TABLE I. Norepinephrine (NE) Concentration in Rabbit Heart Various Times after Administration of Su-5864 (12.5 mg/kg I.V.).

Time, hr	No. of animals	Conc. of norepinephrine, μg/g
0	11	1.78 (1.31-2.31)
1	4	1.17 (.91-1.34)
2	2	.69 (.59- .78)
4	6	.39 (.35- .42)
18	3	.27 (.13- .48)
48	2	.85 (.80- .90)
72	2	1.13 (1.11-1.15)
120	2	1.35 (1.29-1.42)
168	2	1.98 (1.82-2.14)

Figures in parentheses represent range of values.

injection some rabbits showed signs of transient apnea and prostration. At various times after drug administration the animals were killed by intravenous injection of air or by chloroform. The tissues were analyzed for serotonin and norepinephrine by methods previously described(2,3).

Results. Norepinephrine content of rabbit heart declined progressively for about 4 hours after guanethidine administration, reaching a level 15% that of normal (Table I). The norepinephrine level remained at this low value for at least 18 hours, had partially recovered in 48 hours, and reached normal value in about 7 days. The norepinephrine level in spleen also declined about 60% in 18 hours. However, norepinephrine levels in brain or adrenal medulla were not affected. In addition, there was no decrease in level of serotonin in brain over a period of 18 hours.

Guanethidine in the cat caused a decrease in heart norepinephrine level by about 75% in 24 hours but did not lower norepinephrine level in brain or adrenal medulla.

Discussion. Guanethidine, like reserpine, produces a decline in content of tissue norepinephrine in the rabbit and cat. The catecholamine depletion observed after guanethidine (12.5 mg/kg) is slower however than after reserpine(4). The effect on norepinephrine could be due either to release of the amine or to blocking of its synthesis. Both of these possibilities are now being investigated.

The failure of guanethidine to lower content of brain norepinephrine is not surprising as the drug is unlikely to cross the blood-brain barrier readily, due to its extremely low lipid solubility (compound not extracted from buffer pH 7.4 into chloroform).

The data presented here suggest that guanethidine lowers blood pressure by producing chemical sympathectomy through depleting norepinephrine from peripheral nerve endings.

Summary. Guanethidine (Su-5864), a new hypotensive agent, depletes the norepinephrine level in the heart of rabbits and cats. It is suggested that guanethidine lowers blood pressure by producing chemical sympathectomy through depletion of norepinephrine from peripheral nerve endings.

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A Simple Method for Concentration of Live and Formaldehyde-Inactivated Poliovirus. (25703)

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Salk type vaccine has been widely used for immunization against poliomyelitis. Since antigenicity of currently used vaccine preparations is not uniformly satisfactory(1), concentration of poliovirus suspensions to be transformed into vaccine or of formalinized vaccines may provide considerably more potent immunizing agents. Several methods for

concentration of poliovirus preparations have been described. One is based on precipitation with methanol and further purification of the virus-containing material by elution, ultracentrifugation and enzyme treatment(2,3); others use chromatography on cellulose ion-exchange columns(4,5), and on calcium or aluminium phosphate gels(6,7). These procedures, mainly aimed at obtaining pure virus preparations, are not easily adaptable to large scale vaccine concentration. This communication deals with a simple single-step method for concentration and partial purification of poliovirus preparations by precipitation with salts of various bivalent cations. Both active virus and formalinized poliomyelitis vaccine can be concentrated by this method.

