plete in strongly acid solutions. We have found the titration satisfactory provided the initial pH is not below 2, the approximate pH of the di-hydrochloride of arsphenamine.

TABLE I.
Comparative Analysis of Arsphenamine by Gravimetric and Iodine Titration Methods.

Sample	% As (Grav.)	% As (I_2titr.)	Mols. I_2	Ratio G/I_2
A_1	31.57	29.81	7.555	1.059
A_2	31.65	29.84	7.543	1.061
B_1	31.17	29.55	7.584	1.055
B_2	31.31	29.54	7.548	1.060
C_1	31.55	29.70	7.532	1.062
C_2	31.40	29.67	7.560	1.058

Number of analyses to date—12.

We are able to conclude, therefore, that unless the solution is strongly acid, the reaction between arsphenamine and iodine reaches a point of equilibrium when 7.55 ± 0.03 mols. of iodine (theoretical 8.0) have been used. Consequently, by means of the conversion factor $8.0/7.55 = 1.060$ we can convert the amount of iodine actually used in a given titration into the amount which would be used theoretically if the reaction could be carried to completion, from which figure the percentage of arsenic in the sample is calculated.

The method is extremely rapid, the end point is sharp (unless the solution is too acid) using starch as an indicator, and the conversion factor has been found to be applicable to all samples of arsphenamine so far tested. Samples of arsphenamine contaminated with oxidizable sulphur compounds would, of course, give too high values for the arsenic content.

7045 P

Relation Between Colloidal Properties and Toxicity of Arsphenamine and Neoarsphenamine.*

A. BIEDERMANN, E. HANSSEN AND HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota Medical School.

Bauer,[1] Klemensiewicz,[2] Sherndal,[3] Raiziss and Gavron,[4] and others, have demonstrated that both arsphenamine and neoarsphen-

* A part of the funds used in this research were supplied by a grant from E. R. Squibb & Sons, New York; a further portion was supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

amine belong to the group of emulsoid "semi-colloids". Hirschfelder and Wright[5] studied the effects of neoarsphenamine and other semi-colloids on solutions of egg albumin and rabbit plasma and concluded that "the fact that most of these substances react strongly with proteins, producing ultramicroscopic, and in some cases macroscopic, changes in the proteins, lends strong support to the idea that anaphylactoid and febrile reactions following the injection of these substances into the blood stream, are due to definite changes brought about in the hydration and aggregation of the blood proteins".

Raiziss and Gavron determined the degree of colloidality of a few samples of arsphenamine and neoarsphenamine by means of dialysis, using parchment membranes. Such membranes are relatively impermeable, and permitted only a small fraction of the arsenical to pass through.

Dialysis experiments using both arsphenamine and neoarsphenamine with viscose and cellophane membranes of varied permeability indicate that both of these arsenicals contain particles of many sizes ranging from the true crystalloid which will pass through membranes of slight permeability to particles which must be large aggregates since they fail to pass through the most permeable membranes we have employed.

Arsphenamine HCl may be maintained in aqueous solution under nitrogen indefinitely without evidence of deterioration, since the iodine value remains constant and tests for arsenoxide were always negative. Dialysis through viscose membranes does not produce either oxidation or precipitation. Solutions of sodium arsphenamine under nitrogen are stable in the absence of a membrane. When a membrane is interposed sodium diffuses out and the arsenical precipitates within the membrane. Attempts to stabilize the solutions by dialyzing against buffered solutions containing sodium were unsuccessful. Solutions of sodium arsphenamine are, however, perfectly stable when dialyzed against a 1:10,000 solution of sodium formaldehyde sulphoxylate. Solutions of neoarsphenamine likewise develop an acid reaction, and undergo hydrolysis, oxidation

[1] Bauer, H., Chem. Abst. George Speyer Hauses, 1919, Gustave Fischer, Jena.

[2] Klemensiewicz, M. Z., *Bull. soc. chim. de France*, 1920, **27**, 820.

[3] Sherndal, A. E., *J. Lab. and Clin. Med.*, 1933, **7**, 723.

[4] Raiziss, G. W., and Gavron, J. L., *J. Pharmacol. and Exp. Therap.*, 1922, **20**, 163.

