5. We wish to thank S. N. Timasheff of the Eastern Utilization Research Branch, U.S. Agricultural Research Service, for his valuable suggestions.
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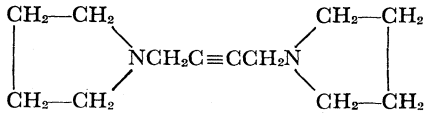
15 March 1956

## Tremor Induced by Tremorine and Its Antagonism by Anti-Parkinson Drugs

During the course of screening drugs in mice, it was observed that 1,4-dipyrrolidino-2-butyne (Tremorine)



produces a profound tremor of the head and limbs lasting for more than 1 hour. In addition, the animals move about slowly, show rigidity, and are less active. Parasympathetic stimulation is marked and characterized by profuse salivation, miosis, diarrhea, and bradycardia. A profound fall of body temperature also occurs. Sustained tremor is a rare phenomenon of drug action (in our experience, less than 10 of 10,000 drugs tested).

Because of the possible usefulness of such a drug in testing for anti-Parkinson agents, further studies have been carried out. In mice, doses of 20 mg/kg given orally, intraperitoneally, intravenously, or subcutaneously are effective, the tremors appearing within 10 to 30 minutes, depending on the route, and lasting 3 to 4 hours. Such drugs as atropine, scopolamine and other anti-Parkinson agents control the tremor and cholinergic effects completely in one dose of 2 to 10 mg/kg. They are effective either given prior to Tremorine or after full tremor effects are evident. Methantheline will control the parasympathomimetic effects but does not affect the tremor; thus the central and peripheral actions of the drug can be separated. Central depressants such as the barbiturates, mephenesin, alcohols, anticonvulsants, and analgesics are ineffective against the tremor in doses less than those causing marked ataxia, sedation, or hypnosis. Hexamethonium and TEA were also ineffective. These results emphasize the specificity of the antagonism of Tremorine tremor by the anti-Parkinson drugs in contrast to nicotine tremor which is ephemeral, has both central and peripheral components, and may be controlled by a wide variety of agents (*1*).

Tremorine in dogs has effects similar to those observed in mice. In the dog, a dose of 5 mg given intraperitoneally gives a full picture of Tremorine action. In the monkey, tremor is not as marked as it is in dogs, but it is easily seen and the Parkinsonlike facial changes are very striking. Tremorine action in the dog and monkey develops within the first hour. The tremors last for 24 hours or more and parasympathetic stimulation lasts for 2 or 3 days. Repeated doses of atropine or other anticholinergic agents will control these effects. Tremorine is often fatal in dogs and monkeys because of respiratory complications if adequate treatment is not given.

Twenty analogs of Tremorine were tested. None produced tremor. This suggests a high degree of chemical specificity for the production of these effects.

The site of action of Tremorine is under investigation. The tremor occurs in decerebrate rats, mice, and rabbits. Tremor also appeared in a chronic completely decerebellate dog, superimposed on the much coarser and slower tremor normally present in this animal (*2, 3*).

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2. This experiment was conducted in the laboratory of R. S. Snider at Northwestern University Medical School.
3. The detailed pharmacological studies on this drug are in preparation.

7 March 1956

## Another Case of Anomalous Pregnancy in a White Rat

Evans (*1*) reported a few specific instances of anomalous pregnancies in rodents and suggested that the phenomenon might be more common than a review of relevant literature would indicate. In four unequivocal cases (one in a rat, two in mice, and one in a guinea pig), the animals, although they were segregated from the time that pregnancy was observed until the time that the young were of weaning age, delivered a second litter.

In our laboratory, female rats are invariably isolated in heavily meshed individual cages as soon as pregnancy is observed. A case of anomalous pregnancy was noted in 1955, but detailed records are not available. However, another unequivocal case of anomalous pregnancy was observed and recorded in 1956. A healthy seven-pup litter was delivered by albino rat XX (Wistar strain) on 21 Jan. 1956, each member of which has survived to date. On 11 Feb. 1956, this rat produced a healthy eight-pup litter, each member of which was still alive at 16 days of age. The first litter consisted of three males and four females, and the second consisted of three males and five females.

