lipid of the nerve sheath is penetrated relatively slowly by the amides.

Conclusions. 1. The anesthetic potencies of three anesthetics and their amide analogues have been determined and compared.

2. The amide analogues are relatively ineffective as anesthetics upon intact nerve trunks.

3. The partition coefficients between Ringer's solution and chloroform and between buffered isotonic saline of these anesthetics and their amide analogues have been determined.

4. The differences in partition coefficients between these anesthetics and their amide analogues and their pharmacological actions have been discussed.

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Application of Ion Exchange Resins to the Purification of Certain Viruses. (17639)

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The application of ion exchange resins to the separation of closely related chemical substances by adsorption or exchange of ions(1) suggested a possible means of separating viruses from the host tissues upon which they are cultivated. Since virus particles and many host tissue components may be selectively adsorbed by a number of common adsorbents depending upon the pH of the suspending medium, their separation might be effected by passage of an infected tissue suspension through a column of ion exchange resin.

Methods. Amberlite cation exchange resin, XE-64*, between 140 and 180 mesh was used. The resin was placed in a beaker fitted with a mechanical stirrer and converted from the acid to the sodium form with 4% NaOH. After thorough washing with distilled water, the resin was then converted to a mixed sodium-acid form by the gradual addition of concentrated HCl to the resin suspension until the pH of the supernatant was at the desired point between 5.8 and 8.8. Pyrex glass chromatographic columns were then charged with the resin which was supported in the columns on coarse sand and glass wool layered over perforated glass plates. The amount of wet resin employed was twice the volume of virus suspension to be purified. The charged columns were rinsed with distilled water, drained, sterilized in an autoclave and chilled before use.

The viruses used in these experiments were: Japanese encephalitis (Nakayama strain), Eastern equine encephalomyelitis (strain 86), Western equine encephalomyelitis (Rockefeller strain), and rabies virus (CVS strain) obtained from the Virus and Rickettsiae Strain Collection at the Army Medical Department Research and Graduate School. The tissue suspensions were prepared from normal and infected chick embryos and mouse brains homogenized in a Waring Blendor with sufficient phosphate-buffered physiological saline (pH 7.7) to make 5 to 20% suspensions. Following centrifugation at 2000 r.p.m. for 10 minutes to remove coarse tissue particles, aliquots of the cleared suspensions were pipetted into the resin columns. With the aid of vacuum the fluid was pulled through the columns in 15 to 30 minutes. Samples were saved from the original centrifuged suspensions and from the percolates for titration of virus infectivity and for determination of total nitrogen by the micro-Kjeldahl method. The infectivity of preparations was determined by intracerebral titrations using groups of

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* Manufactured by Rohm and Haas Co., Philadelphia, Pa.

TABLE I.
pH and Total Nitrogen Content of Tissue Suspensions Before and After Passage Through Ion Exchange Resin of Varying Supernatant pH.

20 % tissue suspension	pH of resin supernatant	pH of tissue suspension		Total N. mg/ml	
		Before	After	Before	After
Chick embryo	5.8	7.1	6.9	1.21	.08
	6.8	7.1	7.7	1.21	.13
	7.8	7.1	8.1	1.21	.23
	8.8	7.1	9.8	1.21	.20
" "	5.8	7.2	6.9	1.23	.10
	6.8	7.2	7.7	1.23	.18
	7.8	7.2	8.0	1.23	.17
	8.8	7.2	9.9	1.23	.24
Mouse brain	5.8	7.2	7.1	2.22	.02
	6.8	7.2	7.7	2.22	.20
	7.8	7.2	7.9	2.22	.35
	8.8	7.2	9.9	2.22	.65
" "	5.8	7.1	7.2	2.18	.09
	6.8	7.1	7.8	2.18	.28
	7.8	7.1	8.3	2.18	.35
	8.8	7.1	9.7	2.18	.77

5 mice for each serial 10 fold dilution performed in the usual manner with the results expressed as LD_{50}.

