tailed account of these observations will be published elsewhere (*2*).

With regard to the mechanism of meiosis in triploid females, we suggest that early in oogenesis an endomitotic division takes place and produces oocytes with 84 chromosomes. Homologous chromosomes do not generally synapse. Instead, the sister chromosomes produced in the endomitosis become associated with one another by chiasmata and form 42 "pseudo-bivalents." After the two meiotic divisions, ova with 42 chromosomes are produced. Sperm from males of *A. jeffersonianum* or *A. laterale* stimulate these to develop although they do not contribute chromosomes to the triploid nucleus. The eggs develop into large-celled females with 42 chromosomes per somatic cell.

The occasional quadrivalent in the oocytes of triploid females could result from sufficient separation of sister chromosomes produced in the endomitosis to allow occasional synapsis homolog with homolog instead of sister with sister. However, the fact that no trivalents have been seen suggests that sister chromosomes resulting from the endomitosis do not separate widely.

The high incidence of chiasmata in triploid females cannot result in new genetic combinations since the chromosomes between which chiasmata form are presumably identical.

Similar meiotic and reproductive mechanisms have been described in the Lumbricidae by Muldal (*9*) and in the Enchytraeidae by Christensen and O'Connor (*10*). In those polyploid members of the Lumbricidae which reproduce parthenogenetically, Muldal (*9, 11*) has observed a premeiotic endoduplication which leads to the formation of pseudo-bivalents at first meiosis in the eggs. The chromosomes which form these pseudo-bivalents are joined to one another by chiasmata, but no multivalents have been seen. Muldal (*11*) suggests that the chromosomes are unpaired at the time of the endoduplication and that subsequent "pairing" takes place between sister chromosomes.

Christensen and O'Connor (*10*) described natural mixed populations of *Lumbricillus lineatus* consisting of diploid and triploid individuals. The triploid forms reproduce parthenogenetically, do not produce sperm, and lack seminal vesicles. Their spermathecae however, always contain spermatozoa. Sperm from diploid *L. lineatus* are necessary for the activation and normal cleavage of the eggs of triploids but do not contribute chromosomes to the triploid nucleus. Christensen and O'Connor described the relationship between triploid *L. lineatus* and diploid *L. lineatus* as "obligatory co-existence." A similar relationship would seem to prevail between the triploid females and diploid males of the *A. jeffersonianum* complex.

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14 November 1963

Gamma Hydroxybutyrate and Gamma Butyrolactone: Concentration in Rat Tissues during Anesthesia

Abstract. *Gamma-hydroxybutyric acid, when administered to animals or human beings, causes sleep. It is convertible to gamma-butyrolactone, which also produces sleep. Tissue concentrations in rats after administration of these two compounds show that the induced sleep is related to the concentration of the lactone in the brain.*

Gamma-hydroxybutyrate, a normal metabolite in brain (*1*), is apparently unique among natural intermediates in that it has anesthetic properties (*2*). Knowledge of the distribution of this compound in blood or tissues during anesthesia might be of use in understanding its mechanism of action. A primary question is whether the form of the compound directly related to sleep is the lactone or the anion. The ease with which lactone formation occurs, together with the markedly greater anesthetic properties of γ-butyrolactone (*3, 4*), suggests that the lactone might be the active form of the compound. This experiment was designed to expose any differences in the distribution and disposal of the two forms of the same compound in the tissues.

