Radius core, moment inertia ⊕, ρ ~~top~~ mantle + assumed $\rho = \rho(r) \Rightarrow$ distrib. E.g. Bullen, p. 89

From Lyttleton, $k_s = \frac{1}{\rho}\left(\frac{\partial \rho}{\partial p}\right)_s = \frac{1}{a+bp}$

$$\therefore \frac{d\rho}{dr} = \left(\frac{\partial \rho}{\partial p}\right)_s \left(\frac{dp}{dr}\right) = -k_s \rho^2 g$$

$$= -\frac{\rho^2}{a+bp} g$$

$$k = \frac{1}{\rho}\frac{d\rho}{dp} = \frac{1}{a+bp}$$

$$\therefore \frac{d\rho}{\rho} = \frac{dp}{a+bp}$$

Set $x = a+bp$, $dx = b\,dp$

$$\frac{d\rho}{\rho} = \frac{dx/b}{x} = \frac{1}{b}\frac{dx}{x}$$

$$\int_{\rho_0}^{\rho} \frac{d\rho}{\rho} = \frac{1}{b}\int_{x_0}^{x} \frac{dx}{x}$$

$$\ln\frac{\rho}{\rho_0} = \frac{1}{b}\ln\left(\frac{x}{x_0}\right) = \frac{1}{b}\ln\left(\frac{a+bp}{a+bp_0}\right)$$

$$\frac{\rho}{\rho_0} = \left(\frac{a+bp}{a+bp_0}\right)^{\frac{1}{b}}$$

$$\left(\frac{\rho}{\rho_0}\right)^b = \frac{a+bp}{a+bp_0}$$

But $p_0 \simeq 0$.

$$\left(\frac{\rho}{\rho_0}\right)^b = 1 + \frac{b}{a}p$$

$$\therefore \boxed{p = \frac{a}{b}\left\{\left(\frac{\rho}{\rho_0}\right)^b - 1\right\}}$$

Very roughly, $a \simeq 1.5 \times 10^{12}$ dynes cm^{-2}

$b = 3.5$ (dimensionless)

$\therefore$ for density at $\oplus$ center,

$$\frac{\rho}{\rho_0} = \left(1 + \frac{b}{a} p\right)^{\frac{1}{b}} = \left(1 + \frac{3.5 \times 3.6 \times 10^{12}}{1.5 \times 10^{12}}\right)^{\frac{1}{3.5}}$$

$$= (1 + 8.4)^{\frac{1}{3.5}} = (9.4)^{\frac{1}{3.5}} \simeq 1.9$$

~~For $\rho \sim 17$ gm cm^{-3}, need $\rho_0 \sim 6$ gm cm^{-3} according to Sytleton, whereas Fe has 79 gm cm^{-3}.~~

~~$\rho_0 \simeq 7$ gm cm^{-3}, too small~~

~~But p might be slightly larger.~~

$$\cancel{\frac{dp}{dr} = -\rho g = \frac{a}{b}\frac{d}{dr}\left\{\left(\frac{\rho}{\rho_0}\right)^b - 1\right\}}$$

$$\cancel{= \frac{a}{b} b \left(\frac{\rho}{\rho_0}\right)^{b-1} \frac{d\rho}{dr} = a\left(\frac{\rho}{\rho_0}\right)^{b-1} \frac{d\rho}{dr}}$$

$$\cancel{= a\left(\frac{\rho}{\rho_0}\right)^{b-1} \frac{dp}{a + bp}}$$

$g = g_0 \frac{r}{R}$ for a homog sphere.

$$\therefore -\rho g_0 \frac{r}{R} \simeq a\left(\frac{\rho}{\rho_0}\right)^{b-1} \frac{d\rho}{dr}$$

$$\therefore -\frac{g_0 \rho_0}{a R} \int_{r_0}^{r} dr \simeq \int_{\rho_0}^{\rho} \rho^{b-2}\, d\rho$$

$$\therefore -\frac{g_0 \rho_0}{a R}(r - r_0) = \left[\frac{\rho^{b-1}}{b-1}\right]_{\rho_0}^{\rho}$$

Could attempt hydrostatic equil. + diff. of bound eq., but only know $g = g_0 \frac{r}{R}$ for homog. ⊕.

Core densities are 10-17 gm cm^{-3}, ∴ uncompressed core densities are 5.2-9 gm cm^{-3}. Fe uncompressed is 7.9 gm cm^{-3}. ∴ argument for Fe core. But some people (Ramsey, Lyttleton) claim better model accounting ∴ a discrepancy).

Comp. core. Seismic data. P + S waves.

$\frac{dp}{d\rho} = v_p^2 - \frac{4}{3} v_s^2$ from theory of elastic wave propagation. Seismographic frequency + amplification ranges. Lowest mode oscillation ⊕ is $P \sim \frac{1}{\sqrt{G\rho}} \sim 1$ hr.

Reflec., refrac. Birch Fig. 2. Discont. ⟹

phase or composition change. Vel vs. p or ρ

is a comp. index. Birch Fig 3. + p. 135. Meteorites.

Core form: melting early, information radioactivity later, gradual

migration Fe to interior. Wrong. Motion. Bullard,

p. 102. Common vs. uncommon.

Pressures $10^{11} - 4 \times 10^{12}$ dynes cm^{-2} too high for

lab. + too low for theory (electron shells stripped)

Ref.: E. Segré, "Nuclei + Particles" Benjamin, N.Y. 1965, pp. 37-38

A chgd. particle ionizes the gas it penetrates. Only part of the energy is used in ionization + in producing superthermal electrons; the remainder is used ~~the~~ in molecular excitation leading to emission of radiation. The avg. energy required per ion pair is "remarkably independent of the charge, mass, + velocity of the particle producing the ionization, but depends on the gas in which the ions are formed." Some of the superthermal electrons (delta rays) have sufficient energy to produce secondary ions.

eV/ion pair

He	Ne	Ar	Xe	H_2	O_2	N_2	CO_2	Air		
42.7	36.8	26.4	21.9	36.3	32.5	36.5	34.3	35.0	Po	α
42.3	36.6	26.4	22.0	36.3	30.9	34.9	32.9	33.8	H^3	β

