mixture to induce even a faint blue color.

The vestibular contents have an immediate dissociating effect on mandelonitrile, but not on amygdalin. No blue color developed when droplets of benzidine reagent, applied to filter paper, were allowed to spread toward adjacent droplets of mandelonitrile, or of amygdalin in concentrated or dilute aqueous solution. But when drained vestibule contents were interposed between reagent and mandelonitrile, so that the reagent as it spread eluted the secretion and carried it toward the mandelonitrile; then a blue color developed instantly on contact. Under the same circumstances, amygdalin showed no signs of dissociation. In its proven susceptibility to immediate dissociation by the contents of the vestibule, the precursor in the reservoir again resembles mandelonitrile rather than the glucoside.

The discharged secretion is an emulsion. The continuous phase is miscible with water, turns blue cobaltous chloride paper pink, and stains blue with benzidine reagent. It is probably an aqueous solution of hydrogen cyanide. The clear droplets of the discontinuous phase presumably contain benzaldehyde (pink stain with Schiff's reagent) together with residual undissociated mandelonitrile and some hydrogen cyanide. The liquid in the reservoir is also an emulsion, the discontinuous phase of which presumably contains the stored mandelonitrile. If the cyanogenic compound were a glycoside, one would expect a single aqueous phase.

The nature of the vestibular catalyst remains unknown. However, the paper-spotted vestibular contents, even after exposure to air at room temperature for 4 hours, liberate hydrogen cyanide from mandelonitrile. Heating to 130°C for 20 minutes destroys this property.

The defensive gland of *Apheloria* is an admirably refined weapon. The storage of mandelonitrile provides the organism with a convenient way of retaining relatively large quantities of hydrogen cyanide in a stable form. In the absence of catalyst, and with no opportunity for the hydrogen cyanide to escape, but little dissociation would be expected (*12*). At discharge, the addition of the vestibular catalyst forces the gradual liberation of hydrogen cyanide. The essentials of the mechanism may be no different in other polydesmoid species, but in some the precursor might be a glycoside rather than a cyanohydrin (*2*).

Among millipedes, a two-compartmented gland may be an exclusive feature of the order Polydesmida. The glands of other forms studied, including spiroboloids, spirostreptoids, juloids, and chordeumoids (*4, 13, 14*), have a typical reservoir and an efferent duct with a terminal muscle-operated valvular infolding, but they lack a vestibule (Fig. 5). None of these species are cyanogenic, and the absence of a second compartment in the gland may signify that the compounds they secrete—quinones and phenols (*14, 15*)—are stored as such in the reservoirs and do not require chemical activation when they are discharged.

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9. The initial traces of hydrogen cyanide detected in vestibular contents might reflect slight contamination from the reservoirs during excision of the glands.
10. Chromatoplates coated with Silica Gel G, with benzene:chloroform (5:1) as solvent system, and 2,4-dinitrophenylhydrazine in phosphoric acid as spray reagent after development.
11. Mandelonitrile was undetected in our earlier work (*3*) because it probably dissociated to benzaldehyde and hydrogen cyanide during storage in vitro. In vapor phase chromatography it would have remained undetected even in fresh samples, since at the column temperature used (126°C), it also dissociates.
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Hydrogen-Water Vapor Mixtures: Control of Hydrothermal Atmospheres by Hydrogen Osmosis

Abstract. *Experiments at 700°C and 800 bars total pressure demonstrate positive deviations from ideality for mixtures of hydrogen and* H_2O *gases. The deviations are greater than predicted with Stockmayer's method. The composition of the mixture and the fugacity of hydrogen are controlled by diffusing hydrogen through metallic membranes. The results give the fugacities of both* H_2O *and oxygen.*

An experimental technique has been devised which gives simple and continuously variable control of the fugacities of hydrogen, oxygen, and H_2O in hydrothermal experiments by means of controlled diffusion of hydrogen through metallic membranes. The pressure of pure hydrogen gas is an independent variable in addition to temperature and total pressure.