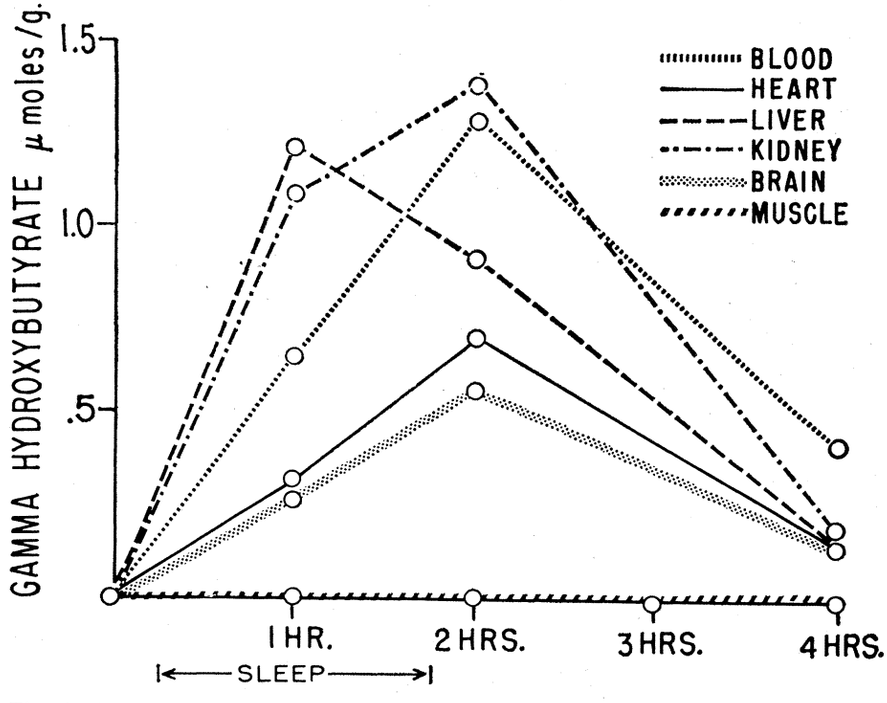

Fig. 1. Gamma-hydroxybutyrate concentration after intraperitoneal administration of sodium γ-hydroxybutyrate.

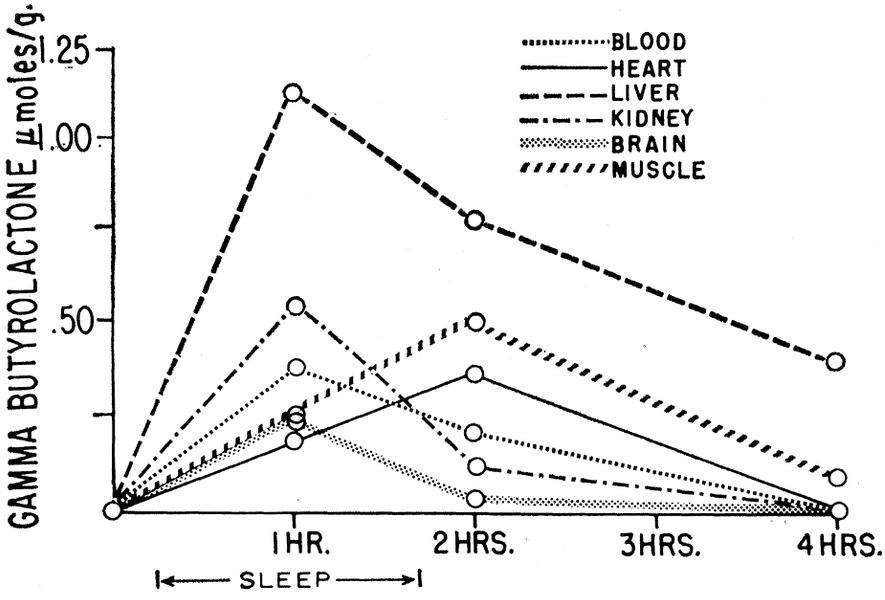

Fig. 2. Gamma-butyrolactone concentration after intraperitoneal administration of sodium hydroxybutyrate.

A solution of the sodium salt of γ-hydroxybutyric acid was prepared by addition of a stoichiometric amount of sodium hydroxide to the lactone at room temperature. A neutral solution of the lactone was also prepared. At room temperature the lactone is stable in water at *p*H 7 for at least 24 hours. Both solutions contained 50 mg of butyrolactone, or the equivalent in sodium salt, per milliliter. Eight white rats weighing 200 g each were given 2.0 ml of the lactone solution or of the sodium γ-hydroxybutyrate solution intraperitoneally (500 mg or 5.8 meq/kg). Four rats were used as controls. At hourly intervals two rats from each group were killed by a blow on the neck. In order to have pooled samples of each tissue, approximately equal amounts of blood, brain, liver, heart, muscle, and kidney from each animal were combined. The tissues were weighed, cooled, ground, and deproteinized by successive treatment with zinc sulfate and barium hydroxide solutions (*5*). The final dilution of the protein-free filtrates was 1 : 5.