Thus, 23 days elapsed between the production of the first and second litters. For at least 6 days before the birth of the first litter, rat XX was securely isolated, and from parturition until the birth of the second litter, it was segregated from all except its own immature offspring. With these, it shared a cage until 2 days before the birth of the second litter.

Evans discusses critically two theories explanatory of such anomalous pregnancies, namely, delayed implantation at the blastocyst stage, which is said to occur sporadically in rodents, and superfetation. Evans thought that the superfetation hypothesis was unlikely in the case of her rat, since the interval between its litters (25 days) was greater than the average gestation period. If the essential criterion for superfetation is a shorter-than-normal gestation period, the superfetation hypothesis does not seem relevant to the case of our rat, where the 23-day interval between the delivery of litters represents, according to Farris (*2*), a gestation period that is well within the normal range.

If the delayed implantation hypothesis were invoked in the case of rat XX, it would have to be assumed that the blastocysts had survived during the first pregnancy, been retained in the uterus during labor, and implanted after delivery of the first litter. Boyd and Hamilton (*3*) discuss lengthened gestation in lactating rodents in relation to physiological delay of implantation, but, in the case of our rat, the period seems improbably prolonged.

Another possibility is tentatively suggested. Rat XX was segregated 6 to 7 days before the birth of the first litter, and the total time between segregation and the birth of the second litter was thus 29 to 30 days. Farris (*2*) says that gestation may be extended a week or more if a female is carrying a large number of young and is suckling a litter. Thus, if it were possible for a pregnant female to be inseminated, this may have occurred just before segregation, and the 29 to 30-day gestation period does not constitute an improbable gestation period for a multiparous rat that is suckling a seven-pup litter. Although the sizes of the litters renders this explanation unlikely, the remote possibility that this rat may have been fertilized by way of the other uterine horn is nevertheless suggested for consideration.

It is finally suggested that the literature on parthenogenetic development in unfertilized rat eggs, as, for example, Austin and Braden (*4*), might be considered in relation to recorded cases of anomalous pregnancies.

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29 February 1956

## Pathology of Egg-Adapted Avian Encephalomyelitis

The viral etiology of a disease of chicks that is characterized by a fine tremor of the upper body, bilateral ataxia, or both was first reported by Jones (*1*) in 1932. Knowledge of avian encephalomyelitis (AE), or epidemic tremor, as summarized by Olitsky and Van Roekel (*2*), has remained limited, principally because of the difficulty of propagating the virus by intracerebral inoculation of chicks.

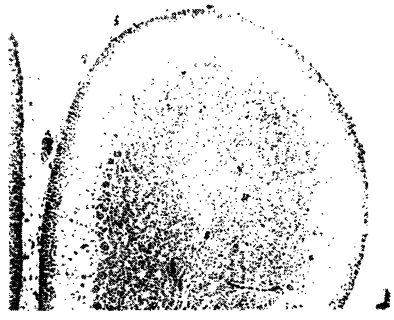

Fig. 1. Cerebellar folium of normal 20-day-old embryo (×38.7).

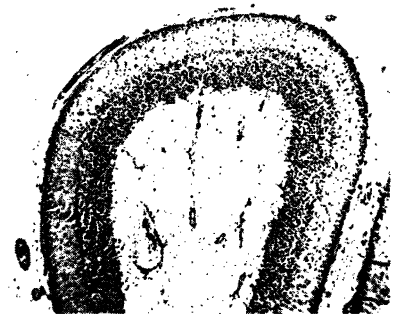

Fig. 2. Cerebellar folium of 20-day-old embryo inoculated with AE virus (×38.7). Severe edema of central white matter.

For control, wing-web vaccination of growing birds with a live chick-propagated virus has been suggested (*3*) for conferring passive immunity upon the offspring. From the academic point of view, AE virus is of interest by virtue of its relation to the polio group of viruses, in the tentative classification of Burnet (*4*), on the basis of its neurotropic character and ether resistance (*5*).