Consider a homogeneous gas phase containing only the constituents H_2, H_2O, and O_2, which is separated from an external source of H_2 by means of a membrane permeable only to H_2. The equilibrium constant for the reaction

$$H_2\ (g) + \tfrac{1}{2}\ O_2\ (g) \rightleftarrows H_2O\ (g)$$

$$K_f = \frac{f_{H_2O}}{f_{H_2} \cdot f_{O_2}{}^{1/2}} \qquad (1)$$

where g is a gas is well known for temperatures to 1500°K (*1*).

In this reaction it is evident from the equilibrium constant that at a given temperature if the fugacities of two of the constituents are specified, the third is determined and the total pressure of the gas mixture must be fixed. Conversely, if the fugacity (f) of hydrogen is arbitrarily controlled by means of osmotic equilibrium with the external reservoir and the total pressure of the gas mixture is also controlled, then f_{H_2O} and f_{O_2} are fixed. Unless the mixture is ideal, however, values cannot be assigned to f_{H_2O} and f_{O_2} without additional information. Several procedures might be followed to evaluate f_{H_2O} and f_{O_2}, but one stands out in simplicity. For nearly all conditions of interest in hydrothermal work f_{O_2} is numerically small and the gas phase can be assumed to be essentially a mixture of H_2 and H_2O. On this assumption it is necessary only to determine the molar proportion of H_2 and H_2O at any given temperature and total pressure. With this data and f_{H_2}, values of f_{H_2O} can be determined because of the Gibbs-Duhem relationship. It follows that f_{O_2} can be determined from the equilibrium constant.

In discussing the H_2-H_2O vapor mixture it is convenient to introduce activities for which the reference states are the pure gases at the specified temperature and total pressure of the mixture.

Experiments have been carried out at a temperature of 700°C and 800 bars total pressure which establish activities of H_2O in H_2-H_2O mixtures. Further experiments were performed to demonstrate control of certain oxidation-reduction reactions and measurement of f_{O_2} for these reactions at the same temperature and total pressure. These experiments were performed in a cold-seal pressure vessel divided into two chambers by a membrane of palladium-silver alloy (Pd_{30} Ag_{70}). One side is connected to a system containing pure hydrogen gas and the other to a system containing argon gas. The hydrogen diffuses through the membrane and mixes with the argon which is at a higher pressure. The part of the system containing pure hydrogen is designated the H-side of the membrane and the other side the AH-side. The membrane is mechanically supported on the H-side to prevent the high pressure on the AH-side from rupturing it. This permits operation at any hydrogen pressures up to the total pressure on the AH-side.

This mechanism provides a way of independently controlling the equilibrium hydrogen pressure, and thus f_{H_2}, at any desired value for a given temperature and total pressure on the AH-side of the membrane. Provision was made to place two sealed capsules on the AH-side of the membrane and thus in the controlled atmosphere. The capsules consist of either palladium-silver alloy or platinum which, at high temperatures, are quite permeable to hydrogen. The capsules are very malleable and deform to eliminate pressure differentials across these additional membranes that enclose the system to be studied.

Mixtures of hydrogen and H_2O were studied by sealing weighed amounts of H_2O in the capsules and allowing hydrogen to diffuse in from the argon-hydrogen mixture at chosen values of f_{H_2}. The final composition of the fluids in the capsules was then determined by measuring the amount of hydrogen after a quench which took about five minutes. This measurement was made by releasing the hydrogen and measuring its volume at the recorded room temperature and barometric pressure. Compositions were approached from opposite directions by making suitable adjustments of hydrogen pressure during the runs. The duration of runs ranged from 24 to 96 hours. The results of the measurements in terms of activities in the system H_2-H_2O are shown in Fig. 1. The measured activity of hydrogen (crosses) is given directly from the conditions of the runs and is merely the ratio $f_{H_2}/f^\circ_{H_2}$, where f_{H_2} is the fugacity of hydrogen in the argon-hydrogen and hydrogen-H_2O gas mixtures and $f^\circ_{H_2}$ is the fugacity of pure hydrogen at a pressure equal to the total pressure on the AH-side. Fugacity coefficients for H_2 were calculated on the basis of the reduced density charts of Schaefer and Thodos (*2*). The dotted lines represent ideal mixing. The dashed lines represent the theoretical functions

