termine antibacterial activity of the compounds. The only variation from this technic was in use of *Difco "penassay"* seed agar adjusted to variable hydrogen ion concentrations and the use of distilled water instead of buffer solutions to dissolve the penicillin and test compounds. Cylinders 8 mm in diameter were used, and *S. aureus* 209P was the test organism. All tests were run in quadruplicate, and an average of the 4 zone diameters was taken for the final reading.

Discussion. A concentration of 20 mg/ml of gentisyl alcohol or of homogentisic acid was necessary to produce antibacterial activity comparable to that shown by 0.1 unit/ml of the working standard of penicillin. Brack[1] reported antibacterial activity with concentrations of gentisyl alcohol of 100 γ/cc over the pH range 5.0 to 8.5. This degree of antibacterial activity was not observed in our experiments. We did note slight activity with this concentration of gentisyl alcohol in the pH range above 8.2. With 2% gentisyl alcohol antibacterial activity was more marked at low and at high pH values. Some oxidation of the compound occurs in the assay procedure, especially in alkaline media, and is evidenced by browning of the zones of inhibition. The possibility that oxidation products might have contributed to the antibacterial activity of gentisyl alcohol noted in these cases has not been investigated. It will be noted that the pH activity curve usually observed with penicillin was obtained for control and comparative purposes in these experiments.

The various results of different laboratories might possibly be due to differences in media, test organism or to a combination of these two important factors, and they suggest that these factors may be critical in the evaluation of the antibacterial activity of gentisyl alcohol or its derivatives.

The slight antibacterial activity exhibited by homogentisic acid has not to our knowledge been described previously. This activity decreased rapidly with increase in the pH of the assay agar.

Summary. Gentisyl alcohol in our experiments was found to have only slight antibacterial activity over a broad pH range. Our results with this compound are at variance with those of Brack[1] but are in agreement with the original report of Raistrick and coworkers.[5]

Homogentisic acid was found to have a definite but slight antibacterial activity.

The effect of pH of the medium on the antibacterial activity of these compounds against *S. aureus* was studied.

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Purification of Psittacosis Virus by Alcohol Precipitation and Centrifugation.

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A number of procedures have been described in which chemical agents such as calcium phosphate,[1] alum,[2] protamine,[3] zinc and magnesium hydroxide[4] are utilized in the concentration and partial purification of influenza

1 Stanley, W. M., *Science*, 1945, **101**, 332.

2 Bodily, H. L., Corey, M., and Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 165.

3 Chambers, L. A., and Henle, W., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 481.

virus. Recent experiments were reported by Cox *et al.*[5] on the treatment of this virus with organic solvents such as methyl and ethyl alcohols and the possibility of applying the same procedure in the purification of other viruses was suggested. Accordingly, experiments were conducted to determine (1) the effects of methyl and ethyl alcohol on the viability of psittacosis virus and (2) the purification value of this method compared to simple centrifugation.

Materials and Methods. The 6BC strain of virus used in these tests was originally obtained from Dr. K. F. Meyer. Following inoculation with an allantoic passage strain of this virus, the eggs were incubated at 37°C for 4 to 5 days. They were then candled and living embryos chilled at 4°C for 12 to 18 hours, after which the allantoic fluid was harvested aseptically and used as starting material. Usually the harvest was held another 18 to 24 hours at 4°C pending the results of sterility tests.

Infectivity titrations were performed by the single dilution method, *i.e.*, the inoculation of 0.25 ml of a single dilution of each test aliquot into the yolk sacs of 30 embryonated eggs. The eggs were candled at 24 hour intervals thereafter. The LD_{50} is then calculated from the average day of death of the group of embryos.[6]

Total nitrogen determinations were made by the micro-Kjeldahl method.*

The alcohol precipitation method used was comparable to that recently described by Cox *et al.*[5] To aliquots of infected allantoic fluid, cooled to 0°C, absolute methanol or ethanol (previously chilled to –40°C or below) was added dropwise through a capillary pipette until final concentrations of from 5% to 40% by volume were reached. The mixtures were held at 0°C for approximately 3 hours and then centrifuged for 30 minutes at 3500 rpm in a No. PR-1 International Refrigerated centrifuge (0°C) equipped with an angle head rotor. The supernatant fluid was removed with a syringe and discarded. The precipitate was resuspended to original volume in 0.1 molar phosphate buffer, pH 7.4, and gently agitated on a mechanical shaker for 1 hour at room temperature. The suspension was then centrifuged at 1500 rpm for 15 minutes at room temperature in a No. I International centrifuge equipped with a horizontal rotor. The supernatant fluid, removed from the insoluble sediment, was titrated for virus activity in eggs by the method described. No pH adjustments were made.

Results. Following the completion of preliminary experiments on the behavior of psittacosis virus to the alcohol precipitation technic, tests were conducted to determine the effect of varying concentrations of methanol and ethanol on the viability of the virus.

