Effect of Chlorpromazine upon Epileptic and Normal Monkeys.* (22010)

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The widespread use of chlorpromazine in treatment of disorders affecting the nervous system of man suggested the advisability of a study of its effects upon epileptic and normal monkeys.

Method. Chronic experimental epilepsy was produced in 13 *Macaca mulatta* (3-4 kg) by the application of alumina cream to a cerebral cortical area(1). Discs containing alumina cream were applied to a principal sensorimotor area in 10 monkeys; multiple injections of alumina cream were made into an occipital cortical area in 2 monkeys and into an anterior mesial temporal area in the remaining monkey. Cerebral arteries (anterior, middle cerebral or carotid) were ligated in some monkeys in an effort to modify the clinical epilepsy produced. Chlorpromazine in doses of 2 mg/kg or 10 mg/kg was injected intravenously within one minute and the animals observed subsequently for a period of 4 hours during which behavioral, clinical and electroencephalographic studies were made. A total of 40 test injections (spaced at approximately biweekly intervals) were given to the experimental group. Clinical epileptic seizures were activated by vigorous prodding for one minute or by the intramuscular injection of small doses (16 mg/kg or less) of pentamethylenetetrazole (Metrazol®). Five normal unoperated monkeys were treated with chlorpromazine on 14 occasions and served as controls. In this group the effect of vigorous prodding as well as of intramuscular Metrazol (32 mg/kg) was similarly tested(2).

Results. Profound changes in behavior following intravenous injection of chlorpromazine occurred in both epileptic and normal monkeys. Within 1-2 minutes the animals became quieter, less aggressive, lethargic, flaccid and hypokinetic. They were soon unable to maintain a sitting or standing posture, becoming progressively more stuporous; eyelids remained drooped or closed, and increased salivation and tachypnea were frequently noted. On being touched, the monkeys would occasionally make feeble efforts to blink eyelids, open mouth or withdraw a stimulated limb. Jerking myoclonic type movements of the body, tail or extremities sometimes occurred. Epileptic monkeys occasionally exhibited a typical focal or generalized motor convulsion spontaneously during chlorpromazine treatment. Maximum behavioral changes lasted 30 to 60 minutes, subsiding gradually thereafter within the next 4 to 6 hours. Effects were generally more marked and sustained with the larger doses used.

Pronounced electroencephalographic changes occurred within 1-2 minutes after the intravenous administration of chlorpromazine. Diffusely distributed slow waves with frequency of 2 to 5 per second and maximum amplitude of 150 microvolts became dominant. Against this slow wave background, there was usually noted in epileptic monkeys a striking pattern of increased spike and sharp wave discharges (Fig. 1).

Among the epileptic monkeys treated with chlorpromazine, the intramuscular injection of 16 mg/kg or less of Metrazol was uniformly effective in provoking clinical epileptic seizures. In some animals of this group, while under the influence of chlorpromazine, a smaller dose of intramuscular Metrazol was sometimes effective in provoking a seizure than was necessary in the untreated state. Normal control monkeys exhibited no clinical epileptic seizures while under the acute influence of chlorpromazine despite the use of relatively large doses of intramuscular Metrazol (32 mg/kg).

Vigorous prodding of epileptic monkeys following chlorpromazine treatment usually resulted in the activation of clinical epileptic seizures. Normal monkeys similarly stimulated while under the effect of chlorpromazine

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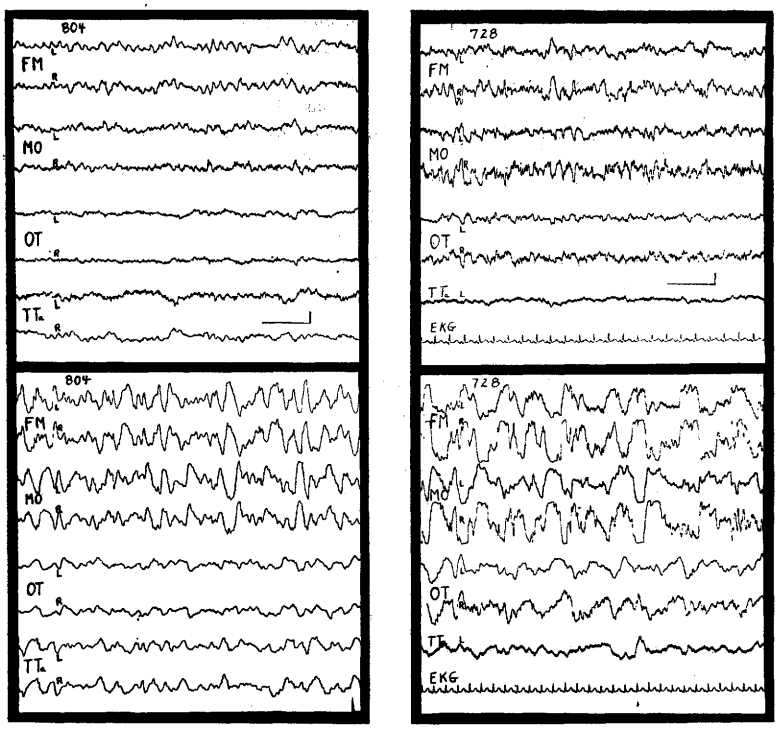

FIG. 1. Electroencephalogram before (top) and ½ hr after (below) intravenous chlorproma zine treatment (2 mg/kg). Normal monkey (804) on left; epileptic monkey (728) on right. F = frontal; M = motor; O = occipital; T = temporal; Ta = anterior temporal; EKG = electrocardiogram. Calibration: 1 sec (horizontal); 50 microvolts (vertical).

never showed evidence of clinical seizures.