$$a_{H_2} = X_{H_2} \exp\left[\frac{X^2_{H_2O}P}{RT}(2B_{12}-B_{11}-B_{22})\right] \quad (2)$$

$$a_{H_2O} = X_{H_2O} \exp\left[\frac{X^2_{H_2}P}{RT}(2B_{12}-B_{11}-B_{22})\right] \quad (3)$$

where X is the mole fraction, B_{11} is the second virial coefficient for hydrogen gas, B_{22} is the second virial coefficient for H_2O, and B_{12} is the second virial coefficient for a H_2-H_2O mixture. The constants given by Beattie and Bridgeman (*3*) were used to calculate the coefficient B_{11}; the equations given by Stockmayer (*4*) were used to calculate the coefficients B_{22} and B_{12}. They are: B_{11} = 18.5 cc/mole, B_{22} = −25.4 cc/mole, and B_{12} = 16.9 cc/mole. The solid lines in Fig. 1 are given by the equations

$$a_{H_2} = X_{H_2} \exp\left(\frac{.066\ X^2{}_{H_2O}P}{RT}\right) \quad (4)$$

$$a_{H_2O} = X_{H_2O} \exp\left(\frac{.066\ X^2{}_{H_2}P}{RT}\right) \quad (5)$$

These were chosen as the simplest functions consistent with the data. When more data have been obtained it may be necessary to add other terms and consequently the empirical constant 0.066 is not directly comparable to the term ($2B_{12}-B_{11}-B_{22}$). It is thought that the empirical Eq. 5 should give a fair representation of the activity of H_2O in H_2-H_2O mixtures for pressures up to about 1000 bars at 700°C. It must be remembered that the second virial coefficients in Eq. 2 and Eq. 3 and the empirical

Table 1. Results demonstrating control and measurement of oxygen fugacity*.

P_{H_2} (bar)	f_{H_2}	f_{H_2O} (bar)	log f_{O_2} (bar)		
			Experimental data observed†	Ideal	Data (*5*)‡
			Reaction I		
4 ± 3	4 ± 3	638 ± 2	−16.2 ± 0.8	−16.2 ± 0.8	−16.4 ± 0.7
			Reaction II		
12 ± 7	12 ± 7	635 ± 3	−17.3 ± 0.6	−17.3 ± 0.6	−17.5 ± 0.7
			Reaction III		
320 ± 23	343 ± 25	500 ± 13	−20.63 ± 0.07	−20.7 ± 0.1	−20.5 ± 0.8
			Reaction IV		
575 ± 75	648 ± 85	300 ± 60	−21.56 ± 0.30	−21.9 ± 0.4	−21.3 ± 0.8

* T = 700°C; *P* total = 800 bars; log K_f = 10.45. Reaction I, nickel-nickel oxide; II, quartz-fayalite-magnetite; III, magnetite-wüstite; IV, wüstite-iron. † Experimental data for H_2-H_2O from this report. The uncertainties given in this column and the next column are based on the widths of the brackets in f_{H_2}. Ideal: from experimental data for f_{H_2} but assuming ideal mixing for H_2-H_2O. ‡ Data (5): Eugster and Wones (*5*). Where more than one set of constants is given by these authors, the set calculated from thermochemical data was chosen. The uncertainties given in this last column are on the basis of a maximum uncertainty of about 4 percent in the standard free energies of reaction.

