(vol/vol) (*2*). Most of the globulins in the supernatant were then precipitated by addition of an equal volume of saturated (at 21°C) ammonium sulfate solution. The C′2 in the supernatant was precipitated by solid ammonium sulfate (20 g for each 100 ml of solution). The crude C′2 was dissolved in 0.2*M* KI, (1/10 original serum volume), to dissociate the rivanol-protein complexes (*3*), and the insoluble rivanol-iodide complex was separated by centrifugation. The supernatant was dialyzed against *p*H 5.8 0.01*M* phosphate–0.06*M* NaCl buffer, and chromatographed on carboxymethyl Sephadex (Fig. 1). The active fractions (approximately 0.1 percent of the original serum protein) were pooled, adjusted to *p*H 7, and concentrated by ultrafiltration. Partially purified C′2 loses activity on storage in the cold. Stability of the C′2 solutions is enhanced by addition of agents chelating heavy metals. Since it does not interfere with the Ca^{++} and Mg^{++} requirements for reaction of C′1 and C′2 respectively, calcium disodium ethylenediaminetetraacetate (EDTA) was particularly useful.

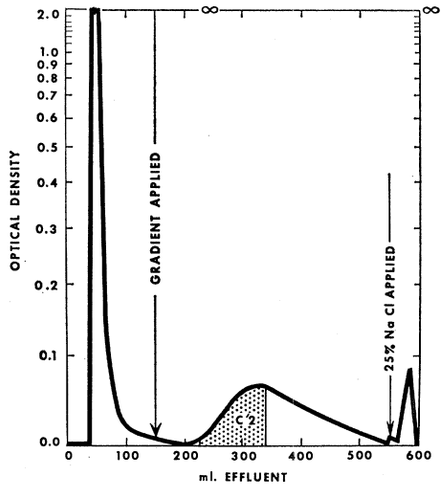

Fig. 1. Chromatography of human C′2 on carboxymethyl Sephadex. The starting buffer was *p*H 5.8, 0.01*M* phosphate–0.06*M* NaCl; the limit buffer to form linear gradient was *p*H 5.8, 0.01*M* phosphate–0.18*M* NaCl. Optical densities were measured at 280 mμ.

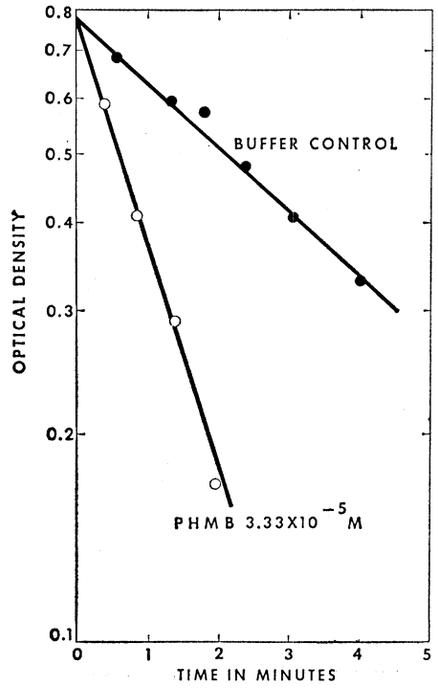

Fig. 2. Effect of PHMB on the intermediate complex EAC′1a,4,2 at 37°C. Concentration of EAC′1a,4,2 at a given time was measured by adding the sample to a source of C′3 (EDTA treated human serum containing cysteine) and measuring the hemoglobin released at 541 mμ.

The purified C′2 was incubated at 0°C for 1 hour with various concentrations of PHMB. The mixture was neutralized by cysteine, and the residual C′2 activity was determined by a modification of the method of Borsos *et al.* (*4*), the reagents being prepared from human serum rather than from guinea pig serum. Essentially all of the C′2 was inactivated by PHMB at a concentration of $5 \times 10^{-5}M$. At a concentration of $2.5 \times 10^{-5}M$ of PHMB, 51 percent of C′2 was inactivated, whereas at lower concentrations no inactivation was observed. Activity was not restored by incubation with a tenfold excess of cysteine. The reagent also inactivated C′2 bound (Fig. 2) in the complex EAC′1a,4,2 (*1*). The PHMB did not reduce the activity of either the C′1 or the C′4 bound in the complex. Although cysteine blocked the effect of PHMB on inactivation of C′2 in the complex, concentrations of cysteine as high as $5 \times 10^{-4}M$ did not affect the thermal inactivation of complexed C′2. This result suggests that PHMB causes inactivation by a mechanism different from that proposed (*5*) for the normal inactivation of C′2 in the complex. Experiments with antibody to C′2 may clarify this point.