∴ except for He, Ar, Xe, all of gases have ~34 eV/ion pair

This gives, for these 2 plates,

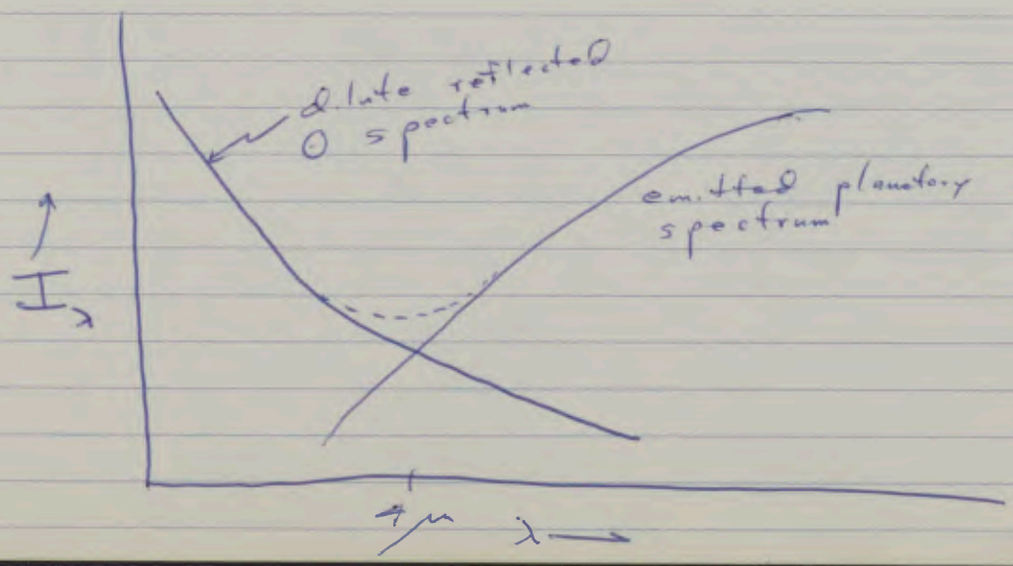
$T_{rot} = 257°K$ + $T_{rot} = 434°K$,

respectively. The pressures depend somewhat on the choice of $\bar{J}$. For $\bar{J} = 22$, these P's are 1.8 and 5.6 atm, resp.

Significance of results. High P ⇒ high T. Venus cover at 7830 Å. Determination of α_{CO_2}. Search for H_2O. Poss. double maximum, suggesting 2 cloud layers.

Planetary temperatures:

Could be measured by determining the Wien peak, or by taking ratios of intensities at neighboring wavelengths. These methods do not depend on absolute calibration, but they do require high-sensitivity detectors, since λ-intervals are used. In practice, absolute ~~energy~~ intensity determinations are used. Usually thermocouples or thermistors, + almost always in the 8 - 13 μ window. These temps are really brightness temps. Unless the sources are ~~black~~ gray bodies, measurements elsewhere might give diff. temps. E.g., for ♂,



Pressure-induced dipole transitions

Here line-width is a function of the time for establishing the transient dipole moment. $\therefore \gamma$ a function of velocity, i.e., of T, alone, and independent of pressure. $\therefore \gamma \propto p^{1/2}$ — the usual Doppler one is removed. $\therefore$ the shape of a pressure-induced line is pressure-independent at a fixed temperature. The absorption coefficient, K_0, on the other hand, is $\propto p^2$, because the number of emitters cm^{-3} $\propto p$, and the number of stimulations sec^{-1} is $\propto p$. For pressure-induced transitions, the collision time $\ll$ time between collisions. $\Delta E \Delta t \sim \hbar$ $\Rightarrow \Delta E$ much larger. $\therefore$ pressure-induced transitions are very broad — the is, a almost continuum at microwave frequencies. At microwave frequencies it is translational rather than vibrational or rotational transition which are occurring.

$CO_2 - N_2$ 9

H_2 24

CH_4	1.16, 1.7 μ	< 10 cm-atm
C_2H_4	1.68 μ	< 2 cm-atm
C_2H_6	1.20, 1.73 μ	< 1 cm-atm
NH_3	1.51 μ	< 2 cm-atm

Jovian planets later.

CH_3Cl spectrum.

$$\bar{\nu} = \frac{1}{\lambda} = \frac{\nu}{c}.$$

Units of wave nos. or cm^{-1}.

Boltzmann distribution of states: if a lg. no. (e.g. N_0) of particles are placed in a given potential fld, how many will occupy which energy level, if the states are quantized? Answer given by the Boltzmann distrib. which states that the no. parts. occupying a state w. energy E_i is related to the no. occupying some other state E_j, which has less energy, by

$$N_i = N_j e^{-(E_i - E_j)/kT}.$$

Sometimes $\exists$ several states with the same energy, i.e. degeneracy. The population of the energy level is then greater. Thus if g_i is the multiplicity of the ith level, (i.e., no. states w. energy E_i) and (as is usually the case) lowest energy level is a singlet state,

$$N_i = g_i N_0 e^{-(E_i - E_0)/kT}.$$

$$\frac{N_i}{g_i} = \frac{N_j}{g_j} e^{-(E_i - E_j)/kT}$$

Pressure-induced: Permitted: time between collisions determines line shape

Line width function of time for establishing dipole moment $\Rightarrow f(v) \Rightarrow f(T) \not\Rightarrow f(p)$.

$p^{1/2}$ dependence on γ removed. ∴ shape of p-induced line is p-indep at fixed T. Coll. to << time betw. colls.

∴ ΔE ($\Delta E \Delta t \sim \hbar$) bigger ∴ p-induced quite broad at 1 r + almost a continuum at pressure.

$k(cm^{-1}) \propto p^2$. Nonetheless $\propto p$; no stimulation $sec^{-1} \propto p$.

$CO_2 - N_2$ $\underset{1.1\,\mu m}{\underline{q}}$; $H_2 - \psi$.

At pressure transitional mode is radiating (not rot or vib)

Vibration of a single particle is given by the simple diff. eq.

$$m\ddot{x} = -kx,$$

where k is the force const. & has units of dynes/cm. The sol. is

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m}}.$$

The potential energy is parabolic

$$U = \frac{1}{2}kx^2$$

if the equilib. pos. is taken as the zero of potential energy.

