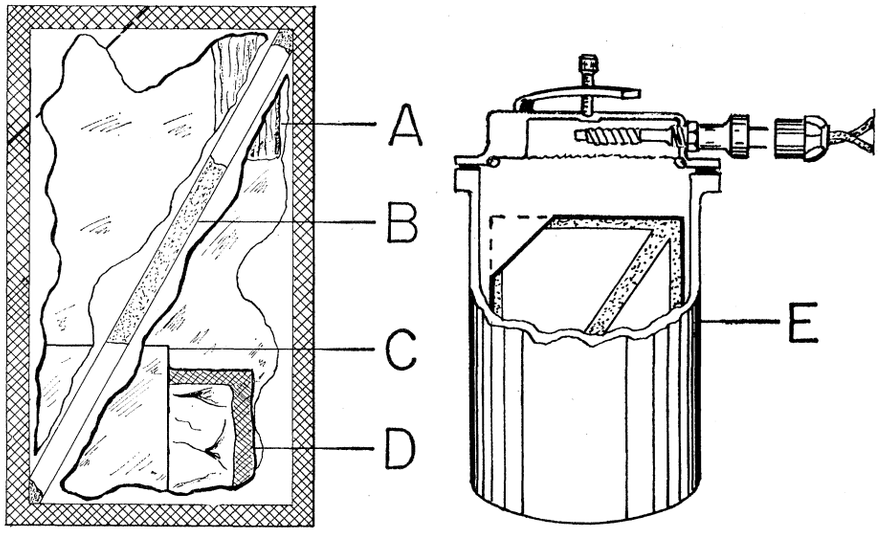

Fig. 1. (Left) A cutout view of the hydrogen generator. *A*, Coarse filter; *B*, heat seal; *C*, fine filter; *D*, absorbent paper bag containing chemicals. (Right) *E*, Position taken by generator in the anaerobe jar.

esses, such as conversion from sodium borohydride (*1*), have been used with moderate success; but expense and the special handling limit their application. We now describe a unit for the controlled generation of a determined volume of hydrogen gas sufficient to produce an effective anaerobic atmosphere within an anaerobe jar (*2*).

The unit is contained in a polyethylene, aluminum-laminated envelope of convenient size for use in the standard Brewer jar. The envelope is diagonally heat-sealed into two approximately equal compartments, and sandwiched between the seal are two pieces of filter paper as shown in Fig. 1. The lower compartment contains 5.0 g of finely divided magnesium granules, 2.5 g of sodium chloride, and 1.0 g of zinc chloride, the chemicals having been mixed in a ball mill. The mixture is sealed in an absorbent paper bag which provides for even water absorption by the contents. The overall reaction appears to proceed as follows:

$$Mg + ZnCl_2 + 2H_2O \xrightarrow{NaCl} MgCl_2 + Zn(OH)_2 + H_2$$

The reaction is initiated by cutting off the corner of the envelope and adding 10 ml of water. The water forms a pool in the upper compartment and flows slowly into the bottom compartment through an absorbent intracompartmental filter. The rate of flow may vary, and it depends on the absorbency of the paper used. Retardation of the flow serves two important functions. First, it delays the reaction for up to 60 seconds, the time required for the water to penetrate the absorbent paper, and the operator has time to cover the anaerobe jar before the reaction begins. Second, a controlled rate of flow from top to bottom compartment of the envelope is established. This is a function of penetration and is important in determining the rate of reaction and subsequent evolution of hydrogen gas. The reaction, therefore, proceeds at a controlled rate. The gas is exhausted at the cut-off corner of the envelope.

The volume of oxygen in the air contained in the jar is about 598.5 ml. A minimum of 1197 ml of hydrogen gas is required if all of the oxygen is to be consumed in the catalytic reaction. The volume of hydrogen gas produced in the first 35 minutes runs between 1400 and 1800 ml. At the end of 4 hours 2000 ml of gas is produced. All measurements were made by the water displacement technique. The platinum or palladium catalyst continues to function until all the oxygen is consumed. Owing to the chemical reaction, temperatures inside the jar range from 26°C to 30°C, thereby being suitable for immediate incubation of the cultures. This method of providing anaerobic conditions has been used in our laboratories and those of the Bacteriology Section of the Maryland State Health Department (*3*), for growing 15 species of *Clostridium*, including *Cl. novyi, Cl. botulinum, Cl. tetani, Cl. sporogenes, Cl. histolyticum*, and *Cl. perfringens*. Also, *Bacteroides fragilis, B. funduliformis, Fusobacterium fusiforme*, and a species of *Veillonella* have been isolated by this technique. Gas samples collected from the units were analyzed by gas chromatography. The only gas produced was hydrogen.

