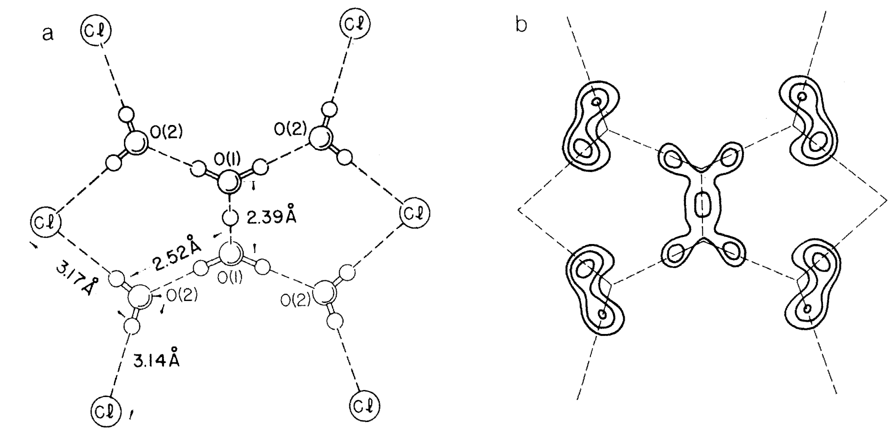

Fig. 2. (a) The $H_{13}O_6^+$ ion and the surrounding Cl^- ions. (b) Portions of a difference map, showing the electron density associated with the hydrogen atoms. Contours are drawn at 0.2, 0.3, and 0.4 electron/Å^3.

of 5.2 for $n = 951$ reflections measured and $p = 88$ parameters adjusted.

The rather high values of the agreement indexes reflect primarily our inability to obtain satisfactory representations of the C_9H_{18} groups, which are severely disordered. The $[(C_9H_{18})_3(NH)_2Cl]^+$ cation (Fig. 1) somewhat resembles three "C" clamps attached to a single object—the encapsulated Cl^- ion. It has the crystallographic symmetry $mm2$ (C_{2v}), one of the mirror planes passing through one of the clamps and the other lying perpendicular to them. However, for one of the $(CH_2)_9$ "clamping" groups to lie on a mirror plane, the conformations about two of the C–C bonds would have to be eclipsed, which would lead to rather severe H··H repulsions. Moreover, Fourier maps show regions of considerable electron density on both sides of the mirror plane, indicating that the symmetry results from a disorder involving a number of different, nonplanar conformations of the chain. The disorder is apparently very complicated, involving a large number of conformations. The model on which we finally settled requires twofold disorder for four of the chain carbon atoms and large, anisotropic thermal parameters for three others; even so, a difference map indicated residual electron density ranging up to 0.55 electron/Å^3 in some regions of this chain. The other two $(CH_2)_9$ chains were less troublesome, and we were able to obtain a fairly reasonable fit by assuming disorder for only two of the atoms in each chain.

The protonated water cluster $H_{13}O_6^+$ has crystallographic symmetry $2/m(C_{2h})$, with the central O··H··O hydrogen bond lying across a center of symmetry (Fig. 2a). The O··O distance, 2.39 ± 0.02 Å, is among the shortest such distances yet observed. It would be expected to correspond to a symmetric hydrogen bond, with the hydrogen atom located midway between the two oxygen atoms and with its potential function represented by a curve with a single minimum. A difference electron density map (Fig. 2b) supports this model; however, it cannot rule out the alternative that the hydrogen atom is disordered over two sites slightly displaced to either side of the symmetry center, and hence is better represented by a double-minimum function. As Hamilton and Ibers (*2*) have pointed out, "Clearly it is always possible to propose a degree of asymmetry that will be undetectable." But such a proposal serves little practical purpose; and in view of the extremely short O··O distance and the identical environments (due to crystallographic symmetry) of the two O(1) atoms, we believe that this cluster should be added to the list of examples of symmetric hydrogen bonds.