Now consider 2 parts connected by a spring:

$$m_1\ddot{x}_1 = k(x_2 - x_1)$$

$$m_2\ddot{x}_2 = -k(x_2 - x_1)$$

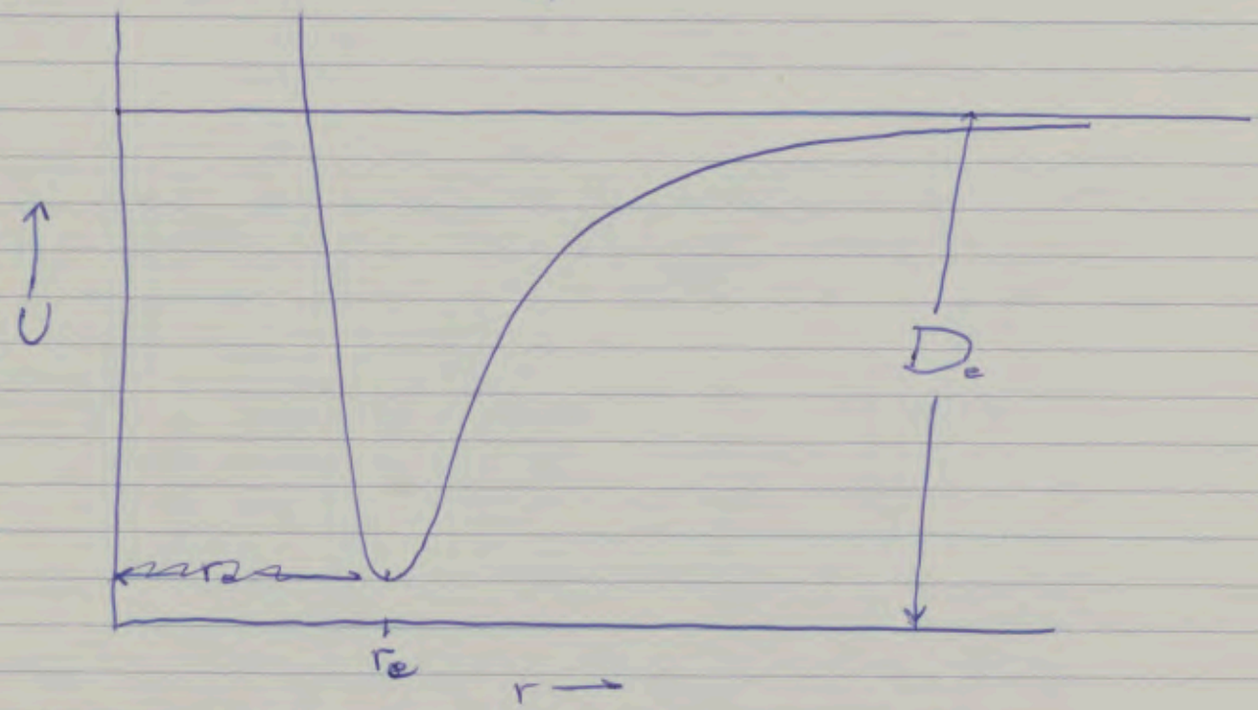
The sol. is

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

where μ is the reduced mass, given by

$$\frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2}.$$

Now for a diatomic mol., we are interested in the ΔU as a function of the distortion of the bond of the molecule from its equil. distance. Except for H_2, $\exists$ no calc. of $E = E(r)$ from the interactions between the bonding electron + the atomic nuclei. The gen. shape of the pot. energy curve can, however, be sketched. One knows, e.g., that if a bond is stretched far enough it will break, i.e., mols. dissociate. Also, bonds strongly resist compression, as is revealed, e.g., by the rel. incompressibility of solids $\therefore$, one expects



r_e = equilib position, D_e = dissociation energy.

$\exists$ an ad hoc analytic expression for this, the Morse pot:

$$U(\Delta r) = D_e\left(1 - e^{-\beta \Delta r}\right)^2$$

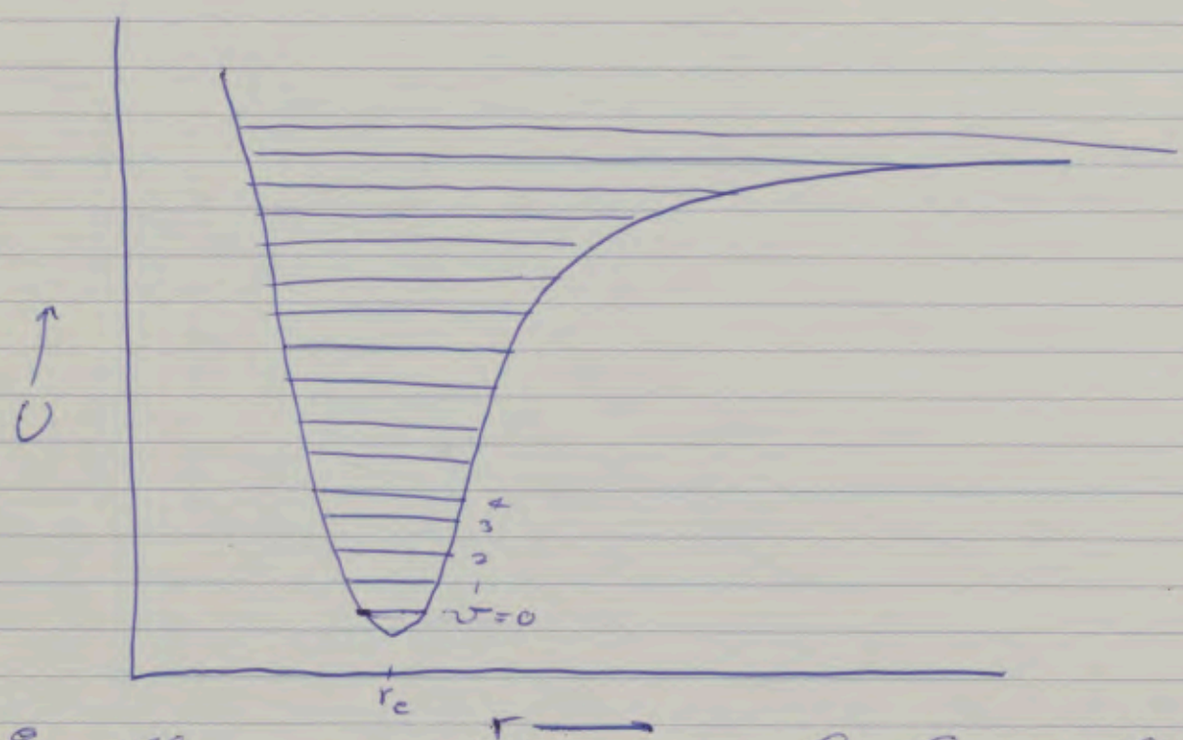
β is a function of the molec. species.

