antagonistic (compensatory stage), depending upon the experimental timing. From the effects of these interacting mechanisms it may be concluded that: refractoriness may be converted into susceptibility to poliomyelitis and vice versa, by factors influencing the adrenal-testis equilibrium either by direct action or by interference with pituitary function. The latter mechanism, which seems to be responsible for the effects of certain seasonal and dietary factors upon susceptibility(2,3), should be taken into consideration as one of the prospective means for disease prevention.

Summary. 1. A significant proportion of castrated male hamsters inoculated with MEF_1 virus intraperitoneally developed paralytic poliomyelitis, whereas non-castrated controls were completely refractory to the virus by this route. 2. Enhancement of susceptibility observed immediately after castration was found to be reversed during the compensatory stage 2 weeks later. 3. The effect of castration upon susceptibility to experimental poliomyelitis was synergistic with cortisone during the initial stage, but antagonistic during the compensatory stage.

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Relationship between Behavioral Changes and Brain Cholinesterase Activity Following Graded Intracerebral Injections of DFP. (22680)

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Previous studies have shown that forced circus movements may be induced by unilateral electrostimulation(1,2,3,4) or ablation (5,6,7,8) of various parts of the neuraxis. Forced circling may also be produced by unilateral intracarotid injections of the anticholinesterase diisopropylfluorophosphate (DFP)(9,10,11,12) as well as by micrometer syringe injections of DFP and CNS stimulants into specific brain structures.† It has been disclosed that the intracerebral administration of 0.01 ml solutions containing from 0.06 to 0.16 mg of DFP does not result in significant spread of this substance to adjacent cerebral gray matter.† This finding opened the possibility to determine (1) the level of cholinesterase (ChE) activity at which stimulation of specific CNS structures occurs and (2) the relationships between the dose of DFP, the level of ChE activity and the production of forced circling. The caudate nucleus was selected for this study because of its comparatively high level of ChE activity(10,12) and the known role it plays in the forced circus movements induced by micrometer injections into this structure.†

Methods. In all experiments DFP was administered into the right caudate nucleus by means of a micrometer syringe attached to a stereotaxic apparatus. Solutions of DFP were made by diluting weighed amounts with water so that injections of 0.01 ml would deliver between 0.0001 mg to 0.32 mg. In order to inject greater doses undiluted quantities of DFP were employed because DFP is immiscible in this volume of water in amounts greater than 32 mg. The dose of undiluted DFP was determined by appropriate adjustment of the micrometer syringe and in all such cases the volume was less than 0.01 ml. The entire dose range of DFP employed was from 0.0001 mg to 10 mg. Controls were obtained by injections of 0.01 ml of physiological saline. Mock injections were also made in order to determine whether DFP was de-

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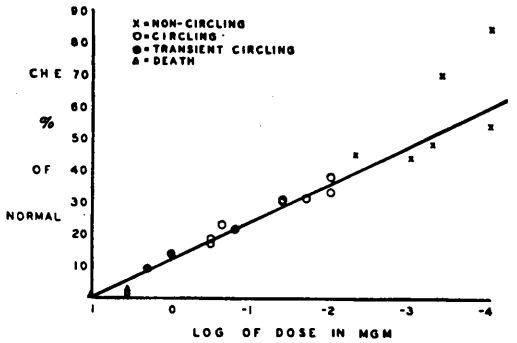

FIG. 1. Relationship between dose, % ChE activity and the behavioral response to inj. of from 0.0001 to 10 mg of DFP into the right caudate nucleus. Circling was always contraversive (*i.e.*, away from the inj. side). Note that one experimental value is shown at naught ChE activity. Also observe that 2 values for circling animals are almost superimposed.