One of the advantages of the Brewer jar is that it does not need to be evacuated; thus the carbon dioxide in the atmosphere is not removed. Nor, owing to the acidic nature of the reaction, is any of the carbon dioxide present in the atmosphere absorbed. Other replacement techniques are recommended for organisms requiring as much as 10 percent carbon dioxide.

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4. We thank R. Lenham for technical assistance.

26 January 1965

Complement: Inactivation of Second Component by p-Hydroxymercuribenzoate

Abstract. p-*Hydroxymercuribenzoate inactivates the second component of complement whether it is in solution or is fixed to a sensitized erythrocyte together with the first and fourth components. Inactivation by the drug is blocked but not reversed by cysteine. Partial purification of the second component of complement is described.*

The possible role of sulfhydryl groups either in preserving the structural integrity of components of complement (C′1, C′2, etc.), or in the participation of these components in immune hemolysis, has been investigated with the reagent *p*-hydroxymercuribenzoate (PHMB). While this reagent had no significant effect on the activities of C′1, C′4, and the C′3 complex, it did inactivate the second component of complement, C′2, both in solution and when fixed to the cell in the complex EAC′1a,4,2 (*1*).

The C′2 for this investigation was obtained from human serum. The C′3 activity was removed from the serum by precipitation with 1 percent rivanol

(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.

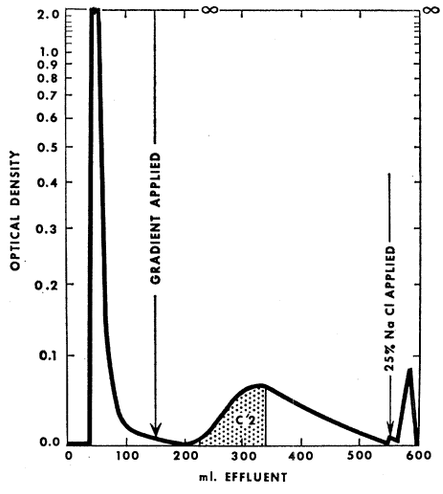

Fig. 1. Chromatography of human C′2 on carboxymethyl Sephadex. The starting buffer was *p*H 5.8, 0.01*M* phosphate–0.06*M* NaCl; the limit buffer to form linear gradient was *p*H 5.8, 0.01*M* phosphate–0.18*M* NaCl. Optical densities were measured at 280 mμ.

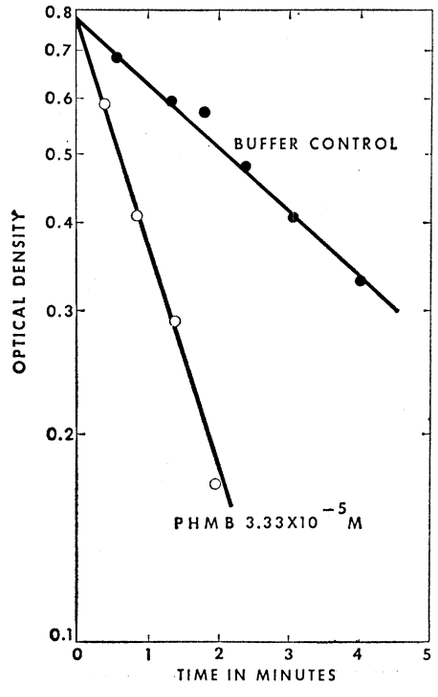

Fig. 2. Effect of PHMB on the intermediate complex EAC′1a,4,2 at 37°C. Concentration of EAC′1a,4,2 at a given time was measured by adding the sample to a source of C′3 (EDTA treated human serum containing cysteine) and measuring the hemoglobin released at 541 mμ.

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

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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.