Now the q.m. sol. of the diatomic harmonic oscillator is

$$E_v = (v + \tfrac{1}{2})\, h\nu, \quad v = 0, 1, 2, \ldots$$

where $\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$,

i.e. the classical energy levels are quantized, Planck's $E = h\nu$ is used, + $\exists$ a zero-pt. energy.



Equally spaced energy levels, each w. its own quantum no. v, + some value of v above which diss. occurs.

∃ restrictions on the vibrational transitions which can occur. A vibrating molecule cannot interact w. e.m. rad. unless an oscillating dipole moment accompanies the molec. vibration. Qualitatively the oscillating dipole couples w. the $\vec{E}$ field of the e.m. rad. so energy can be exchanged between mol. + rad. Turns out that homonuclear diatomic molecules have 0 dipole moment for all r, + ∴ have no vibrational (Lapt p-induced) spectra. Heteronuclear diatomic mols have $p = p(r)$ + ∴ can absorb + emit energy by making ~~vib.~~ transitions among its vib. energy levels.

Even for these mols, ∃ a further restriction, which is rigorously applicable only to harmonic oscillators, viz,

$$\Delta v = \pm 1.$$

Now the energy spacing between vibrational energy levels is typically

$$\sim 2 \times 10^{-13} \text{ erg/molecule} \simeq 3000 \text{ cal/mole}.$$

For $g_i = g_0 = 1$, we compute no. mols in $v=1$ state cf. no. in $v=0$ state at 25°C.

$$\frac{N_{v=1}}{N_{v=0}} = e^{-2\times10^{-13}/298\times1.38\times10^{-16}} = 0.008.$$

∴ less than 1% in $v=1$; + even fewer in higher energy levels. ∴ in expts not much above rm. temp. transitions beginning in ground state dominate. If we find a $v=1$ ground state transition, we know something about temps.

φ ?

There do exist hot bands, e.g. the CO_2 bands at 9.4 + 10.4μ, which have much smaller ΔE + ∴ are excited at much ~~high~~ lower T's. For ordinary ΔE, T ≳ v=1 excited is

$$\frac{2\times10^{-13}}{1.38\times10^{-16}\times T} \sim 2$$

$$T \sim \cancel{1.4\times10^{-3}}\ 700\ ^\circ K.$$

∴ at surface of ☿ + ♀, v=1 excitation begin to become imp't. But hot bands very imp't there.

Value of force const. k can be derived from one spectral feature + used to derive others. E.g., HCl absorbs at 2890 cm^{-1} = 3.47μ.

$$\therefore \nu = 3\times10^{10}\times 2.89\times10^{3} = 8.67\times10^{13}\ \text{cycles/sec.}$$

$$\therefore \Delta E = h\nu = 6.62\times10^{-27}\times 8.67\times10^{13}$$

$$= 5.83\times10^{-15}\ \text{erg.}$$

This must correspond to transition between v=0 + v=1; i.e.,

$$\Delta E = \frac{h}{2\pi}\sqrt{\frac{k}{\mu}}$$

$$\mu \approx m_H.$$

$\therefore k = 4.8 \times 10^5$ dynes/cm.

Then can derive high T excitation bands properly. The k's are a measure of bond strength. E.g.,

H_2	5.2×10^5 dynes/cm
CO	18.7
O_2	11.4
N_2	22.6
$NaCl$	1.2

Actually, the harmonic oscillator ~~has~~ expression

$$U = \tfrac{1}{2} k (\Delta r)^2$$

is only an approximate representation. It is observed that weak spectral absorption features exist corresponding to $\Delta v = +2, +3, \ldots$ Also these <u>overtone</u> absorption are not exactly 2, 3 ... times the frequency of the <u>fundamental</u> $\Delta v = +1$ absorption. Very roughly intensity of overtone ~ 1% intensity fundamental.

Refs.: G. Herzberg, Spectra Diatomic Mols., 1950
I. r. + Raman Spectra, 1945
both van Nostrand

W. Brügel, Intro. to I. r. Spectroscopy, Wiley, 1962

G. Barrow, Intro. to Mol. Spectroscopy McGraw-Hill, 1962.

B. Bak Elementary Intro to Mol. Spectra North-Holland, 1962.

We now consider rotation of a linear diatomic molecule, although the theory developed is equally applicable to linear polyatomic mols; e.g., CO_2, + C_3O_2. We will assume that rot. is uncoupled w. vibration; i.e., the <u>rigid-rotor</u> approximation.

To see simply how the physics proceeds we simply apply Bohr's fundamental condition that the ang. mom. be quantized in units of

$$\hbar = h/2\pi .$$

Total ang. mom. is

$$\sum_i m_i v_i r_i = \sum m_i r_i^2 \left(\frac{v_i}{r_i}\right)$$

distance from c.m.

$$= I\omega$$

where $I = \sum m_i r_i^2$ is the moment of inertia of the system. Calling the rot. quantum no. J, we have

$$I\omega = J\hbar \quad , \quad J = 0, 1, 2, \ldots$$

$\therefore$ the allowed energy levels are

$$E_J = \frac{1}{2} I\omega^2 = \frac{\frac{1}{2}(I\omega)^2}{I}$$

$$= \frac{1}{2I}\hbar^2 J^2$$

$$E_J = \frac{h^2}{8\pi^2 I} J^2 .$$

The correct eq., obtained by sol. of the Schrodinger eq. is only slightly diff.:

$$E_J = \frac{h^2}{8\pi^2 I} J(J+1).$$

∴ the ang. momenta are given by $\sqrt{J(J+1)}\,\hbar$, here J's are integers. For spectroscopic work, it is convenient to express the energy levels in terms of wave numbers:

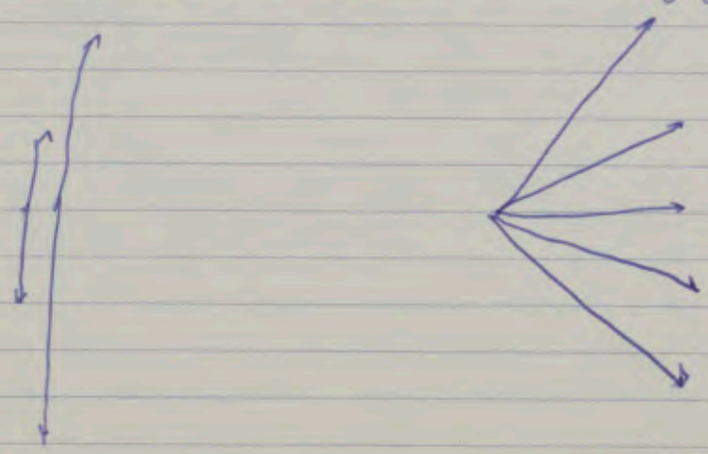
$$\tilde{E}_J = \frac{h}{8\pi^2 c I} J(J+1) \quad cm^{-1}.$$

$$\equiv \tilde{B}\, J(J+1) \quad cm^{-1},$$

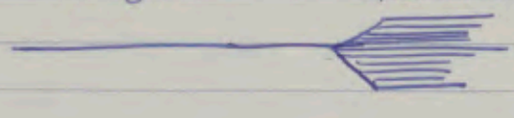
where $\tilde{B} \equiv \frac{h}{8\pi^2 c I}$ cm^{-1}

is a frequently occurring const. in molec. spectroscopy.

Now ∃ a no. of rot. states corresponding to a given rot. energy E_J. Since components of ang. mom. in a given direction are quantized in units of $\hbar$, ∃ $2J+1$ states w. energy $\tilde{B}\, J(J+1)$. E.g.,



This $2J+1$ -fold degeneracy of the Jth energy level results in energy level diagram of the form

J	$\tilde{E}_J$	Allowed energies	Energy levels if degeneracy removed	Multiplicity
5	$30\bar{B}$			11
4	$20\bar{B}$			9
3	$12\bar{B}$			7
2	$6\bar{B}$			5
1	$2\bar{B}$			3
0	$0\bar{B}$			1

$\tilde{E}_J = \tilde{B}\, J(J+1)$ cm^{-1}, where $\tilde{B} = \dfrac{h}{8\pi^2 c I}$ cm^{-1}

$\Delta J = \pm 1$.

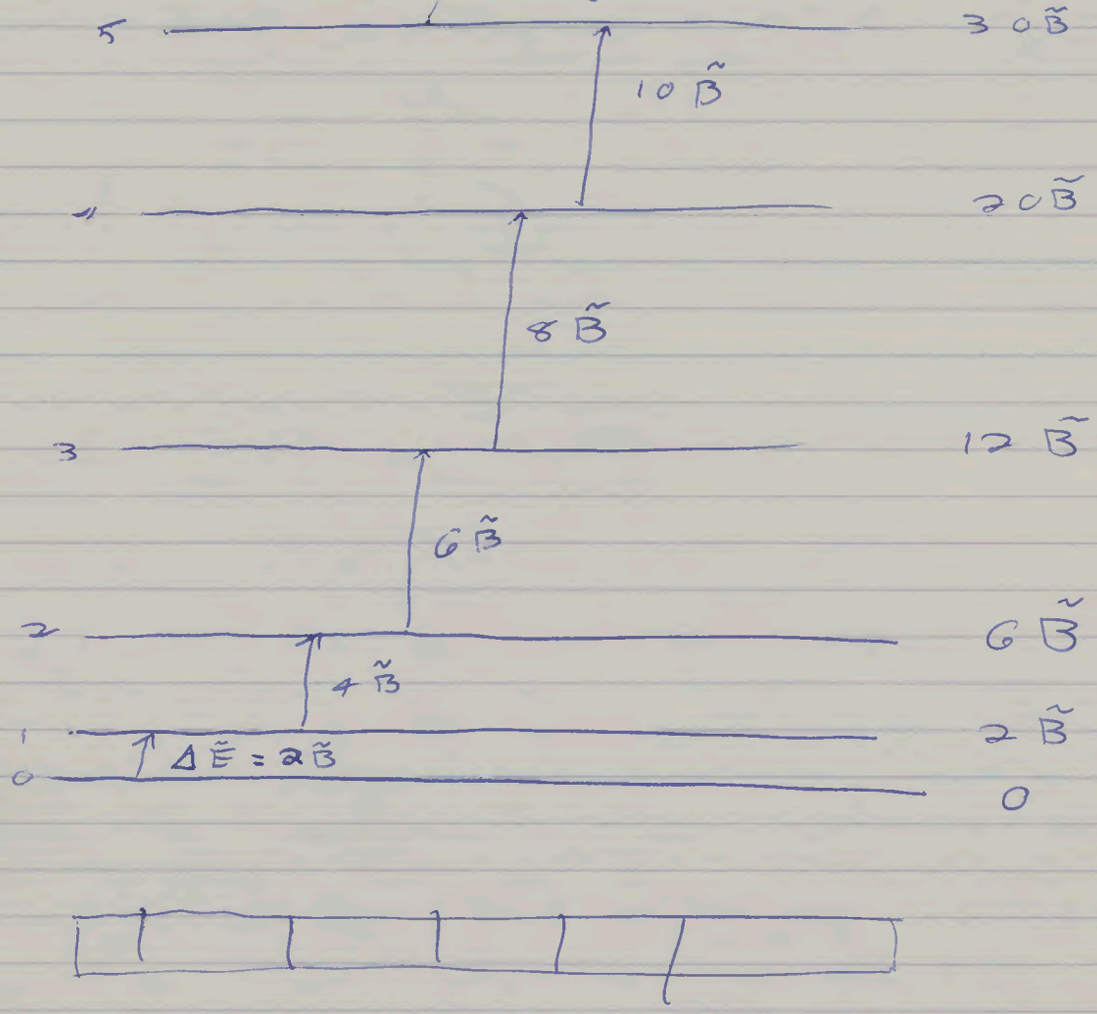
$\therefore$ a transition is

$$\tilde{\nu} = \tilde{B}(J+1)(J+2) - \tilde{B}\, J(J+1)$$

$$= 2\tilde{B}(J+1).$$

Kaplan: B'ly, I sm.