In previous studies in this laboratory (*6*) with a highly chick-adapted strain (*7*), AE virus was shown to be transmissible to adult birds by both the intraperitoneal and the intraocular route and to maintain itself in the allantoic cavity of developing chicken embryos for 6 to 7 days. Many attempts, however, to demonstrate multiplication of the virus by the egg technique have failed. In analogy with the experience in adult birds, AE virus, in the form of 0.05 ml of a 10-percent-chick-brain suspension in buffered saline at *p*H 7.2, was injected into the eye of 11-day-old embryos. Egg-adaptation was thereby achieved (*5*), as was demonstrated by serial passages in eggs, reproduction of the disease in chicks with egg-propagated virus, neutralization of the virus in eggs and chicks by AE immune serum, and specific pathologic alterations in the embryos.

Intraocular injection of embryos was made by cutting a circular window over the border of the air cell, applying a drop of sterile mineral oil to the shell membrane, and exerting a sharp jab in the direction of the eye with a 1.5-in., 20-gage needle attached to a 0.25-ml tuberculin syringe. After the window was sealed with Scotch tape, the eggs were incubated at 37°C for an additional 9 days. During this period, the eggs received no further turning, except for daily candling. Losses during the first 4 days after inoculation were considered incidental deaths and amounted to about 11 percent of the inoculated eggs.

By the use of a pooled suspension of embryonic eye and brain, the virus has undergone 11 passages by the intraocular route with an average titer of $10^{-5}$. Material from the fourth passage was also inoculated into the allantoic cavity of 9-day-old embryos and incubated for an additional 11 days. This series has undergone nine passages with an average titer of $10^{-2}$. Beginning about 6 days after inoculation, embryos inoculated by either route exhibited decreased movement and, occasionally, retardation of growth. When they were opened on the 20th day of incubation, most embryos were alive, as was established by a persistent heartbeat, but were either partially or completely immobilized. Control embryos, which were not inoculated or were inoculated by either route with buffered saline, eye-brain suspension, or allantoic fluid from normal embryos, failed to show this syndrome.

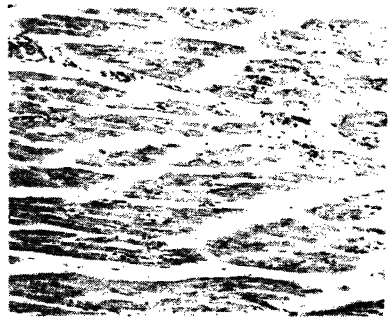

Fig. 3. Skeletal muscle of normal 20-day-old embryo (×79).

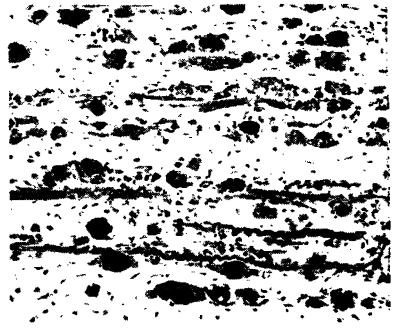

Fig. 4. Skeletal muscle of 20-day-old embryo inoculated with AE virus (×79). Severe muscular dystrophy.

Histopathologic examination of embryos in five to six coronal sections disclosed changes that were uniform in character but variable in intensity and location and consisted of encephalomalacia and muscular dystrophy. Of 48 embryos inoculated with AE virus, 45 exhibited significant alterations in the central nervous system and 43 in the skeletal musculature.

The nervous lesions were characterized by severe focal edema to the point of total destruction of the ground substance, with the margins of the lesions occasionally displaying early gliosis, vascular proliferation, and pyknosis. There was little evidence of ischemic necrosis. The anatomic areas of predilection were, in order of falling frequency, cerebellum (Figs. 1 and 2); striata; diencephalon; mesencephalon; nuclei of cranial nerves III, V, and VIII; and lumbar cord.

The muscular changes were represented by eosinophilic swelling and necrosis, fragmentation, and loss of striation of affected fibers, with rare sarcolemmal proliferation and heterophil infiltration (Figs. 3 and 4). The occipital and upper cervical musculature was chiefly involved, especially in the dorsal aspects.

Of 21 control embryos, seven had mild focal edematous lesions in the distal white matter of the central cerebellar