Table 1. Nominal areas of satellite shadow when near center of disk. The area accepted by the image diaphragm was 4.55×10^7 km^2 on 14 December 1964.

Satellites of Jupiter	Areas (km^2)	
	Umbra	Total
Io	5.82×10^6	1.24×10^7
Europa	2.66×10^6	1.25×10^7
Ganymede	8.56×10^6	3.72×10^7
Callisto	1.71×10^6	5.00×10^7

This noise was somewhat higher than usual owing to slight drift occasioned by observation through light cirrus, but is still probably conservative in its implication of instrumental sensitivity. Accordingly, an upper bound can be placed on the brightness temperature of the shadowed region.

From straightforward geometry the nominal areas of satellite shadows when they are being cast at the center of the Jovian disk can be calculated for the Galilean satellites, and both the umbral and the umbral-plus-penumbral (total) shadows have been determined (Table 1). Because the orbital eccentricities involved are small, and because Jupiter is never far from full phase, Table 1 is an adequate representation of conditions on the night of the observations. The area projected on Jupiter of the image aperture is also shown. If f_s is the fraction of this area occupied by satellite shadow and we express the upper bound of the observation as the brightness of a normal disk background (128.5°K) plus the brightness of a blackbody at 105°K, we can write

$$B\ (T_s) = f_s^{-1}\ B\ (105^\circ) + B\ (128.5^\circ) \quad (1)$$

where T_s is the intensity-weighted average brightness temperature of the shadow. It is not known exactly whether the emission enhancement comes primarily from the umbra or whether it is well spread over the total shadow. Interpreting either way one obtains upper limit temperatures of 138° and 133.5°K, respectively. The 1962 observations of Ganymede, when similarly interpreted, gave temperatures of 184.5° and 155.5°K, while the Europa data yielded 191° and 162°K. There is absolutely no doubt of the reality of the earlier observations, as they consist of individual measurements of very high signal-to-noise ratio repeated many times. That they coincided with the position of the satellite shadows was also visually well monitored. I thus conclude that the effect was present but that it is not now present, if interpretation in terms of an infrared transparency change in Jupiter's atmosphere (*1, 2*) is not to be abandoned. On the other hand, if the hot shadow effect has an explanation based on magnetohydrodynamics, then the absence or presence of the effect may depend upon the particular satellite involved, though it is difficult to understand how this could be the case and still allow the phenomenon to be so closely associated with the intersection of the satellite shadow cone and the Jovian reflecting layer.

Explanation in terms of a time to reach equilibrium seems ruled out by insufficient variation between the orbital velocities of the Galilean satellites.

It would be interesting to see if the hot shadow effect comes and goes with the anomalous tilting of ammonia lines in Jovian spectra, an equally unexplainable phenomenon.

Note added in proof: On the night of 4 February 1965, the shadow of Europa was observed, and no enhanced emission was found. Thus the hot shadow effect is inescapably variable in time.

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1 February 1965

Botulinum Toxin, Type A: Effects on Central Nervous System

Abstract. *This study has demonstrated that type A botulinum toxin has a depressant effect on the cortical electrical activity of anesthetized and unanesthetized monkeys. Simultaneous recordings of vital signs indicated a relative lack of change in the electrocardiogram, respiration, blood pressure, and heart rate during this time. The change in the electroencephalogram appeared cyclic in nature and independent of dose or time. All animals exhibited signs of respiratory failure characterized by a gradual interference with neuromuscular transmission at the diaphragm, and subsequently died an anoxic death.*

One of the most potent active substances known to man is the toxin produced by the anaerobic bacterium *Clostridium botulinum*, which forms spores. The exceptional potency of this toxin has generated wide interest in the study of the mechanism of its toxic action (*1*).

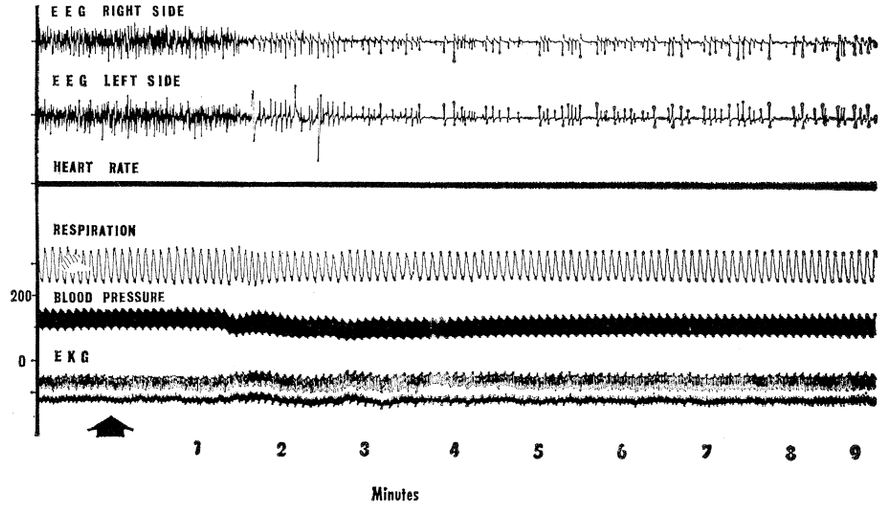

Fig. 1. Immediate effect of a lethal injection (5 LD_{50}'s) of botulinum toxin on EEG, heart rate, respiration, blood pressure, and EKG in the anesthetized monkey.

Dickson and Shevsky (*2*) have shown that botulinum toxin acts peripherally by interfering with impulse transmission at the neuromuscular junction. Possible interference with the release of acetylcholine has been reported (*3*), but the exact mechanism of this block remains obscure.