The O(1)··O(2) distance of 2.52 ± 0.01 Å also represents a very short hydrogen bond, but our difference map (Fig. 2b) clearly indicates that it is asymmetric, with the hydrogen atom covalently bonded to O(1). The angles at O(1) are O(1)··O(1)··O(2), 111°, and O(2)··O(1)··O(2), 132°. The O(2)–H··Cl^- hydrogen bonds are of normal length; however, they are undoubtedly vital to the stability of the $H_{13}O_6^+$ ion.

The $H_{13}O_6^+$ ion represents the largest protonated cluster of water molecules yet characterized. Examples of smaller clusters include $H_9O_4^+$ (*3*), where a central oxygen atom is surrounded by three other oxygen atoms at distances of 2.50, 2.59, and 2.59 Å; $H_7O_3^+$ (*3, 4*), a nonlinear array $H_2O··H_3O··H_2O$ with O··O distances ranging from 2.47 to 2.54 Å; and $H_5O_2^+$, which has been observed in a variety of crystals (*5*) with O··O distances ranging from 2.41 to 2.57 Å.

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Hyperbaric Hydrogen Therapy: A Possible Treatment for Cancer

Abstract. *Hairless albino mice with squamous cell carcinoma were exposed to a mixture of 2.5 percent oxygen and 97.5 percent hydrogen at a total pressure of 8 atmospheres for periods up to 2 weeks in order to see if a free radical decay catalyzer, such as hydrogen, would cause a regression of the skin tumors. Marked regression of the tumors was found, leading to the possibility that hyperbaric hydrogen therapy might also prove to be of significance in the treatment of other types of cancer.*

Over a period of years Dole and his collaborators (*1*) have studied the radiation chemistry of polyethylene (PE) and discovered a pronounced catalytic effect of molecular hydrogen in catalyzing the decay of the alkyl radicals, $-CH_2\dot{C}HCH_2-$ in the solid PE at room temperature. For example, Waterman and Dole (*2*) found that at 24°C an ambient hydrogen pressure of 400 torr increased the first order decay constant of the alkyl radical by about tenfold. Furthermore, the catalytic effect was the result of the hydrogen dissolved in the PE. Wen, Johnson, and Dole (*3*) showed that the tenfold increased catalytic activity of hydrogen at 600 torr in single crystalline mats of PE as compared to that in bulk PE was the result of a tenfold greater solubility of hydrogen in the single crystalline PE.

Free radicals have been thought to be involved in cancerous growths (*4*); we now report the effect of hydrogen gas on cancer (*5*). We chose as experimental animals hairless albino mice on whose skin squamous cell carcinomas had been produced by prolonged intermittent exposure to ultraviolet light (*6*). In a high-pressure vessel (*7*) at the steady state the gas consisted of 2.5 percent oxygen and 97.5 percent hydrogen at a total pressure of 8.28 atm. This gas composition and pressure were ideal for our work because (i) the oxygen percentage was outside the explosion limit of mixtures of O_2 and H_2, thereby eliminating a serious hazard; (ii) the oxygen partial pressure was equal to that of the atmosphere, thus permitting animals (or human beings) to live in such an atmosphere; and (iii) the high hydrogen pressure ensured a relatively large concentration of dissolved hydrogen for the catalytic effect and probably considerable penetration of the hydrogen to all parts of the body. The solubility of hydrogen is greater the higher the hydrogen partial pressure, in accordance with Henry's law.

The details of a typical experiment follow: The mice, along with food and water, were placed in the chamber, and the chamber was sealed. Inside the chamber was a CO_2 scrubber containing soda lime; circulation through the scrubber was created by a small induction motor fan; the temperature was maintained at 32°C. Pure helium was passed into the chamber over a period of 30 minutes until the internal pressure reached 8.3 atm. The chamber was then flushed with a mixture of 97.5 percent H_2 and 2.5 percent O_2 at that pressure. At this pressure the partial pressure of oxygen was equal to that of a normal atmosphere, but the percentage of oxygen was only 2.5, which is well below explosive levels. It has been shown by Dorr and Schreiner (*8*) that hydrogen and oxygen gas mixtures containing less than 5.3 percent oxygen will not burn even when an electric spark is introduced. After complete exchange of the helium by hydrogen, a flow rate was established which would just replace the metabolic utilization of oxygen.