$\therefore$ rot. lines are spaced by an amount $2\tilde{B}$.



$\tilde{B}(J+1)\,[J+2-J]$

Characteristic frequency is then

$$\tilde{\nu} = 2\tilde{B}(J+1)$$

$$= 2\frac{h}{8\pi^2 c I}(J+1)$$

$$= \frac{2\times 6.62\times 10^{-27}\times 15}{8\pi^2\times 3\times 10^{10}\times 3\times 10^{-39}}$$

$$= 2.8\times 10^{1} = 28\ \text{cm}^{-1}$$

$$\therefore \lambda = \frac{1}{\tilde{\nu}} = 3.6\times 10^{-2}\ \text{cm}.$$ Typical microwave frequency

Enough to see that microwave lines of $\lambda \gg 1$ cm are not to be expected. This is, even better, a method of determining I.

Stretching effect of the centrifugal force on bond lengths ∴ on moment of inertia changes the spectra; increase I ∴ decrease $\tilde{\nu}$. More increase for higher J's.

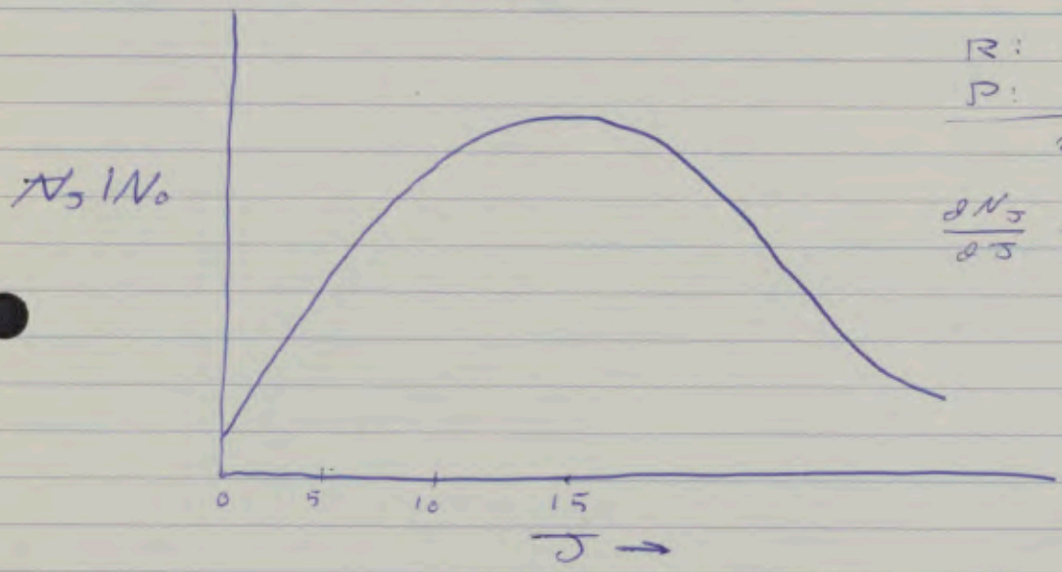
∴, from Boltzmann eq.,

$$N_J = (2J+1)\, N_0\, e^{-B\, J(J+1)/kT}$$

Order of mag. I is $\mu\, m_H \times (1\,\text{Å})^2 \sim 10^{-38}\ \text{gm cm}^{-2}$.

$$\therefore N_J = (2J+1)\, N_0\, e^{-J(J+1) \times 2.0 \times 10^{-3}}$$

∴ at rm. temp., ∃ rel. pop. distrib like



R: $(J+1)$
P: J
$2J+1$

$\frac{dN_J}{dJ} = 0 \Rightarrow J_{max}$

At sm. J increases because of $2J+1$. At lg. J decreases because of exponential.

Here again ∃ restrictions on permissible transitions. Here also the mol. must have a permanent dipole moment. [∃ Pressure-induced transitions] Also ∃ the selection rule

$$\Delta J = \pm 1.$$

Vibration of polyatomic mols.:

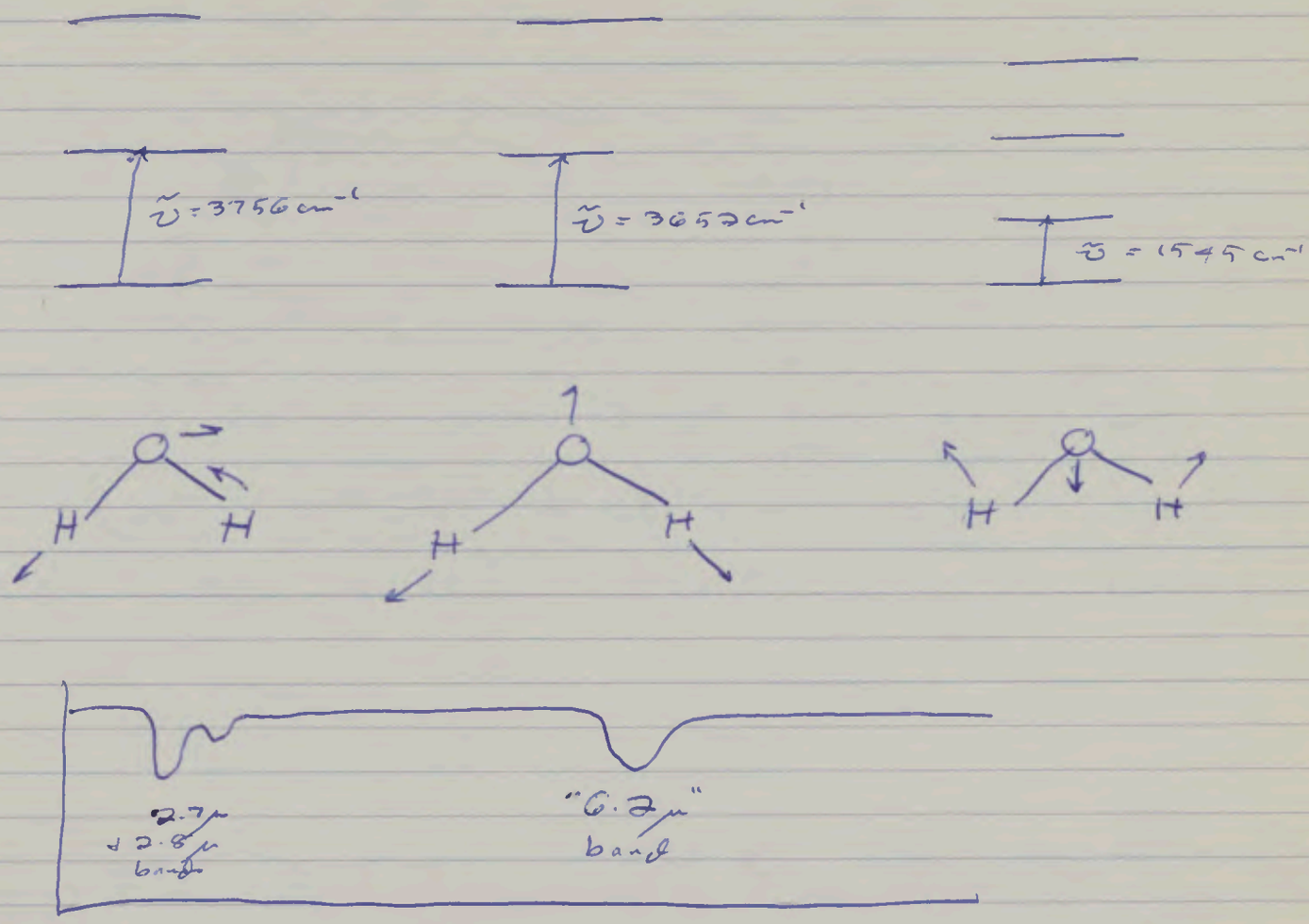
For very loose bonds, each atom has 3 degrees of freedom, so 3n such coordinates for n atoms. Each represents a degree of freedom, + any position or motion can be described in terms of them. As bond strength increases, degrees of freedom decline, if we demand description of the mol. itself. If mol has 3 coordinates for translation + 3 for rotation, degrees of internal freedom left for the atoms are

$$3n - 6.$$

Note this is simply a reclassification. It gives no vibrational modes. If mol is linear, rot. can occur only about the 2 axes ⊥ the molecular axis. ∴ linear mols. have

$$3n - 5$$

degrees of freedom. ∴ H_2O has $9 - 6 = 3$ and CO_2 has $9 - 5 = 4$. For H_2O, ∴ ∃ 3 vibrational energy level patterns (+ overtones).



Actually, high res. vib. spectra show fine structure due to rot. Vib.-rot. spectra. In liquid phase ∃ no well-defined rot. energy levels, + rot. fine structure is not observed.
→ Hornberg

$\Delta v = +1$ is vib. sel. rule for vib.-rot. absorption spectra.

$\Delta J = \pm 1$ " rot. " " " "

+ here both +1 + −1 are imp't, in absorption.

Exceptions are provided by mols. w. an odd electron, like NO. The electron contributes ang. mom. about the molec. axis. This permits $\Delta J = 0$ transitions to occur.

Simplest, + fairly satisfactory approach is to treat mol. as a rigid-rotor, harmonic-oscillator system. Then,

$$\tilde{E}_{v,J} = (v + \tfrac{1}{2}) \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}} + \frac{h}{8\pi^2 I c} J(J+1)$$

$$= (v + \tfrac{1}{2}) \tilde{\omega} + \tilde{B} J(J+1),$$

where $\tilde{\omega} = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}}$

The transition is $v = 0 \longrightarrow v = 1$.

For $\Delta J = +1$; i.e., $J \rightarrow J+1$,

$$\tilde{\nu} = \tfrac{3}{2}\tilde{\omega} + \tilde{B}(J+1)(J+2) - \tfrac{1}{2}\tilde{\omega} - \tilde{B} J(J+1)$$

$$= \tilde{\omega} + 2\tilde{B}(J+1), \qquad J = 0, 1, 2 \ldots$$

For $\Delta J = -1$; i.e., $J \rightarrow J-1$,

$$\tilde{\nu} = \tfrac{3}{2}\tilde{\omega} + \tilde{B}(J-1)J - \tfrac{1}{2}\tilde{\omega} - \tilde{B} J(J+1)$$

$$= \tilde{\omega} - 2\tilde{B} J$$

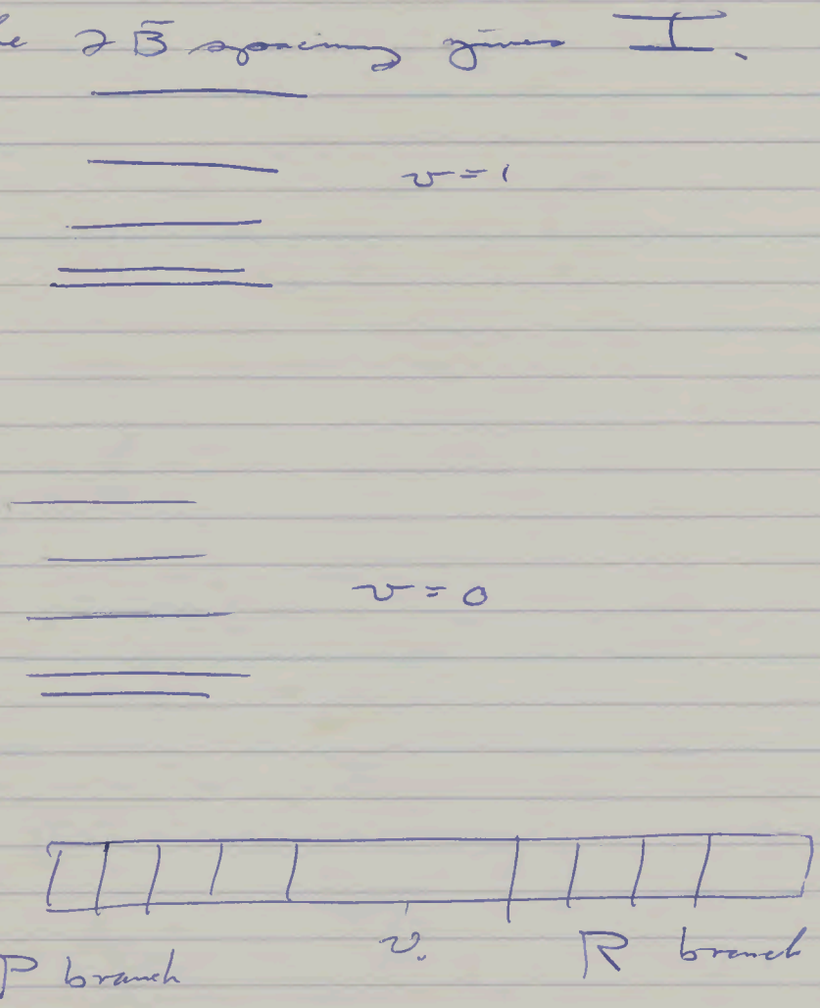
In these expressions, the value for J is rot. q. no. <u>lower</u> state.