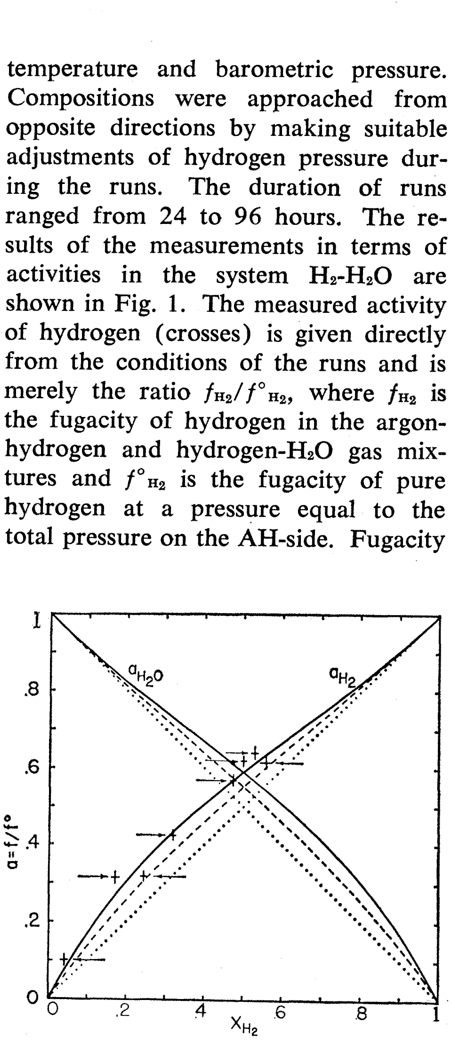

Fig. 1. Activities in the system H_2-H_2O at 700°C and 800 bars total pressure. Experimental points are given for a_{H_2} at composition X_{H_2} (crosses). The direction of approach to equilibrium is indicated by the arrows. Uncertainties in measurements of a_{H_2} are based on uncertainties of 1 percent in f_{H_2}, and 2 percent in $f^\circ_{H_2}$. The uncertainty in X_{H_2} is given by the length of the horizontal bar. Raoult's law is given by the dotted lines. The dashed curves are predictions using Stockmayer's method. The solid curves are given by the empirical Eqs. 4 and 5.

constant in Eq. 4 and Eq. 5 are functions of temperature and the latter cannot be accurately evaluated until the data are extended to other temperatures.

Four oxidation-reduction reactions were studied at 700 ± 5°C and 800 ± 15 bars to demonstrate the use of the H_2 diffusion method in controlling f_{O_2}. These reactions are

I. $2Ni(s) + O_2(g) \leftrightarrows 2NiO(s)$

II. $3Fe_2SiO_4(s) + O_2(g) \leftrightarrows 2Fe_3O_4(s) + 3SiO_2(s)$

III. $6FeO(s, \text{wüstite}) + O_2(g) \leftrightarrows 2Fe_3O_4(s)$

IV. $2Fe(s) + O_2(g) \leftrightarrows 2FeO(s, \text{wüstite})$

where s is a solid and g is a gas. Eugster and Wones (*5*, Table 1) give equations with a pressure term for the equilibrium oxygen fugacities of these reactions.

The reactions were studied by sealing the solid charges together with H_2O in platinum or palladium-silver capsules which were run at various values of f_{H_2}. Equilibrium was approached by causing the reaction to go one way or the other depending on whether the approach was from the direction of low f_{H_2} or high f_{H_2}. Table 1 gives these results in terms of the pressure of pure hydrogen gas at equilibrium, together with values of the oxygen fugacities calculated from the experimental results in this report for H_2-H_2O mixtures. These values of f_{O_2} are compared in Table 1 with values obtained, assuming ideal mixing for hydrogen-H_2O, and values calculated from equations given by Eugster and Wones (*5*).

For the case of ideal mixing, the fugacity of oxygen is given by

$$f_{O_2} = \left(\frac{P\nu^\circ_{H_2O}\nu^\circ_{H_2} - \nu^\circ_{H_2O}f_{H_2}}{K_f f_{H_2}\nu^\circ_{H_2}} \right)^2$$

where ν° is the fugacity coefficient at pressure P. The experimental data for H_2-H_2O mixtures were taken into account by calculating fugacities from activities (Fig. 1), and by using these with the equilibrium constant of formation for H_2O from Eq. 1.