Discussion. In general the behavior of the epileptic and normal monkeys following the intravenous administration of large doses of chlorpromazine did not differ significantly, and resembled that described by Das *et al.*(3) for normal monkeys. Striking EEG changes noted in chlorpromazine-treated monkeys consisted of the prompt appearance of diffuse high amplitude slow frequencies. In the epileptic monkeys, there was, in addition, increased spike and sharp wave formations. This latter effect may be similar in type to that often encountered in man, where somnolence, or natural or drug induced light sleep increases the appearance of spike discharges(4). Chlorpromazine did not protect any epileptic monkey against the seizure precipitating effect of small doses of Metrazol. Some of these animals appeared to be even more susceptible since they required a smaller threshold dose to provoke clinical seizures. Normal monkeys, however, always remained refractory to intramuscular Metrazol even with the relatively high dose of 32 mg/kg (convulsant dose for normal untreated monkeys: 48 mg/kg or greater). Significant alteration in the clinical response of epileptic monkeys to activation of seizures by vigorous prodding was not observed in these experiments.

Conclusions. 1. The rapid intravenous administration of chlorpromazine (2-10 mg/kg) to epileptic and normal monkeys produced prompt profound behavioral changes characterized by hypokinesia, flaccidity, diminished responsiveness, reduced aggressiveness, lethargy and somnolence. 2. High amplitude slow wave activity (2-5/second) became dominant in the EEG up to several hours after intravenous injection of chlorpromazine; epileptic monkeys showed, in addition, increased high amplitude spike and sharp wave forms. 3. Epileptic monkeys under the acute influence of chlorpromazine retained their clinical convulsive responsiveness to intramuscular Metrazol (16 mg/kg or less). Clinical epileptic

response to vigorous prodding was not significantly changed. 4. Normal control monkeys under the acute influence of chlorpromazine did not exhibit clinical convulsions and remained refractory to treatment with intramuscular Metrazol (32 mg/kg) and to vigorous prodding stimulation.

1. Kopeloff, L. M., Chusid, J. G., and Kopeloff, N., *Neurology*, 1954, v4, 218.

2. Chusid, J. G., Kopeloff, L. M., and Kopeloff, N., *J. Appl. Physiol.*, 1953, v6, 139.

3. Das, N. N., Dasgupta, S. R., and Werner, G., *Bull. Calcutta School Tropical Med.*, 1954, v1, 5.

4. Gibbs, F. A., and Gibbs, E. L., *Atlas of Electroencephalography*, Addison-Wesley Press, Cambridge, Mass., 1952, v2, 19.

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In vitro and *in vivo* Neutralization of the Virus of Visceral Lymphomatosis. (22011)

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It has been reported(1) that the progeny of hens which had received a series of injections of the virus of visceral lymphomatosis were much more resistant to challenge with this virus than were the progeny produced by the same hens before the injections were made. The suggestion was made that the basis for this increased resistance was the transfer of antibodies from the dam to the egg and thence to the chick. This is known to occur in other diseases of chickens.

Tests reported here provide evidence that the tumor inciting properties of the virus of visceral lymphomatosis are neutralized by specific antiserum, that serum neutralizing antibodies in chickens are produced by the injection of materials containing the live virus, and that the increased resistance of the progeny is directly related to the increase in specific serum antibodies in dams.

Materials and methods. Antisera for *in vitro* and *in vivo* neutralization tests were obtained from hens that had received a series of injections of preparations containing the virus of visceral lymphomatosis. A description of these preparations and the sequence of injections have been given(1). Normal serum for both tests was collected 6 days before the hyperimmunizing injections were begun. Immune serum for the *in vitro* test was collected from one of the hens 4 days after the last of the first series of injections. Two months later a second series of injections was given intramuscularly. The material injected was live virus preparation similar to that used in the first series. Immune serum for the *in vivo* test was collected 6 weeks after the last of the second series of injections. There was an interval of about 6 months between the first injection and the final collection of immune serum. To provide a sufficient volume of serum for the *in vivo* test the serums from 9 hens were pooled. All serums were obtained from blood collected by heart puncture. The blood was transferred to large centrifuge tubes and refrigerated over night. After centrifugation the serum was transferred to glass vials and sealed. The tubes were stored at –30°C. The source of virus used in both tests was lymphomatous livers of birds in the sixteenth serial passage of strain RPL 12(2). The chicks were from an inbred line of S.C. White Leghorns maintained in isolation for the past 13 years. The incidence of lymphomatosis in isolation has been very low; however, when naturally or artificially exposed, the stock has manifested susceptibility to the disease. *In vitro* neutralization tests were made with constant volumes and dilutions of serums mixed with 4 serial 100-fold dilutions of the virus preparation. Dilutions were made with Simms' salt solution containing 1.5% bovine albumin. Each inoculating dose of 0.2 ml contained filtrate obtained from log –3, –5, –7, and –9 g of lymphomatous liver. All serums were heated to 56°C for 30 minutes,