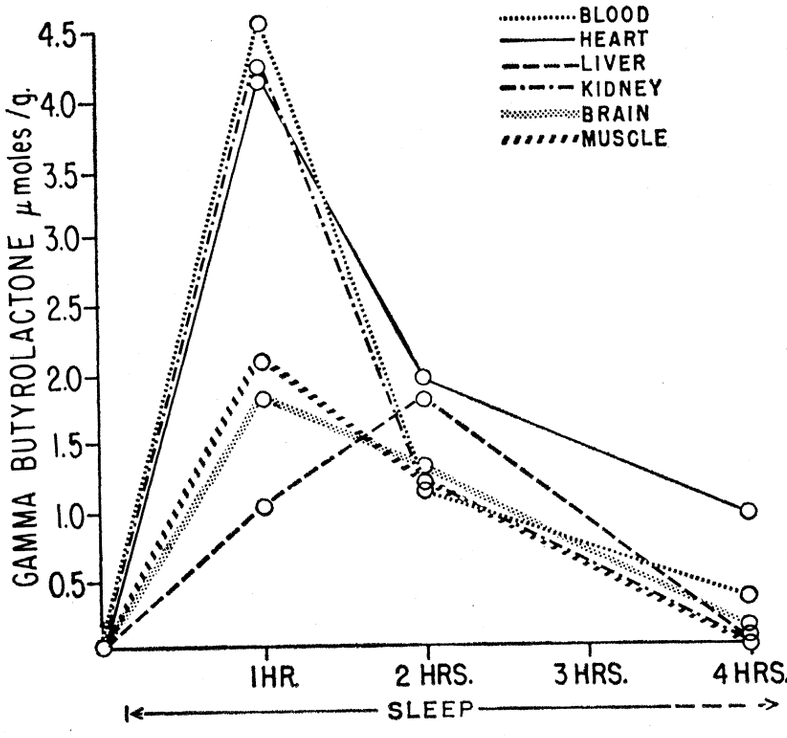

Fig. 3. Gamma-butyrolactone concentration after administration of γ-butyrolactone.

The clear filtrates were analyzed colorimetrically (*1*) for total γ-hydroxybutyric acid and lactone (Fig. 1). The color which developed in the controls was due to glucose which has a molar absorption value by this method of about 1 percent of that of γ-hydroxybutyric acid. The carbohydrate in the tissues and blood does not vary significantly after injection of either the salt or the lactone form as determined by control experiments. The control values were therefore subtracted from all subsequent values and the data represent changes in tissue content.

One hour after the γ-hydroxybutyrate was injected into the rats, approximately 30 percent of the administered acid could be accounted for by measuring the six organs and estimating the total body concentration of anion and lactone. γ-Hydroxybutyrate appeared to be concentrated in the liver initially. The anion content continued to increase until the 2nd hour in all organs except the liver. The concentration in the brain rose to about 0.2 μmole/g by the 2nd hour. Sleep, with lack of response to mild stimulation, began from 5 to 10 minutes after the intraperitoneal injection of γ-hydroxybutyrate, and the animals awoke spontaneously approximately 1 hour and 45 minutes afterward. At the 2nd hour about 40 percent of the administered dose could be accounted for, indicating continued absorption from the peritoneal cavity. By the 4th hour the concentration of anion fell to 0.2 to 0.4 m*M*.

The lactone concentrations in the various tissues after administration of acid or lactone are shown in Figs. 2 and 3. The γ-hydroxybutyrate-treated rats show significant amounts of lactone in all tissues, indicating a rapid conversion in vivo of acid to lactone. The content of γ-hydroxybutyrate and lactone in the liver is the same at 1 hour. At 4 hours there is almost no free acid. The concentration of lactone in the brain rises about 0.2 μmoles/g the 1st hour then falls by the 2nd hour. This is of interest since the animals sleep for 1 hour and 45 minutes.

Administration of lactone results in a very rapid increase of the concentration of lactone in blood, heart, and kidney; the increase is slower in the other organs (Fig. 3). The concentration in

the brain increases to about 2 μmole/g. The animals do not awaken for at least 5 hours. There is no significant change in the content of γ-hydroxybutyric acid in any tissue except brain. In the brain, the free acid concentration increases at 2 hours to 0.3 μmole/g; at 4 hours it is 0.37 μmole, about equal to the lactone which remains. In the lactone-treated animals the sleeping interval is parallel to the concentration of the lactone rather than anion in the brain, for in the 1st hour after lactone administration there is no significant rise in anion, yet the animals are asleep. On this basis it appears that the sleep-inducing properties of γ-hydroxybutyrate are related to its conversion to the lactone form in the brain. If sleep were related to the amount of γ-hydroxybutyrate in the brain, the animals given γ-hydroxybutyrate should have been asleep for more than two hours since this was the time of the highest measured values for free acid. The data, after administration either of lactone or free acid, are consistent in indicating the lactone form as the active intermediate.