On termination or interruption of the hyperbaric hydrogen treatment, the chamber was thoroughly flushed with a helium and oxygen mixture containing 3 percent oxygen at the pressure of 8.3 atm. After complete removal of hydrogen, the chamber was flushed with air and then slowly depressurized to 1 atm. To keep the mice from getting decompression sickness as well as distension from intestinal gas, the decompression was extended over about a 3-hour period.

In one control experiment the mice were allowed to stay in the chamber at the high pressure of the helium and oxygen mixture for 9 days in order to make sure that the observations described below were a real effect of the hydrogen and were not brought about simply by the high pressure. In each of the experiments three mice were inserted into the high-pressure chamber and three were kept as controls in their usual cage.

After a first 10-day period of exposure of the mice to the hydrogen-oxygen therapy it was found qualitatively (i) that the tumors had turned black, (ii) that some had dropped off, (iii) that some seemed to be shrunk at their base and to be in the process of being "pinched off," and (iv) that the mice appeared to suffer no deleterious consequences. Items (i), (ii), and (iii) were not observed in the case of the mice maintained either in the helium and oxygen hyperbaric experiment or in the control mice kept at room temperature and pressure.

The same mice were then kept for a second period of 6 days in the hyperbaric hydrogen-oxygen mixture, and a continual remission of the multiple squamous cell carcinomas was observed. An additional experiment with a second group of three mice in the hyperbaric hydrogen chamber for 10 days confirmed the results obtained with the first group of mice which had been exposed to the hyperbaric hydrogen atmosphere. At the same time all control animals as well as experimental animals placed in helium and oxygen mixture continued to display marked hyperplasia of the epidermis with concomitant hyperkeratosis. No evidence of any regression of the multiple carcinomas was observed, whereas in the case of the mice exposed to the hydrogen and oxygen therapy all displayed degeneration of the multiple carcinomas.

Table 1 contains data for the dimensions of a number of representative tumors as measured by a caliper on mice of the three different treatments—control mice, mice treated with helium and oxygen, and mice treated with hyperbaric hydrogen. The values in parentheses were measured after the treatment in each case. The data demonstrate the marked effect of the hyperbaric hydrogen treatment. In addition, a microscopic examination of a section of an area of the skin underneath a tumor that had fallen off showed none of the typical whorls of squamous cell carcinomas.

A general trend was observed in the animals with the greater number of lesions (> ten per animal) in that it took longer to bring about complete regression of the carcinomas than it did with those having fewer carcinomas. One might hypothesize that the hyperbaric exposure to a reducing atmosphere interferes with the respiration and metabolism of the cancer cells, functions which are different from those of normal cells. The previously overwhelmed immune system could then better cope with the squamous cell carcinomas. The limiting feature of the regression rate may be the organism's inherent ability to reject cancer cells. This possibly explains why animals with the greatest number of growths took slightly longer to bring about regression of the carcinomas.

Table 1. Dimensional changes (in centimeters) of a number of representative tumors over a 10-day period for (i) three control mice, (ii) three mice treated in the $He-O_2$ atmosphere, and (iii) three mice given the hyperbaric hydrogen treatment. Values in parentheses are those at the end of a 10-day period (9-day period for the $HE-O_2$ treated mice).

