dence of gastric ulceration in those receiving cortisone as well as ethionine as compared to the less than 50% incidence in those given ethionine only, suggests that cortisone accelerates development of these lesions. Neither the mode of production of gastric lesions nor the mechanism by which cortisone influences their development, is apparent. The high rate of metabolic activity in the gastric mucosa as in the pancreas is presumably an important factor in explaining the vulnerability of these organs to injury by ethionine. Prolonged administration of adrenal corticoids(15) and hypothalamic stimulation(16) increased gastric acid and pepsin secretion, and cortisone may aggravate ulcer formation in this manner.

Summary. Typical pancreatic changes were induced by ethionine, which were not altered by addition of cortisone. The high incidence of gastric ulceration and hemorrhage found with ethionine alone was doubled in rats receiving cortisone also.

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Relationship Between Inactivation of Poliovirus by Phenol and Appearance of Ribonuclease-Labile Infectivity.* (25111)

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Gierer and Schramm(1) found that infectivity remaining after treatment of tobacco mosaic virus with phenol could be destroyed with ribonuclease in contrast to the stability to ribonuclease of original untreated virus. Similar results were subsequently reported for Mengo encephalitis virus(2), poliovirus(3,4, 5), West Nile encephalitis virus(3), Eastern equine encephalomyelitis virus(6), and Semliki Forest virus(7). In the above studies aqueous virus preparations were serially extracted with phenol. Our results of an investigation of some factors involved in use of phenol for obtaining ribonuclease-labile infectivity from poliovirus are given below.

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Materials and methods. Polioviruses. Wild type virus of the Brunhilde strain (antigenic type 1) and of the Akron strain (type 1) and the cr^i mutant(8) of Akron were used. *Reagents.* Solutions of phenol saturated with water at 0° were prepared; these were 8.03 M phenol (a) by titration with standard NaOH and (b) by quantitative organic hydroxyl determination(9). Solutions of phenol were also prepared by dissolving a weighed amount of phenol, dried over P_2O_5, to volume in high-cystine (0.20 mM L-cystine) altered Eagle's

maintenance medium ($hcAE_m$)(8). The pH was adjusted with HCl and/or NaOH while bringing to volume. Phenol concentration in such a solution was calculated from phenol determinations performed on similarly dried and weighed samples of phenol by above methods (a) and (b). Solutions of crystalline ribonuclease (Nutritional Biochemicals Corp., Cleveland, O.) at 0.10 mg/ml in water were prepared. *Treatment of virus with phenol.* Water-saturated phenol solutions were used for tests in which virus was mixed with a relatively small volume of phenol solution whereas solutions of phenol in $hcAE_m$ were used for tests in which the relative volume of phenol solution was large. In the former case phenol concentration in the reaction mixture is expressed as $\frac{100v_p}{v_p + v_v}$ per cent water-saturated phenol where v_p is volume of water-saturated phenol mixed with v_v volume of virus stock, while in the latter case the molar concentration is given; molar concentration = (0.0803) (per cent water-saturated phenol). Temperature was 0°; where reported, pH was determined immediately after starting and just before stopping the reaction. Timing was as follows: exposure was started by rapidly and thoroughly mixing virus and phenol; exposure was stopped by adding approximately equal volume of ethyl ether at 0° and mixing vigorously. The ethereal phase was discarded and the aqueous phase was extracted twice more with cold ether. Residual ether was removed with N_2. To the virus was added one-ninth volume of ribonuclease solution (see above). This was then incubated 15-30 minutes at *ca* 26°. Ribonuclease was replaced by water in the control. Kidney cells from cynomolgus monkeys (*Macaca irus*) or rhesus monkeys (*M. mulatta*) were dispersed with trypsin using essentially the technic of Youngner(10) and were grown in 60 mm diameter petri plates on lactalbumin growth medium of Melnick(11). Kidney cell cultures were maintained during viral action on altered Eagle's maintenance (AE_m) medium(8) containing L-cystine at 0.050-0.20 mM; this medium was used without agar for formation of stocks to be treated with phenol and with agar for plaque formation.