In both cases the results are quite similar. Nonideality in the system H_2-H_2O is not strongly reflected in log f_{O_2}. The results for reaction III demonstrate that this method for controlling f_{O_2} can have high precision, potentially even better than that indicated.

This method permits simple control of f_{O_2} in hydrothermal experiments with good, and potentially excellent, precision and accuracy. There are several advantages over the "oxygen buffer" technique described by Eugster (*6*), and Eugster and Wones (*5*), perhaps the chief one of which is that P, T, and f_{O_2} may be varied independently and continuously. At the same time f_{H_2O} is determined, where previously some assumption was required for its evaluation. The activity of H_2O can be controlled by this method in hydrothermal studies which are not complicated by appreciable amounts of other components in the gas phase and in which hydrogen acts as an inert component in the gas (*7*).

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Hypersensitivity to a Synthetic Polypeptide: Induction of a Delayed Reaction

Abstract. *A multichain copolypeptide, p(Tyr,Glu)-pAla--pLys, induced in guinea pigs a prolonged state of delayed hypersensitivity not associated with detectable circulating antibodies. Cross reactions of the delayed type were observed with chemically related polypeptides.*

The phenomenon of delayed hypersensitivity is assumed to be either an expression of "cellular" hypersensitivity independent of circulating antibody or to incur the obligatory participation of humoral antibodies of high affinity (*1*). For the second alternative an antibody ought to be able to form a stable union with the homologous antigen at concentrations of the uncombined antibody which are too low to be detected by available methods. Studies of delayed hypersensitivity to proteins have been summarized recently (*2*). It seems (*3*) that a functionally larger antigenic determinant controls the specificity of the delayed reactions than that which promotes the union of antigen with soluble antibody. The availability of chemically well-defined substances that would be capable of eliciting pure delayed hypersensitivity persisting for long periods would thus be of interest for the elucidation of the nature and specificity of this phenomenon.

We report the induction, in most guinea pigs tested with a multichain synthetic polypeptide, of a durable specific delayed hypersensitivity that was not associated with detectable circulating antibodies. The use of this antigen was suggested by recent reports (*4–6*) indicating the potent and specific antigenicity of certain synthetic polypeptides. When used alone, these polypeptides are capable of inducing both delayed and immediate sensitivity (*5*, *6*), whereas, when they are used as an antigen-antibody complex they are capable of inducing a pure delayed sensitivity in a few test animals (*6*).

The antigen used in this study, denoted *p*(Tyr,Glu)-*p*Ala--*p*Lys, is a multichain copolymer in which side chains of poly-DL-alanine, attached to the ε-amino groups of poly-L-lysine, were elongated with peptides containing L-tyrosine and L-glutamic acid (*4*). This substance elicited precipitating antibodies in rabbits (*4*), and immunological tolerance could also be obtained in these animals (*7*). Our two samples were 35 and 210, with average molecular weights of 35,000 and 33,200 respectively, and residue molar ratios of Lys:Tyr:Glu:Ala of 1 : 1.8 : 3.7 : 24 and 1 : 1.8 : 2.4 : 22, respectively. No differences were found in the immunological behavior of these samples.

A mixture (0.8 ml) of equal volumes of antigen in physiological saline and Freund adjuvant (Difco) was injected into the four footpads of white guinea pigs weighing 250 to 450 g. Intradermal tests were performed in the range of 30 to 2000 μg of antigen and were observed at 2, 4, 24, and 48 hours. Control skin tests with saline in experimental animals and skin tests with antigen in normal animals were all negative.

Negative skin reactions were observed in 17 animals which received 1.5 to 2.0 mg of antigen in the Freund incomplete adjuvant 10 days before testing. On the other hand, positive skin reactions occurred in 29 of 32 animals which received 1.5 to 10 mg of *p*(Tyr,Glu)-*p*Ala--*p*Lys in the Freund complete adjuvant (including killed