Recent clinical reports of botulinum poisoning in humans have suggested that the central nervous system is affected (*4*). Furthermore, Michailov has shown that defects in brain stem reflexes followed the administration of botulinum toxin to experimental animals (*5*). The purpose of this investigation, therefore, was to determine if the toxin has a direct action on the central nervous system and to relate any changes which might occur to those that may occur in other physiological parameters.

We used 26 monkeys of the Cynapithecoid group, *Cercocebus torquatus atys*, divided into two groups. The first group consisted of 12 monkeys anesthetized with Pentothal Sodium. Simultaneous polygraphic recordings were made of respiration and blood pressure, together with electrocardiograms (EKG), and electroencephalograms (EEG).

Respiratory rate and the chest lead of the EKG were recorded from a pair of needle-tipped electrodes placed subcutaneously in the chest wall. Blood pressure was continuously monitored by means of a Statham strain gauge and an E&M physiograph recorder. Electric activity of the brain was recorded on a model 5 Grass polygraph by means of silver wire electrodes that were placed on the dura of each cortical hemisphere.

A second group of 14 monkeys was used to study the effects of botulinum toxin on the unanesthetized intact animal. Electric activity of the brain was recorded by means of electrodes placed on the dura by standard stereotaxic techniques. Heart rate, respiration, and EKG were also monitored.

Both groups of animals received doses of type A botulinum toxin (*6*) varying from 1 to 50 LD_{50}'s (*7*). The toxin was administered intravenously by means of a catheter placed in the femoral vein. Both the anesthetized and unanesthetized monkeys exhibited marked changes in the EEG following the injection of the toxin, the changes being characterized by diminution or complete cessation of cortical activity

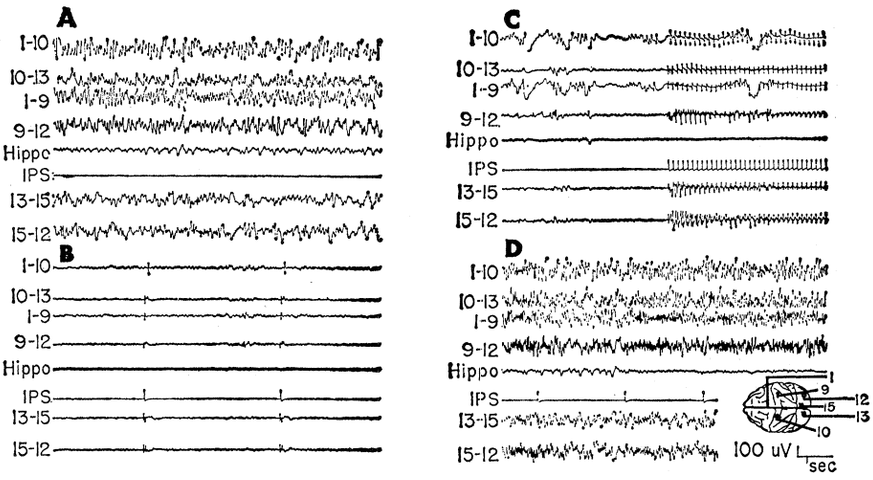

Fig. 2. Cyclic effect of a lethal injection (5 LD_{50}'s) of botulinum toxin on EEG in the unanesthetized monkey. *A*, Control; *B*, 90 seconds after injection, cortical activity ceases completely; *C*, 45 minutes after injection, cortical activity gradually returns; *D*, 7 hours after injection, cortical activity apparently returns. Hippo, hippocampus (right side); IPS, intermittent photic stimulation. Locations of electrodes in insert: 1, mid-frontal; 9, motor-sensory (right side); 10, motor-sensory (left side); 12, visual area (right side); 13, visual area (left side); 15, mid-line visual.

(Figs. 1 and 2). These initial changes consistently occurred within 5 minutes after the injection and, in the unanesthetized animals, were accompanied by loss of consciousness. No significant change in blood pressure, heart rate, EKG, or respiration was discernible during the same period in either group of monkeys (Fig. 1).

Approximately 30 to 60 minutes after the injection, a gradual return of cortical activity and consciousness was noted (see Fig. 2). The loss and recovery of this activity and the conscious state reoccurred as often as three or four times during the course of each experiment at intervals that varied from 3 to 24 hours after injection.

During the periods of EEG silence there was loss of withdrawal in response to pinch as well as loss of the lid reflex. The patellar reflex was hyperactive.

Independent of the cyclic changes in the EEG and at approximately 12 to 24 hours after the injection of the toxin all animals developed progressive respiratory difficulty, terminating in complete apnea. This respiratory failure has been characterized by interference with impulse transmission over the neuromuscular junction of the diaphragm (see *8*).

Respiratory center, phrenic nerve, and the diaphragmatic musculature all appeared relatively unaffected by botulinum toxin (*9*). The exact mechanism of this block is unknown, but the block may be due to interference with release of acetylcholine.

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6. Each gram of partially purified spray-dried toxin contains 3.3×10^9 LD_{50}'s for mice (injected intraperitoneally). Crystalline toxin contains 3.3×10^{10} $MIPLD_{50}$ per gram (mouse intraperitoneal lethal dose). Therefore, the material used to prepare the experimental working solutions was approximately 10 percent pure.
7. One monkey intravenous LD_{50} = 50 mouse intraperitoneal LD_{50}'s (mouse units). Initial concentration of toxin, as determined by mouse bioassay, was 30,000 mouse units per milliliter of phosphate-buffered gelatin. For intravenous injection this was diluted in normal saline to a final concentration of 10 mouse units per milliliter.
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10. In conducting this research the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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