Results. Phenol concentration. In early experiments with 4% water-saturated phenol in reaction mixture great variation in the extent of drop in plaque titer from mixture to mixture was encountered. Testing a wide range of phenol concentrations indicated that, with this method of mixing the virus with small volume of concentrated phenol, 4% is a critical concentration (Fig. 1). With this method, however, some inactivation may have been due to presence in parts of mixture, during mixing, of phenol concentrations higher than the final. This was tested using the method of mixing the virus with a relatively large volume of dilute phenol in $hcAE_m$ (Table I); these data indicate that inactivation due to momentarily high phenol concentrations during mixing virus with concentrated phenol is of appreciable magnitude. (Compare Table I with Fig. 1, especially 4.97% with 5%, respectively; the somewhat greater variation from mixture to mixture encountered in Fig. 1 was probably due to (a) variation in magnitude of this special inactivation at mixing time, (b) low accuracy in measuring volume of concentrated phenol mixed with virus, and (c) variation in pH, which may have varied as much as from 5 to 7½.) The data in Table I show accurately and quantitatively the dependency of inactivation on concentration.

Reaction mixtures were sampled after exposure times of 0.5 to 240 minutes. Over this range the surviving virus fraction in a mixture was constant (Fig. 2). At pH's 4 ± 0.2, 6.2 ± 0.2, and 7.4 ± 0.2 the minimal phenol concentration required to drop the titer to 10^{-3} of the original was 0.36, 0.40, and 0.44 M, respectively (estimated from data in Table I).

Ribonuclease-labile infectivity. The low (ca $<10^{-5.5}$ of original) phenol-surviving virus fractions, but neither the original virus nor the high surviving fractions, could be inactivated by ribonuclease. This ribonuclease-labile infectivity was present 0.5 minute after start of reaction and its titer was essentially constant to at least 240 minutes after the start.

Discussion. The population of infectious Brunhilde poliovirus particles used for experi-

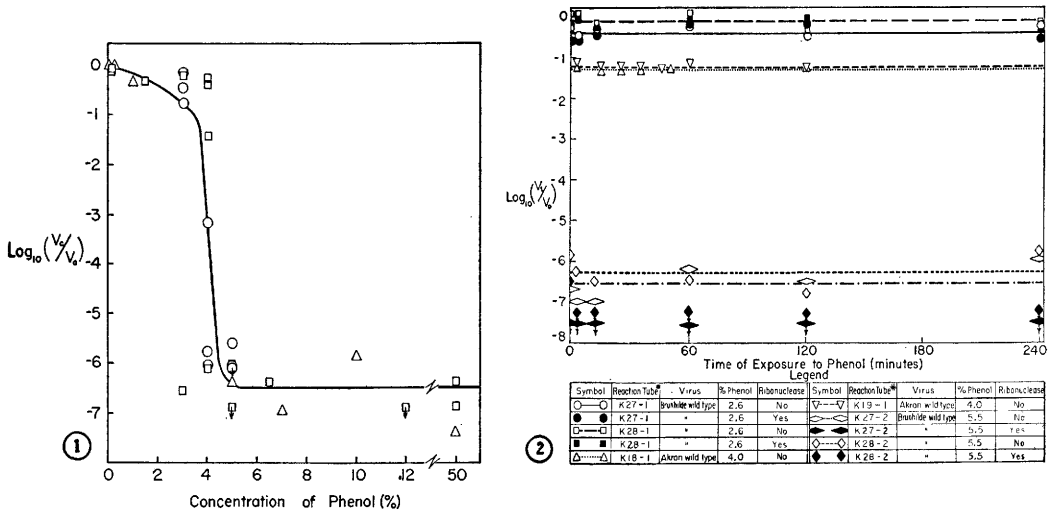

FIG. 1. Inactivation of poliovirus at 0° by phenol as a function of concentration of phenol, using the method of mixing the virus with relatively small volume of water-saturated phenol. V_c is titer (plaque-producing particles/ml) after exposure to concentration c of water-saturated phenol for 20 min.; V_o is titer without exposure to phenol. Each point represents a different reaction tube and data are from 3 experiments, △ for experiment K10 using the Akron *cr*^{1} mutant, ○ for K22 using Akron wild type virus, and □ for K26 using Brunhilde wild type. An arrow (↓) indicates that no virus was found, a point so indicated thus representing maximal titer. No ribonuclease was used. In reaction tubes with water-saturated phenol at concentrations *ca* >7% the aqueous phase was saturated with phenol (diphasic reaction mixture).