Animals (No.)	Tumors (No.)	Width	Length	Thickness	Comments
			Control mice		
1	1	1.62 (1.78)	1.19 (1.45)	1.2 (1.21)	Tumors healthy with normal appearance
	2	0.40 (0.50)	0.46 (0.43)	0.35 (0.56)	
2	1	0.97 (1.19)	0.8 (1.13)	0.38 (0.75)	
	2	0.35 (0.43)	0.31 (0.34)	0.20 (0.55)	
3	1	0.30 (0.38)	0.35 (0.43)	0.16 (0.19)	
	2	0.43 (0.41)	0.31 (0.35)	0.28 (0.45)	
			Mice treated with helium and oxygen		
1	1	0.25 (0.35)	0.47 (0.50)	0.15 (0.21)	Same as controls
	2	1.10 (1.2)	0.80 (0.70)	0.33 (0.2)	
2	1	0.27 (0.40)	0.22 (0.37)	0.14 (0.25)	
3	1	0.25 (0.5)	0.30 (0.55)	0.10 (0.22)	
	2 3	1.1 0.47 (1.83)	0.95 0.45 (1.3)	0.22 0.18 (0.25)	Tumors 2 and 3 merged
			Mice treated with hyperbaric hydrogen		
1	1	1.78 (1.7)	1.45 (0.96)	1.21 (0.2)	Tumor gone; scab healing
	2	0.23 (0.4)	0.25 (0.24)	0.20 (0.17)	Brown, necrotic, constricting at base
2	1	0.43	0.34	0.55	Tumor gone
	2	1.19 (0.68)	1.13 (0.51)	0.75 (negligible)	Scab only remaining
3	1	1.04 (0.73)	1.0 (0.60)	0.83 (0.24)	Almost fallen off
	2	0.45 (0.32)	0.42 (0.34)	0.46 (0.60)	Enlarging black scab constricted at base

These experiments represent the necessary initial observations. Whether the observed effects are permanent, whether intermittent exposure to hydrogen gas is equally effective, whether the observed results are quantitatively proportional to the hydrogen exposure time, and whether any deleterious effects are caused by the hydrogen are questions that remain to be answered. In any future work involving hyperbaric hydrogen, explosion hazards of hydrogen and oxygen mixtures should be scrupulously avoided.

The exact mechanism of the hydrogen effect should be elucidated if possible. For example, in the radiation chemistry studies mentioned above, no hydrogen catalytic effect could be observed on the decay of the allyl-type free radicals in irradiated PE, such as $-CH_2CHCH{=}CHCH_2-$, or on the decay of the $-CH_2OCHOCH_2-$ free radical in irradiated polyoxymethylene. The possibility exists that the hydrogen effect observed here is the result of a completely different mechanism. For example, the hydrogen might act to scavenge the $\cdot OH$ radical by means of the exothermic reaction

$$H_2 + \cdot OH \rightarrow H_2O + H\cdot \quad (\Delta E \sim -12\ \text{kcal/mole})$$

followed by the $H\cdot$ radical scavenging the O_2^- radical ion by the reaction

$$H\cdot + O_2^- \rightarrow HO_2^-$$

This sequence of reactions might prevent the reaction of O_2^- with H_2O_2, which Fridovich (*9*) has described as "the most damaging reaction that O_2^- can undergo" because this reaction results in the formation of the $\cdot OH$ radical, "the most potent oxidant known to mankind" (*9*).

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Implication of *Phlebotomus* Sand Flies as Vectors of Bartonellosis and Leishmaniasis as Early as 1764

Abstract. *A written account implicating* Phlebotomus *sand flies as vectors of Carrion's disease and cutaneous leishmaniasis in Peru was published by Cosme Bueno in 1764. Bueno's report precedes other publications implicating sand flies in the transmission of human pathogens by nearly a century and a half.*

Evidence that *Phlebotomus* sand flies transmit *Bartonella bacilliformis* (Carrion's disease) and *Leishmania* spp. remained circumstantial for many years. During the present century these minute flies were initially incriminated as vectors of Carrion's disease in 1913 (*1*). *Bartonella bacilliformis* was transmitted experimentally in 1928 to *Macaca mulata* by exposing the monkey to wild-caught sand flies collected in an area where the disease was endemic (*2*). The first published reports suggesting *Phlebotomus* sand flies as potential vectors of human pathogens (*Leishmania tropica* and sand fly fever virus) appeared in 1905 (*3–5*).