posited in the brain merely upon insertion of the needle. To assure the delivery of solutions to exactly the point desired the bevel of a 27 gauge needle was ground off. This caused the direction taken by the drop to be parallel to the long axis of the needle. The needle was cleared with a stylus before each injection. Animals which had shown signs of bleeding during the experiment or at autopsy were discarded. Only male albino rabbits of approximately the same weight (average 2238 g) were selected for this study so that their cranial dimensions would be as uniform as possible. *e.g.*, the Bregma to Lambda skull length in all of these animals was close to 18.35 mm. A local anesthetic (0.5% pontocaine) was administered subcutaneously over the dorsum of the skull and the scalp was laid open. The animals were then lightly etherized and a hole drilled through the cranium with a dental burr 2 mm anterior to the transverse suture and 2 mm lateral to the longitudinal suture. The injection into the caudate nucleus was made at this point at a depth of 9 mm from the external skull surface. Ether administration was then stopped and the animal was released from the stereotaxic instrument and observed for at least 1 hr. The animals were then sacrificed by an intravenous injection of air and the ChE activities bilaterally determined in the caudate nuclei, dorsomedial thalami and motor cortices by the titrimetric method of Aprison, Nathan and Himwich(10). All ChE determinations were run "blind." In a second series of experiments 0.2 mg of atropine sulfate was administered with 0.16 mg of DFP and in a third one 1 mg of atropine sulfate was injected i.v. after forced circling was induced by the deposition of 0.16 mg of DFP into the nucleus caudatus.

Results. Injections of from 0.01 to 0.32 mg of DFP in solution into the caudate nucleus resulted in every case in contraversive forced circus movements which persisted for longer than 15 minutes (range from 18 min. to 1 hr. and 44 min.). As seen in Fig. 1 the ChE activity of the right caudate nucleus was below 40% of the control value in each of these circling animals. The ChE activity determinations on the left caudate nucleus, however, revealed values which were higher than 50% of normal and in general with doses of from 0.01 to 0.16 mg there was no significant evidence of spread of the injected DFP either to the opposite caudate or to the rest of the brain. With greater doses (0.23 to 0.32 mg) a spread of this substance throughout the brain was evident. Nevertheless the ChE activity values were always more than 50% of the control in parts of the brain other than the right nucleus caudatus. No other effects of DFP such as miosis or head nystagmus(9,11) were observed as a result of the intracerebral injection of this substance.

With doses of 1 and 2 mg of undiluted DFP contraversive circling was less than 8 minutes in duration and only intermittent. Doses of 3 and 10 mg did not cause circling and resulted in the death of the rabbit within 7 to 60 minutes after the intracerebral injections. In all of these animals the ChE activity in the left caudate nucleus was less than 35% of normal and in those that died it was near zero; the other brain areas in fatal cases had decreased to less than 13% of normal values.

On the other hand, injections of less than 0.01 mg of DFP did not cause any apparent change in the behavior of the animal and the ChE activity values were greater than 40% of normal. As shown in Fig. 1 the distribu-

tion of ChE activity of the right caudate nucleus in this noncircling group was greater than with the circling animals. No significant ChE activity reduction (less than 4%) resulted from the mock injections.

Addition of 0.2 mg of atropine sulfate to the injected solution of DFP prevented the appearance of forced circus movements. Moreover, the i.v. administration of 1 mg of atropine was effective in stopping the circus movements induced by previous intracerebral injections of DFP (0.04 to 0.16 mg).

Discussion. The results indicate that the optimum dose for the production of contraversive circling by intracerebral administration of DFP into the caudate nucleus is between 0.01 and 0.32 mg and that no significant spread of DFP from such injections may be expected with doses from 0.01 to 0.16 mg. Greater concentrations lead to an increasingly greater degree of spread of this substance, a finding which suggests that beyond a certain dose the quantity lost is increased progressively either by way of the circulation or cerebral spinal fluid. Because of this spread it was not possible to limit the reduction of ChE activity to a single brain structure to near zero by this method. Such low values for the caudate nucleus, however, have been reported when DFP is administered by means of the common carotid artery whereas other areas of the brain do not suffer a similar profound decrease in enzyme activity(10,13). Under these conditions electrographic(12,14) and other evidence(7) has been advanced to show that the nucleus caudatus is physiologically active. Since it was necessary to excise the entire caudate nucleus in order to obtain enough tissue for analysis, it is possible that the ChE activity of the caudate nucleus at the injection site was more reduced than reported in this study for circling animals. On the other hand, when DFP is administered intracarotidly the lenticulostriate vessels would cause a more uniform distribution of this substance to the caudate nucleus(12) and consequently an analysis of this whole structure may lead to lower values than was obtained by intracerebral injections.