Materials and methods. Poliovirus suspensions were prepared in monkey kidney cell monolayers using medium 199. Crude virus preparations were passed through Seitz clarifying pads, grades K3 and K7, then filtered through Seitz sterilizing (STl) pads. These Seitz filtrates were used for precipitation with salts. Formalinized monovalent vaccine pools were prepared according to methods described earlier(8,1). Most of the work was carried out using cobalt chloride as precipitating agent. Salts of Ca^{++}, Ba^{++} and Mn^{++} were also employed. The procedure with $CoCl_2$ is outlined briefly as follows: A Seitz-filtered virus suspension is alkalinized to pH 8.8-9.0 by careful addition of NaOH (0.1 M to 2 M alkali according to initial volume processed). Cobalt chloride, 1 M solution, is added with stirring to final concentration of approximately 1 mg Co^{++}/ml virus suspension. A flocculate appears almost immediately and settles down in a few minutes. Complete clarification of the supernatant fluid is obtained after standing about 1 hour at 4°C, or by centrifugation 2-3 minutes at about 1000 rpm. The supernatant is decanted, and the precipitate (bluish in color) is taken up in small volume of McIlvaine's buffer pH 3.0 (about 4 vol. of 0.1 M acetic acid and 1 vol. of 0.2 M disodium phosphate) and dialyzed several hours against approximately 100 vol. of buffer. Degree of concentration of live virus is determined by titration in monkey kidney cell culture tubes using 6 to 8 tubes/log dilution. Concentration of formalinized vaccine is carried out similarly. However, a slightly higher initial pH (9-9.3) is required for satisfactory precipitation. Clarification of precipitate is achieved by dilution 1:4 with citric acid/phosphate buffer, pH 4.2-4.4. Partial purification and removal of Co^{++} is obtained by dialysis against citric acid buffer, pH 5.0-6.0. Degree of concentration of the antigen is determined by potency testing in guinea-pigs using the method of Gard *et al.* (9) or in chicks according to De Somer *et al.* (10) or by *in vitro* tests measuring combining capacity with neutralizing antibody(11,12).

Results reported here were obtained by using cobalt as precipitating agent in the form of 1 M $CoCl_2$ solution. However, salts of Ca^{++}, Ba^{++} and Mn^{++} gave essentially similar results in preliminary trials. Table I shows results obtained with two 1000 ml samples of type 2 poliovirus suspensions and Table II illustrates concentration of type 1 poliovirus vaccine. In both, $CoCl_2$ was used for precipitation. The method enabled concentration of live poliovirus about a thousand-fold, and that of monovalent vaccine pools, over one-hundred-fold, with only negligible losses of virus or antigen. So far, live virus preparations of types 2 (MEF-1) and 3 (Saukett) and monovalent vaccine pools types 1 (Brunhilde) and 3 were concentrated. Work is in progress to assess degree of purification of these concentrated preparations as well as the mechanism of reaction. A detailed de-

TABLE I. Concentration of Type 2 Poliovirus Suspensions Using Cobalt Chloride as Precipitating Agent.

		Before conc.	After conc.	Degree of conc.	% recovery
Sample 1	Vol (ml)	1000	1.0	1000	
	Infectivity titer*	7.4	10.3	Ca. 1000	Ca. 99
" 2	Vol (ml)	1000	5.0	200	
	Infectivity titer*	7.3	9.6	Ca. 200	Ca. 99

* Expressed as negative logarithm of 50% tissue culture infective dose end point.

TABLE II. Concentration of Type 1 Monovalent Poliomyelitis Vaccine by Cobalt Chloride.

	Before conc.	After conc.	Degree of conc.	% recovery
Vol (ml)	1000	7.0	Ca. 140	
Potency in guinea-pigs (extinction point)	2.0	4.2a*	Ca. 140	100
		3.3b*	20	15

* Expressed as negative logarithm of 50% tissue culture infective dose end point. (a) and (b) represent results of 2 separate assays of potency on same concentrate; (a) potency determined shortly after concentration; (b) potency determined after concentrated undialyzed material has been stored at 4°C for 3 mo.

scription of various methods and results will be published elsewhere.

Comments. The method may have various advantages because of its simplicity, and the results obtained seem very promising. The possibilities in use of this procedure may be as follows: a) reduction of large volumes of virus suspensions used in preparation of formalinized vaccine on an industrial scale; b) preparation of antigens for use in complement fixation or other serological reactions; c) concentration of antigen in vaccine preparations of low antigenicity and d) if concentration is accompanied by purification, removal from the vaccine of potentially harmful substances originating in destroyed monkey kidney cells. All these will have to be tested experimentally. When processing larger volumes of material, continuous flow or Sharpless centrifugation may be used instead of batch treatment. It appears that this method can be adapted with ease to concentration of other viruses grown on tissue culture.

Summary. A simple and efficient method for concentration of poliovirus preparations is described. Bivalent cations, and particularly cobalt chloride, are used as precipitating agents, and the precipitate is resuspended with acid and/or by dialysis. Concentration of both live virus preparations as well as formalinized vaccine (Salk type) may be obtained in this way. The method permits concentration of live poliovirus about one thousand-fold and that of vaccine over one-hundred-fold, with negligible losses of virus or antigen.

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