FIG. 2. Lack of dependence of inactivation of poliovirus by phenol and of titer of ribonuclease-labile infectivity present after treatment with 5.5% water-saturated phenol on time of exposure at 0° to phenol in range of about 0.5 to 240 min. The method of mixing the virus with relatively small volume of water-saturated phenol was used. V_t is titer (plaque-producing particles/ml) after time t of exposure to phenol; V_o is titer without exposure to phenol. An arrow (↓) indicates that no virus was found, a point so indicated thus representing a maximal titer.

ments summarized in Table I was heterogeneous in phenol-stability. Though no particle in this population was completely phenol-stable, some were stable at higher concentrations than were others; for example, at pH 7.4 ± 0.1 with 0.40 M phenol, about 1% of

TABLE I. The Inactivation of Poliovirus at 0° by Phenol as a Function of Concentration of Phenol and of pH of Reaction Mixture.

Phenol concentration M: Added to virus	Phenol concentration M: Final in reaction mixture	Phenol concentration % water-saturated phenol*	pH during reaction	$\text{Log}_{10}\left(\frac{V_c}{V_o}\right)$ Exp. 1	Exp. 2	Exp. 3 Tube 1	Exp. 3 Tube 2	Arithmetic mean
.298	.295	3.67	4.0 ± .2	−1.84	−1.52	−1.34	nd	−1.57
			6.2 ± .2	− .52	− .36	− .03	"	− .30
			7.4 ± .1	− .46	−1.72	−2.14	"	−1.44
.404	.399	4.97	4.0 ± .2	−4.87	−4.58	−(>5.29)	"	−(>4.91)
			6.2 ± .2	−3.72	−3.10	−2.95	"	−3.26
			7.4 ± .1	−2.07	−2.30	−1.83	"	−2.07
.500	.494	6.15	4.0 ± .2	−2.20†	−(>5.18)	−(>5.71)	−(>5.71)	−(>5.53)
			6.2 ± .2	−5.17	−(>4.97)	−(>5.71)	−(>5.70)	−(>5.39)
			7.4 ± .1	−5.35	−4.94	−(>5.71)	−5.40	−(>5.35)

* Included to facilitate direct comparison with Fig. 1 and 2.

† Probably technical error: not included for calculating mean.

nd = not done. V_c and V_o as in Fig. 1. Method of mixing the virus with relatively large vol of solution of phenol in hcAE_m was used. No ribonuclease was added. Wild type Brunhilde virus was used throughout. Controls without phenol were kept under same conditions at 3 pH's; they suffered no loss of infectivity.

the infectious Brunhilde particles were stable. If at least the major part of the phenol-stable ribonuclease-labile poliovirus infectivity is *produced* by phenol treatment, as it apparently is in the case of Mengo encephalitis virus(3), then the finding that this infectivity is present in its maximal titer (maximal for assay system employed) within not more than 0.5 minute, while the original ribonuclease-stable virus population disappears within not more than this same period of time, suggests that both this disappearance and the appearance of the phenol-stable ribonuclease-labile infectivity may be the result of a single fast reaction.

Summary. The influence of phenol concentration, time, and pH on phenolic inactivation of poliovirus at 0° was studied. A minimal phenol concentration of about 0.5 M was required for removal of all the original ribonuclease-stable poliovirus infectivity. This minimal requirement was influenced slightly by pH; it was lower at pH 4.0 than at pH 6.2 and lower at pH 6.2 than pH 7.4. Amount of inactivation was independent of time of exposure to phenol over range of 0.5 to 240 minutes. Poliovirus populations studied were heterogeneous with respect to phenol-stability, some particles requiring higher phenol concentrations than others for inactivation. Under conditions giving phenolic inactivation of all ribonuclease-stable infectivity, the ribonuclease-labile infectivity was present 0.5 minute after start of reaction and its titer remained essentially constant for at least 4 hours.

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Pharmacological Studies with Nialamide, A New Antidepressant Agent. (25112)

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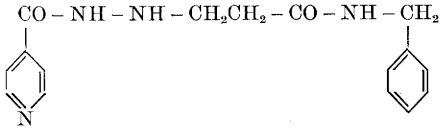
The introduction of monoamine oxidase (MAO) inhibitors as antidepressants has opened up a new concept in chemotherapy of mental disorder. As a result of favorable modification of psychic state of patients during its clinical trial as a tuberculostat, iproniazid was investigated intensively in depressed patients(1) and had marked antidepressant properties. Since side effects such as postural hypotension(2) and hepatotoxicity(3) were encountered, it was necessary to search for agents which lacked these attributes but retain the therapeutic value. From a large series of carboxamido alkyl hydrazines(4) investigated for this purpose, nialamide,*



was chosen for further studies because its out-

* Generic name of Niamid, Chas. Pfizer & Co.