Results. In Table I are shown the nitrogen content and pH values of normal chick embyro and mouse brain tissue suspensions (20%) before and after passage through resin columns adjusted as described over a pH range from 5.8 to 8.8. It will be seen that in both pairs of experiments there was a reduction in the nitrogen of the percolates amounting to 65 to 99%. Greater reductions in nitrogen were obtained at the lower pH values. Nevertheless, in experiments with infected tissues, the pH was adjusted to 6.8 or higher in order to keep within the optimum pH stability zones with respect to infectivity of the viruses studied(2,3,4).

The results of experiments in which infected tissue suspensions were passed through resin columns are shown in Table II. Suspensions of Japanese encephalitis virus obtained from infected chick embryos showed a reduction of 71 to 88% in total nitrogen after one passage through resin columns, while the infective titer was not reduced significantly except in one experiment. Somewhat less purification was obtained in the case of suspensions of Eastern equine encephalomyelitis virus obtained from infected chick embryos. These showed a reduction in total nitrogen of 50 to 76% and a reduction in infectivity titer of 0.1 to 0.6 log. The fact that somewhat more nitrogen was removed from Japanese encephalitis virus suspensions than from suspensions of Eastern equine encephalomyelitis virus with little or no loss in virus infectivity may have been due to differences in size or physico-chemical characteristics of the viruses under the conditions of these experiments.

Similar results were obtained with infected mouse brain suspensions. In these experiments 63 to 93% of the original total nitrogen content was removed while the reduction in infectivity titer ranged from zero with Japanese encephalitis virus to 1.3 log in the case of Eastern equine encephalomyelitis virus.

It should be noted that the reduction in virus infectivity titer was not in all cases directly proportional to the reduction in total

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TABLE II.
Infective Titer and Total Nitrogen Content of Virus Infected Tissue Suspensions Before and After Passage Through Ion Exchange Resin.

		Infective titer		Total nitrogen		
		Log dilution		Mg/ml		
	pH of resin supernatant	Before	After	Before	After	% reduction
	From chick embryo					
Japanese encephalitis—20%	7.8	6.6	6.6	0.96	.17	82
" " "	7.8	7.3	6.5	1.15	.25	78
" " "	7.8	6.4	6.5	0.80	.10	88
" " "	7.8	6.6	6.8	1.17	.24	79
" " "	7.8	7.5	7.4	1.10	.22	80
" " "	8.8	6.6	6.5	1.17	.34	71
Eastern equine encephalomyelitis—20%	8.8	9.1	8.5	1.24	.38	69
" " " "	7.6	9.0	8.6	1.17	.59	50
" " " "	7.6	9.6	9.5	1.12	.49	56
" " " "	7.6	8.6	8.5	1.28	.31	76
	From mouse brain					
Japanese encephalitis—10%	8.8	7.4	7.5	1.12	.15	87
Eastern equine encephalomyelitis—20%	7.6	9.2	7.9	2.22	.16	93
Western equine encephalomyelitis—10%	7.6	9.0	7.8	1.27	.47	63
Rabies—5%	7.0	6.8	5.6	0.65	.17	74
" 10%	6.8	7.2	6.6	1.26	.25	80

nitrogen content. These differences may have been a result of variations in the flow rate through the columns.

Summary. Ion exchange resin techniques offer a simple and effective method for the partial purification of the neurotropic viruses which have been studied.

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Immunological Studies of Embryo-propagated Hamster-adapted Newcastle Virus in Chickens. (17640)

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A California strain of Newcastle disease virus, No. 11,914, adapted to the Syrian hamster(1), has proved effective in the form of hamster brain suspension in immunizing chickens against the virulent unmodified virus of this California strain(2), and against virulent Connecticut and Colorado strains(3). These successful immunizations prompted further experiments to test the value of this hamster-adapted strain after one passage in 8-day-old embryonated eggs. Vaccine used for this experiment was prepared from the 73rd hamster passage. Fifteen 5-week-old Syrian hamsters (average weight 40 g) were injected intracerebrally with 0.05 cc of a 10%

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