A recent note by Gooneratne (*6*) quoted an 1884 report by Mitford (*7*) on cutaneous leishmaniasis (Aleppo boil) in the Middle East; the disease was thought to be caused by "some mineralogical impregnation of the water, or some minute insect that inhabits it." Although in this case the possible participation of some insect was considered, its exact role in the transmission of the Aleppo boil was not clearly indicated. The first solid evidence that sand flies were involved in the epidemiolo-

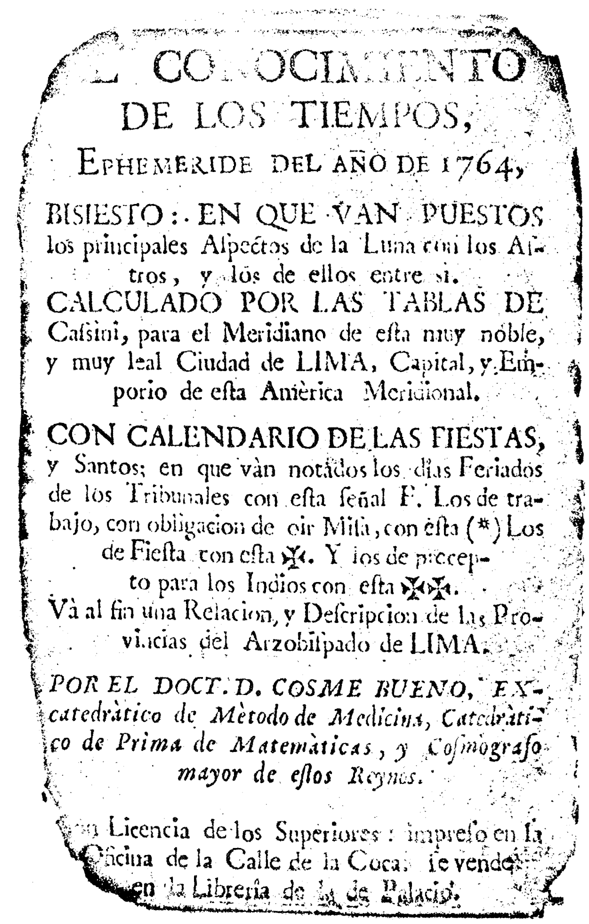
[E]L CO[N]OCI[MIE]NTO
DE LOS TIEMPOS,
EPHEMERIDE DEL AÑO DE 1764,
BISIESTO: EN QUE VAN PUESTOS
los principales Aſpectos de la Luna con los Aſtros, y los de ellos entre si.
CALCULADO POR LAS TABLAS DE
Caſsini, para el Meridiano de eſta muy noble, y muy leal Ciudad de LIMA, Capital, y Emporio de eſta Amèrica Meridional.
CON CALENDARIO DE LAS FIESTAS,
y Santos; en que vàn notados los dias Feriados de los Tribunales con eſta ſeñal F. Los de trabajo, con obligacion de oir Miſa, con eſta (*) Los de Fieſta con eſta ✠. Y los de precepto para los Indios con eſta ✠✠.
Và al fin una Relacion, y Deſcripcion de las Provincias del Arzobiſpado de LIMA.
POR EL DOCT. D. COSME BUENO, Excatedràtico de Mètodo de Medicina, Catedràtico de Prima de Matemàticas, y Coſmografo mayor de eſtos Reynos.
Con Licencia de los Superiores: impreſo en la Oficina de la Calle de la Coca; ſe vende en la Libreria de ... de Palacio.

Fig. 1. Front cover of *El Conocimiento de los Tiempos*, a kind of almanac published in Lima, Peru, under the direction of Cosme Bueno during the 18th century. A single copy of this publication is available in the Biblioteca Nacional, Lima. This copy was partially burned during a fire on 10 May 1943.