Some experiments have shown that iodoacetamide, at concentrations up to $5 \times 10^{-4}M$, does not inactivate C′2 in solution or C′2 bound in the complex EAC′1a,4,2. This data may indicate that PHMB inactivates C′2 by reaction with groups other than sulfhydryls (*6*), or that sulfhydryl groups of differing reactivity to PHMB and iodoacetamide are present in C′2.

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References and Notes

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7. I thank A. Ransby, Jr., for technical assistance. Supported by the Elisabeth Severance Prentiss Foundation and by grant No. 02312 from NIH.

16 November 1964

Hot Shadows of Jupiter

Abstract. *On the evenings of 26 October and 15 December 1962, while the disk of Jupiter was being scanned for thermal emission in the 8- to 14-micron wavelength region, a large enhancement was discovered in the emission from shadows cast on Jupiter by the Jovian satellites Ganymede and Europa. However, on the evening of 14 December 1964, the shadow of satellite Io was observed and no enhancement was detected. The effect is thus variable with time.*

On the evening of 14 December 1964, at the east-arm Cassegrain focus of the 200-inch (508-cm) Hale telescope, I observed the shadow cast on Jupiter's surface by the innermost Galilean satellite Io. The observations were collected with an improved-model photometer of the same basic design that was used to collect previous observations of Jupiter (*1*). The photoconductor-filter thresholds were about 8 and 14 microns. The area of Jupiter's image admitted onto the radiation-sensitive cell was 2.5 seconds-of-arc across; thus, the blur from atmospheric turbulence (astronomical seeing) permitting, the resolution was better than twice that used in 1962 (*1*). Jupiter was 44 seconds-of-arc across.

The shadow was under continuous observation beginning from its ingress onto the visible disk until it reached a position quite close to the center of the disk. The shadow itself was alternated in the image diaphragm-aperture with adjacent regions of Jupiter's disk "North," "South," "East," and "West" in search of differences in signal level other than that from limb darkening. However, none was detected. Had there been a difference as large as the existing noise-level brightness temperature of 105°K, it would have been detected.

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*).

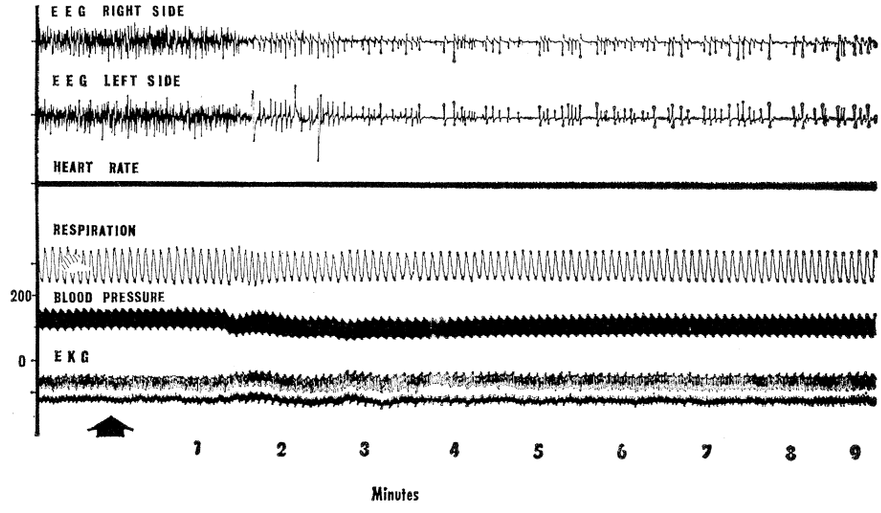

Fig. 1. Immediate effect of a lethal injection (5 LD_{50}'s) of botulinum toxin on EEG, heart rate, respiration, blood pressure, and EKG in the anesthetized monkey.