This study also indicates that the ChE activity of one of the two caudate nuclei must be reduced to at least 40% of control value if circling is to be expected. There is no reason to believe that other brain areas need be affected by DFP to induce circling by intracerebral injections into this structure (see also reference†). The similarity between forced circus movements obtained in this study and those resulting from vestibular excitation with DFP(15) suggests a functional relationship between these two systems as proposed by Muskens(16). That death occurs when the total brain ChE activity is 10% of normal or less has been previously reported(17). Interestingly it occasionally took as long as an hour for massive doses of DFP to cause death when injected by micrometer syringe. This prolonged period would seem to offer a special advantage in the study of nerve gas antidotes when lethal doses are employed.

Atropine has been shown to correct the forced circus movements induced by DFP (injected via common carotid artery)(9) and it is thought therefore that DFP whether injected intracarotidly or intracerebrally has its effects by a cholinergic mechanism. The finding that intracerebral injections of DFP may induce circling in the rabbit supports the conclusions of other investigators that DFP administered intracarotidly causes circling through its central effects(13). None of the observable peripheral signs of the adversive syndrome(9,11) such as ipsiversive miosis are evident with intracerebral injections of DFP. The similarity between responses obtained with electrostimulation of different brain areas and micrometer syringe injections of DFP† reveal the possibility that this last procedure may be used as an additional method to study experimentally CNS cholinergic drugs and their antagonists.

Summary. The relationship between the amount of DFP injected into the right caudate nucleus and the resulting decreased cholinesterase activity has been investigated in regard to the appearance of forced circus movements. DFP in doses of less than 0.01 mg had no observable behavioral effect and did not lower the ChE activity to 40% of the control value. Doses of from 0.01 to 0.16

mg, however, caused persistent contraversive forced circling and decreased the cholinesterase value of the nucleus caudatus to from 21 to 40% of normal. There was no evidence of significant spread of the DFP within the brain in this group of animals. The administration of atropine sulfate either prevents or stopped these forced circus movements. Persistent forced circling could also be induced with larger doses of from 0.23 to 0.32 mg of DFP and evidence of spread of the DFP was then observed. In this group the injected nucleus caudatus showed ChE activity values of from 18 to 20% of the control whereas remaining parts of the brain revealed symmetrical reduction in ChE activity between 48 to 82% of normal. When still greater amounts of DFP were injected (1 to 10 mg) the spread of this substance progressively increased so that eventually ChE activity was decreased bilaterally and transient circling or death resulted.

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Sensitivity of *Staphylococcus aureus* to Penicillin in Various Media. (22681)

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The composition of the culture medium is of importance in testing the sensitivity of microorganisms to penicillin. The media may contain substances which react with penicillin chemically, thereby reducing its effective concentration. Substances having —SH groups such as thioglycollate, cysteine and glutathione behave in this manner(1,2,3,4). Acetic acid, too, inactivates penicillin by chemical interaction(5). Vitamins such as folic acid and vit. B_6 antagonise the effect of penicillin (6), and ascorbic acid also shows a deleterious effect(7). Glucose may diminish the effect of penicillin when added to cultures of *Staphylococcus aureus*(8,9). Furthermore, the activity of penicillin was decreased by addition of serum to the basal medium(10). On the other hand, certain substances, such as nicotinamide, exhibit synergistic effects with penicillin(8,11).

The present work deals with the effects of the addition of various carbohydrates to culture media, on the sensitivity of *Staph. aureus* to penicillin.