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Cerebellar Ataxia in Hamsters Inoculated with Rat Virus

Abstract. *Chronic ataxia associated with hypoplasia of the cerebellum was induced by intracerebral inoculation of newborn hamsters with various strains of rat virus. The condition usually became recognizable within 3 weeks of inoculation and remained static as the animals matured. Preliminary studies suggest that hypoplasia is induced by the rat virus acting on the outer germinal layer of the cerebellum at a critical time in its ontogenic development.*

Rat virus has produced a wide spectrum of disease states in the Syrian hamster. Rat 12 strain (*1*), adapted by previous animal passages, has produced an acute, fulminant, rapidly fatal disease (*2*) in neonates, a mongoloid dwarfism (*3*) associated with periodontal disease (*4*) in older sucklings, and infections of the uterus, placenta, and fetus (*5*) in pregnant hamsters. In none of these earlier studies were infections of the central nervous system apparent.

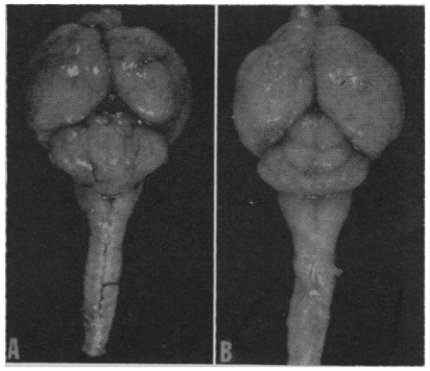

Fig. 1. The cerebellum of a normal (*A*) and an ataxic (*B*) hamster.

In more recent experiments with freshly isolated strains of rat virus inoculated intracerebrally in newborn hamsters, cerebellar ataxia has been regularly encountered. Ataxia was first observed in studies with two new strains, 171 and 312, isolated from two different lots of the Moloney leukemia virus (*6*).

Initially, neither of these strains appeared to be pathogenic for hamsters, since they produced no overt disease during the suckling period after combined intraperitoneal and intracerebral inoculation of newborn animals. It was then decided to keep the animals under observation for prolonged periods to determine whether neoplasms would develop. While no tumors appeared during periods up to 8 months, ataxia developed within 4 to 5 weeks of inoculation, and the animals remained smaller than littermates given control intracerebral injections of tissue culture fluid at birth. The ataxia was characterized by an unsteadiness of gait, quick oscillatory movements of the body and, particularly, by instability of balance. The affected animals frequently and spontaneously fell over on their backs and righted themselves with variable difficulty as they progressed around their cages. This ataxic state generally affected all inoculated littermates to the same degree, and persisted without significant change in severity as the animal matured.

The anatomic basis for the ataxia was a selective action of the rat virus upon the outer germinal layer of the cerebellum. Pathologic studies (*7*) demonstrated a lysis of these cells late in the 1st week after inoculation and, subsequently, failure of the mature cerebellar granular layer to develop. Consequently, the cerebellum presented an overall picture of severe hypoplasia, in which the neonatal appearance and relationships were retained. Characteristically, the cerebellum of the ataxic hamster appeared as a small arciform structure, above which the corpora quadrigemina were completely uncovered (Fig. 1).

During five continuous passages in rat embryo tissue culture, the virulence of the 171 and 312 strains of rat virus remained stable and continued to produce ataxia upon intracerebral inoculation. The age of the hamsters at the time of injection was a major determinant of the ability of the virus to induce the cerebellar lesion. When the inoculation was made within 24 hours of birth, virtually all animals of a litter developed ataxia. When the virus was administered on the 2nd day after birth or later, no ataxia or other manifestations of disease appeared during the observation periods which extended over 2 months. Both the 171 and 312 strains were similar to the original rat virus strain (*1*) in being resistant to exposure to 20-percent ether overnight and to heating at 60°C for 30 minutes (*6*).

After three passages in rat embryo tissue culture, and three continuous passages in hamsters (*2*), the 171 and 312

Table 1. Results of neutralization tests in which the capacities of three different strains of rat virus (RV) to induce cerebellar ataxia were tested against serum from an RV-immune hamster, after intracerebral inoculation of newborn hamsters.

Hamster serum HI titers*	RV-strain inoculated		
	171	312	13
Normal (HI = 0)	4/4†	3/3	4/4
RV-immune (HI = 1:320)	0/3	0/5	0/4

* Inhibition of hemagglutination. † Number developing ataxia/number inoculated.