Precipitation of allantoic fluid virus with varying concentrations of absolute methanol. To aliquots of infected allantoic fluid, previously centrifuged at 1500 rpm for 15 minutes to remove supposedly only large particles of extraneous material, absolute methanol (–40°C) was added in concentrations of from 5% to 40%, and the samples treated according to the procedure outlined. A control aliquot of virus from each starting virus suspension was held at 0°C during the period of the extraction. Upon completion of the process, each alcohol fraction was titrated along with the control for virus infectivity in embryonated eggs.

The results shown in Table I are typical of the observations made on several repeat experiments. There did not appear to be an optimum concentration of methanol between 5% and 30% which would consistently yield high recovery values of living virus as measured by infectivity tests. Although the results of tests made for mg total N/ml indicate that in most instances better than 90% of the nitrogen had been removed, the loss in infectivity titer varied from 0.2 to

[4] McKinstry, D. W., and Cox, H. R., 1945, Unpublished experiments.

[5] Cox, H. R., Van Der Scheer, J., Aiston, S., and Bohnel, E., *J. Immunol.*, 1947, **56**, 149.

[6] Golub, O. J., *J. Immunol.*, 1948, in press.

*Determinations made by Dr. Benjamin Warshowsky of the Biological Division, Camp Detrick, Frederick, Md.

TABLE I.
Precipitation of Psittacosis Virus from Allantoic Fluid with Varying Concentrations of Methanol.

Lot No.	Type of data	Control	Precipitation by absolute methanol concentration of: 5%	10%	15%	20%	25%	30%	40%
1	LD_{50} (neg. log)	6.2	6.0	5.8	5.9				<2.0
	% recovery		63	40	50				<0.1
	Mg N/ml	0.516	0.017	0.018	0.019				0.057
2	LD_{50} (neg. log)	7.0			6.6	6.5	6.8	6.5	<2.0
	% recovery				40	32	63	32	<0.1
	Mg N/ml	0.447				0.021	0.027	0.035	0.042
3	LD_{50} (neg. log)	6.3			5.8	5.8	5.9	6.0	
	% recovery				32	32	40	50	
	Mg N/ml	0.504			0.034	0.050	0.055	0.049	

TABLE II.
Comparison of Virus Recoveries by Alcohol Precipitation at Different Temperatures.

Temp. of alcohol	% conc. of precipitant	Methanol LD_{50} (neg. log)	Methanol % recovery	Methanol Mg N/ml	Ethanol LD_{50} (neg. log)	Ethanol % recovery	Ethanol Mg N/ml
−40°C	0 (control)	6.4	*100	.588	6.4	100	.588
	10	6.0	40	.012	6.0	40	.024
	25	6.5	100	.054	<2.0	<.1	.066
	40	<2.0	<.1	.090	<2.0	<.1	.064
0°C	0 (control)	6.4	100	.588	6.4	100	.588
	10	5.5	13	.050	5.6	16	.021
	25	6.0	40	.045	<2.0	<.1	.064
	40	<2.0	<.1	.070	<2.0	<.1	.051

* The control LD_{50} is given a value of 100%. The per cent recoveries of precipitated suspensions are all based on this figure. The control for both the ethanol and methanol series is the same virus suspension.

0.5 log, indicating recoveries between 63 and 32%, respectively, of the original virus material. Methanol concentrations of 40% total volume were in all cases definitely destructive to the virus.

Precipitation of allantoic fluid with absolute ethanol as compared to methanol (at −40°C and 0°C). In view of the poor recovery values obtained with methanol (−40°C), it was decided to make comparative tests using absolute ethanol as the precipitant at both −40°C and 0°C. To measured aliquots of the virus chilled to 0°C were added varying concentrations of the alcohol previously chilled to either (1) below −40°C in an acetone-dry ice bath, or (2) 0°C in an ice-water bath. The results are shown in Table II.

It can be seen that when the precipitations were performed at either temperature, a concentration of 25% ethanol was toxic, whereas fair or good recoveries were obtainable with methanol at the same concentration. At the 40% level, both alcohols resulted in a marked inactivation of the virus at either temperature. The probable greater denaturation of virus protein resulting from ethanol activity is in agreement with similar observations made by Liu *et al.*[7] on denaturation of serum proteins. The results of total nitrogen determinations indicated that similar purifications resulted whether ethanol or methanol was used.

[7] Liu, Szu-Chih, and Wu, Hsien, *Chinese J. Physiol.*, 1937, **11**, 315.

In view of the observations made by Lazarus[8] on the comparative susceptibility of psittacosis virus to trypsin, attempts were made to digest the proteins of infected allantoic fluid by preliminary treatment with this enzyme. Although the virus withstood the action of the trypsin, it was not possible to demonstrate any increased purification when the digestion was done prior to alcohol precipitation.

