	
	Tripelennamine				1/3	2/3

solution (0.2 to 0.5 g). In a few instances serum salicylate levels were determined.[17] A value of 1224 γ per ml was found a few minutes after one injection of 0.4 g and 311 γ per ml after 4 hours. Tripelennamine hydrochloride (Pyribenzamine) was administered as an 0.5% solution in doses from 20 mg intramuscularly to 5 mg intravenously on the day before and at 4-hour intervals on the day of induction of the Arthus reaction. Ten mg intravenously caused transient convulsions in some rabbits.

The results of the systemic administration of salicylate or Pyribenzamine on the severity of the passive Arthus reaction are presented in Table I. It is apparent that the severity of the reaction was not appreciably altered when the higher concentrations of antibody were used, or with quantities normally giving a minimal reaction. The readings tabulated are those at the end of 24 hours, in conformity with our previous experience, and with the generally accepted picture of the Arthus phenomenon. However, during the first 6 or 8 hours a difference between the control and salicylate treated groups was noted which does not lend itself to adequate measurement. Usually the control and tripelennamine treated groups had a thicker and slightly wider area of edematous skin than did the salicylate treated group. The reaction at 24 hours more closely approximates the tissue damage associated with the classical Arthus phenomenon and apparently the end reaction is not influenced by the moderate difference in edema at the end of 6 hours.

Bacterial Allergic Reaction. "Bacterial" allergic reactions have been distinguished from the Arthus reaction chiefly because it is not possible to transfer the allergy to bacterial products which results after infection with serum of sensitized individuals.[18] Chase[19] showed that sensitivity of the tuberculin type may be passively transferred with cellular exudates from sensitized animals. The mechanism of this cellular transfer of sensitivity is not yet clearly understood. In these studies it was necessary to determine the initial degree of sensitivity and then subject the same individual to the salicylate or tripelennamine and ascertain the effect on the reaction.

Subjects were adults on the wards of the Presbyterian Hospital recovering from vari-

17 Brodie, B. B., Undenfriend, S., and Coburn, A. F., *J. Pharm. and Exp. Therap.*, 1944, **80**, 114.

18 Rich, A. R., *The Pathogenesis of Tuberculosis*, Springfield, Charles C. Thomas, 1941.

19 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

TABLE II.
Effect of Salicylate and Tripelennamine on Intracutaneous Skin Tests in Individuals Sensitive to Streptococcal Nucleoprotein Fraction C18K.

Subject	Area of erythema			
	Control (inches²)	Salicylate (inches²)	Salicylate level (γ/ml)	Tripelennamine (inches²)
R.A.	.15	.20	321	.23
E.C.	.33	.31	548	.53
L.C.	.30	.50	352	.39
B.C.	.11	.19	534 (toxic)	.17
B.D.	.30	.42		.28
H.H.	.35	.32	278	.58
Y.M.	.05	.14	338	
M.R.	.16	.26		.25
A.S.	.50	.58		.60
P.W.	.40	.10	507 (toxic)	.60
D.	.27	.40		
X.C.	.19	.05		
Y.C.	.07	.12		
I.W.	—	.37		.50

ous diseases, medical students, and children convalescing from rheumatic fever at the Pelham Home for Children.† Intracutaneous skin tests were done on comparable areas of the forearm but individual sites were used only once. The purified protein derivative (PPD) of tuberculin[20] and a nucleoprotein fraction C18K prepared from the hemolytic streptococcus by Heidelberger and Kendall[21] and kindly given for this study by Dr. Heidelberger, were used as antigens. The PPD was used in one of the 2 customary strengths, 0.00002 mg in 0.1 ml and 0.005 mg in 0.1 ml. The C18K was diluted from an analyzed (Kjeldahl) stock solution so that 0.1 ml contained 0.0001 mg nitrogen. Readings were made as frequently as possible, every 6 or 12 hours after injection. The C18K skin test appeared to be at its maximum at 24 hours in all cases except the most severe reaction which went on to become hemorrhagic at 48 hours. The other cases showed well demarcated areas of erythema and edema or induration, occasionally surrounded by a zone of erythema alone. In instances where the zone was present, the areas of both ovals were determined, but only the outer one is listed in Table II. Skin reactions were recorded by cellophane tracings of the lesions and an appraisal of the degree of erythema and induration was made to compare further the reactions in the same individual. Medication was given orally, either a week before or a week after a control test was performed. Salicylate was given as enteric coated aspirin or sodium salicylate in doses of 0.6 to 1.2 g every 4 hours, night and day, giving salicylate levels from 153 γ/ml to 534 γ/ml in a representative group of subjects. Tripelennamine (Pyribenzamine) was given in doses of 25 to 50 mg 3 times a day for a few days before and during the skin reacting period.