[5] Hirschfelder, A. D., and Wright, H. N., *J. Pharmacol. and Exp. Therap.*, 1930, **39**, 13.

and precipitation unless dialyzed against a very dilute solution of sodium formaldehyde sulphoxylate.

Sodium formaldehyde sulphoxylate when added in very dilute concentrations to solutions of arsphenamine HCl, sodium arsphenamine or neoarsphenamine acts as a powerful peptizing agent, markedly increasing the amount of the crystalloid fraction and decreasing the toxicity of the drug.

The crystalloid and colloid fractions of the arsenicals were obtained by dialysis through viscose sausage skin membranes (Visking Corporation, Chicago) or cellophane sheets (Dupont No. 600). Sodium arsphenamine and neoarsphenamine were dissolved in and dialyzed against dilute solutions of sodium formaldehyde sulphoxylate adjusted to pH 9.0. Water solutions of arsphenamine HCl were used. The dialysate was removed at 24 hour intervals and replaced by fresh solution. The amount of arsenical passing through the membrane decreased rapidly in each succeeding period, reaching an equilibrium in 4 to 5 days, when no more arsenical passed through the membrane. The first 24 hour dialysate, kept under nitrogen 5 days was used as the crystalloid fraction. The arsenical remaining in the dialyzing sac after 6 days dialysis was used as the colloid fraction. Toxicity studies on the whole drug were made with solutions maintained under nitrogen for 6 days. Freshly boiled distilled water, cooled under nitrogen was used. Arsphenamine solutions were alkalinized under nitrogen. All solutions were tested for arsenoxide by Rosenthal's[6] method and found negative.

The results obtained are shown in Table I.

TABLE I.

Comparative Toxicity of Crystalloid and Colloid Fractions of the Arsphenamines.

Drug	Fraction	No. Rats	Immediate deaths		Delayed deaths	
			mg./K	% deaths	mg./K	% deaths
Arsphenamine HCL	Whole drug	30			35	25
" "	Crystalloid	34			60	50
" "	Colloid	48	25	100	20	50
Sodium Arsphena-	Whole drug	32			125	75
" mine	Crystalloid	29			125	75
" "	Colloid	13	100	none		
Neoarsphenamine	Whole drug	277			375	50
"	Crystalloid	87			450	50
"	Colloid	56	35	50		

In most of the experiments the toxicity of the crystalloid fraction was less than that of the whole drug. Rats injected with fatal doses of the crystalloid fraction invariably died several days after

[6] Rosenthal, S. M., *U. S. Pub. Health Rep.*, 1932, **47**, 933.

the injection, as did those receiving the whole drug, with typical symptoms of arsenical poisoning.

Rats injected with fatal doses of the colloid fraction showed a reaction immediately after injection. The animals developed severe dyspnea, and in many cases prolonged apnea; there were clonic convulsions of the hind legs; in most cases there was a bloody exudate from the nose and mouth. Many animals died within 15 minutes, almost all within 3 hours. Animals living longer than 3 hours usually recovered.

These experiments indicate that it is the colloid fraction of these arsenicals which is responsible for the immediate toxic reactions, whereas the crystalloid fraction is less toxic than the whole drug and produces only delayed symptoms of arsenical poisoning when injected in toxic amounts.

The authors desire to express their deep appreciation to Dr. A. D. Hirschfelder for his valuable suggestions and constant interest in the progress of the work.

7046 P

Therapeutic Efficiency of Arsphenamine and Neoarsphenamine Fractions.*

E. HANSSEN AND HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota, School of Medicine.

Biedermann, Hanssen and Wright[1] have shown that arsphenamine hydrochloride, sodium arsphenamine or neoarsphenamine may be separated by means of dialysis through viscose membranes into 2 fractions, the one consisting of particles which readily pass through the membrane (crystalloid fraction), the other consisting of particles which fail to pass through the membrane after repeated dialysis until no further arsenical passes through the membrane (colloid fraction). The technic and precautions used are similar to those described in the previous paper. In all experiments with sodium arsphenamine and neoarsphenamine it was found necessary to use 1/10,000 sodium formaldehyde sulphoxylate as a stabilizing agent.

* A part of the funds used in this research were supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

[1] Biedermann, A., Hanssen, E., and Wright, H. N., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 172.