In the course of our studies on the mechanism of the precipitation it became increasingly evident that, at least in the case of psittacosis virus, the sedimentation of the virus during the alcohol procedure was in all probability due to centrifugation (3500 rpm/30 minutes) and not to a virus protein precipitation. It was apparent also that preliminary centrifugation of infected allantoic fluids at 1500 rpm for 15 minutes was sedimenting not only large particulate matter, but a considerable proportion of the virus as well. This was especially true when the harvested allantoic fluid contained large quantities of insoluble material. Aliquots of original infected fluid, not subjected to any preliminary centrifugation, were precipitated with absolute methanol at −76°C and centrifuged at varying speeds from 1500 to 3500 rpm for 30 minutes, after which the sediments were suspended in phosphate buffer and tested for virus activity as previously described. A second virus preparation, previously purified by centrifugation,† was also subjected to the same alcohol procedure. Aliquots of the same

TABLE III.
Comparison of Virus Recoveries by Centrifugation and by Alcohol Precipitation.

Virus	Speed of centrifugation (rpm)	Alcohol precipitation*			Centrifugation control			Uncentrifuged refrigerated control	
		LD_{50} (neg. log)	% recovery	Mg N/ml	LD_{50} (neg. log)	% recovery	Mg N/ml	LD_{50} (neg. log)	Mg N/ml
Original allantoic fluid	1500	7.1	100	.053	7.0	100	.050		
	2500	7.0	100	.052	7.5	100	.034		
	3500	7.1	100	.052	7.4	100	.052	7.0	.548
Partially purified allantoic fluid	1500	6.1	13	.017	6.1	14	.012		
	2500	6.6	40	.011	6.7	50	.011		
	3500	6.9	80	.012	6.8	63	.013	7.0	.016

* All aliquots were precipitated with 25% by volume of absolute methanol.

[8] Lazarus, A. S., and Meyer, K. F., *J. Bact.*, 1939, **38**, 121.

† Preliminary purification was accomplished as follows: original infected allantoic fluid was spun at 4000 r.p.m. for 60 minutes in a refrigerated angle-head centrifuge. The supernatant fluid was discarded and the sediment resuspended to approximately one-half the original volume in phosphate buffer, shaken with glass beads for several minutes, and centrifuged at 2000 r.p.m. for 15 minutes. The supernatant fluid was removed, spun again at 4000 r.p.m. for 60 minutes and the sediment resuspended to the original volume in buffer solution. This procedure removes most of the extraneous protein and cell debris without significant loss of active virus.

parent pools, not treated with alcohol, were included as controls.

Table III shows the results obtained in terms of the virus recovery and N content. It is apparent that when such preparations of original allantoic fluid are subjected to centrifugation alone, at speeds even as low as 1500 rpm, the virus is sedimented and recoverable in its entirety, within the limits of the error of the methods employed. Several virus samples indicated greater than 100% recovery, and it is believed that these are the results of disaggregation of virus clumps normally present in the original allantoic fluid. When a partially purified virus suspension was employed, the centrifugal force necessary to bring down virus activity was definitely increased but the addition of alcohol did not augment the recovery of virus. The N content of the partially purified virus suspension (Table III, under Refrigerated Control) is further shown to be lower than in the original allantoic fluid preparations treated with alcohol. The original fluid, which was subjected only to varying speeds of centrifugation from 1500 to 3500 rpm for 30 minutes, demonstrates as great a reduction in non-viral N as those preparations undergoing alcohol precipitation. Therefore, it must be concluded that the alcohol did not act as a virus precipitant with this agent and that the primary factor in sedimentation and purification by this procedure was the simple centrifugation employed in the process.

Summary. Experiments are reported on the effect of methanol and ethanol on the viability and purification of psittacosis virus. A comparison of this technic with differential centrifugation is presented.

The results suggest that the separation of virus from crude allantoic fluid was dependent on a non-specific adsorption of the virus on particulate matter either present originally in the allantoic fluid or brought out of solution by the alcohol. With virus suspensions first purified by centrifugation, the speed of centrifugation and not the precipitation by alcohol was the determining factor in the recovery of virus.

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Changes in Ascorbic Acid Metabolism of the Rat During Infection with *Trypanosoma hippicum*.*

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Investigation of the biochemical changes occurring in the host during trypanosomiasis has been directed primarily toward the observation of changes in carbohydrate metabolism.[1] There have been some studies, however, which indicate that the ascorbic acid metabolism of the host is affected during infection. Scheff and Csillag[2] showed that the ascorbic acid content of the liver and blood of guinea pigs is lowered during infection with *Trypanosoma brucei.* Ascorbic acid administration was reported by Perla[3] to increase the resistance of guinea pigs to infection with *T. brucei,* but not to alter the course of the infection in mice. These findings sup-

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1 VonBrand, T., *Quart. Rev. Biol.,* 1938, **13**, 41.

2 Scheff, G., and Csillag, Z., *Arch. f. exp. Path. Pharmakol.,* 1938, **188**, 467.

3 Perla, D., *Am. J. Hyg.,* 1937, **26**, 374.