Tests were done 2 or 3 times in the same individual, one time without any medication and the other one or 2 times at weekly intervals with salicylate or tripelennamine administered. The results of the C18K skin tests are seen in Table II, which lists the area of the cellophane tracings of the skin lesions as determined by planimeter. Other characteristics of the lesions such as degree of erythema and induration are not listed since they did not vary appreciably from test to test in the same individual.

Thirteen subjects were tested with C18K with and without salicylate administration.

† We are grateful to Miss Mary C. Kelly and the Pelham Home for Children for their cooperation.

20 Seibert, F. B., Aronson, J. D., Reichel, J., Clark, L. T., and Long, E. R., *Am. Rev. Tuberc.*, 1934, **30**, Supp.

21 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **54**, 515.

Of these only 2 showed diminution of the area of the skin reaction of more than 0.1 square inch during aspirin administration, one of these had marked signs of salicylism and a level of 507 γ/ml. Seven individuals had no difference in area with and without salicylates and 4 had increases of more than 0.1 square inch despite comparable doses of salicylate. With tripelennamine, the C18K reaction was approximately the same as the control reaction in 5 individuals and increased 0.1 square inch or more in 4 individuals. A similar degree of variation is present when the reactions with salicylate are compared with those during tripelennamine administration. Four individuals did not show any change, 2 had lesions which diminished by more than 0.1 square inch and four showed areas which increased by more than 0.1 square inch. With the tuberculin test, 22 subjects were given aspirin; 14 showed no appreciable change from their control skin test, 5 showed a more severe reaction, and three had a less severe reaction.

Discussion. The Arthus reaction is a manifestation of the union of certain antigens with antibodies, and its severity can be related to the amount of antibody uniting with the antigen.[16,22,23,24] Since the severity of the Arthus reaction was not altered appreciably it does not appear that the drugs employed inhibit the union of antigen with antibody *in vivo*. However, the moderately diminished edema at the end of 6 hours in the salicylate treated group may be of interest in view of the demonstrated inhibition by salicylate of spreading due to hyaluronidase.[25]

Tripelennamine has been shown to exhibit a significant antihistamine action.[3,4] Since it did not lessen the severity of either lesion studied, it appears that histamine does not play a significant role in the pathogenesis of necrotizing allergic reactions.

Summary. A study was made of the effect of salicylate and of a synthetic antihistamine compound, tripelennamine hydrochloride, on necrotizing allergic reactions of the Arthus and "bacterial" types. The Arthus reaction was induced passively in rabbits by quantitative methods. "Bacterial" sensitivity to a streptococcus nucleoprotein fraction and to tuberculin was observed in human subjects. Neither type of reaction appeared to be altered by the administration of salicylate or tripelennamine. However, in the early development of the Arthus phenomenon, salicylate treated animals exhibited less edema at the site of the lesion.

[22] Opie, E. L., *J. Immunol.*, 1924, **9**, 231.

[23] Culbertson, J. T., *J. Immunol.*, 1935, **29**, 29.

[24] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1941, **40**, 127.

[25] Guerra, F., *J. Pharm. and Exp. Therap.*, 1946, **87**, 193.

16150

Further Observations on the Cultivation of Strains of Poliomyelitis Virus in Developing Eggs.*

JOHN B. ENRIGHT AND EDWIN W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, School of Medicine, Stanford University, Stanford, Calif.

In an earlier paper[1] we reported the successful cultivation of the murine SK strain in developing eggs. In the present paper we wish to summarize observations made since then on this and other strains.

Murine SK Strain. The SK strain has now

* Supported by the Howard Frost Poliomyelitis Research Fund.

[1] Schultz, E. W., and Enright, J. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 8.