∴ ∃ 2 sets of absorption lines spaced in energy by $2\tilde{B}$. ∃ a gap at the band center, corresponding to the absent $\Delta J = 0$ transition, all of which would have had frequency $\tilde{\omega}$.

The low-frequency set is the P branch
" high " " " " R "

When $\Delta J = 0$ is allowed, it is the Q "branch"

The $2\tilde{B}$ spacing gives I.



Kaplan: line spacing $\Rightarrow$ sm $2\tilde{B} \Rightarrow$ sm. $I \Rightarrow$ hydrogenated mols.

For vib-rot spectra have 2 Boltzmann eqs.

for the P and R branches:

P branch:

$$N_J = J N_0 e^{-BJ(J+1)/kT}$$

R branch:

$$N_J = (J+1) N_0 e^{-BJ(J+1)/kT}$$

$\therefore$ sum of the two $\Rightarrow (2J+1) N_0 e^{-BJ(J+1)/kT}$, correct statistical weights.

What J is most populated at a given T?

P branch

$$\frac{dN_J}{dJ} = 0 = N_0 e^{-BJ(J+1)/kT}$$

$$+ J N_0 e^{-BJ(J+1)/kT}\left[-\frac{B}{kT}(2J+1)\right]$$

$$\therefore 1 = \frac{B}{kT}(2J^2 + J)$$

$$\therefore \quad \frac{2B}{kT} J^2 + \frac{B}{kT} J - 1 = 0$$

$$J_{max} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$= \frac{-\frac{B}{kT} \pm \sqrt{\frac{B^2}{k^2T^2} + \frac{8B}{kT}}}{\frac{4B}{kT}}$$

– sign gives neg. J_{max}, unphysical.

$$\therefore \quad J_{max} = -\frac{1}{4} + \frac{kT}{4B}\sqrt{\frac{B^2}{k^2T^2} + \frac{8B}{kT}}$$

$$= -\frac{1}{4} + \frac{1}{4}\sqrt{1 + \frac{kT}{2B}}$$

$\frac{B}{k} \sim 0.3$ in cgs units. $\therefore \frac{kT}{2B} >> 1$ at ordinary temps.

$$\therefore \quad J_{max} \simeq -\frac{1}{4} + \frac{1}{4}\sqrt{\frac{kT}{2B}}$$

For $T \simeq 300°K$, $J_{max} \simeq -\frac{1}{4} + \frac{1}{4}\sqrt{\frac{300}{0.6}}$

$$= -\frac{1}{4} + \frac{1}{4}\sqrt{4.9 \times 10^2} = -\frac{1}{4} + \frac{1}{4}(22)$$

$$= 5.3 \simeq 5.$$

R branch.

$$\frac{\partial N_J}{\partial J} = 0 = N_0 e^{-BJ(J+1)/kT}$$

$$+ (J+1) N_0 e^{-BJ(J+1)/kT} \left[-\frac{B}{kT}(2J+1) \right]$$

$$\therefore 1 = \frac{B}{kT}(2J^2 + 3J + 1)$$

$$\therefore \frac{2B}{kT} J^2 + \frac{3B}{kT} J + \left(\frac{B}{kT} - 1\right) = 0.$$

$$\therefore J_{max} = \frac{-\frac{3B}{kT} \pm \sqrt{\frac{9B^2}{k^2T^2} - \frac{8B}{kT}\left(\frac{B}{kT} - 1\right)}}{\frac{4B}{kT}}$$

$$\frac{k^2T^2}{16B^2}\left(\frac{8B}{kT}\right) = \frac{1}{2}\frac{kT}{B}$$

$$= -\frac{3}{4} \pm \sqrt{\frac{9}{16} - \frac{1}{2} + \frac{1}{2}\frac{kT}{B}}$$

$$= -\frac{3}{4} + \sqrt{\frac{1}{16} + \frac{1}{2}\frac{kT}{B}}$$

$$\simeq -\frac{3}{4} + \sqrt{\frac{1}{2}\frac{kT}{B}}$$

For $T \simeq 300\,K$, $\frac{B}{k} \sim 0.3$,

$$J_{max} \simeq -\frac{3}{4} + \sqrt{4.8 \times 10^2} = 22 - \frac{3}{4} \simeq 21.$$

For vib.-rot. spectra we again have

$$N_J = (2J+1)\, N_0\, e^{-BJ(J+1)/kT}$$

$\rightarrow \frac{dN_J}{dJ} = 0$ for P + R

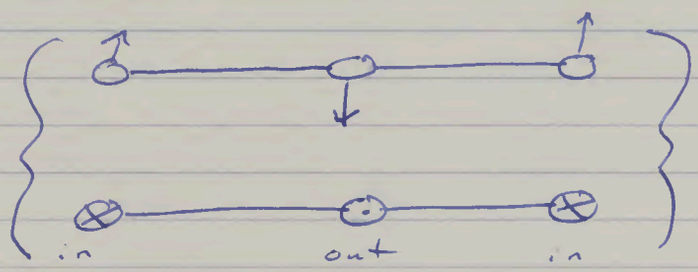
Actually spacing between lines not const., due to coupling between rot. + vib.

If one had available a spectrometer of ∞ resolving power, one could determine experimentally, for a specified amount, w, of absorbing substance, the fractional absorption A_ν, of the incident radiation at any frequency. In practice, because of the finite width of the slit, one integrates over frequencies. The observed A_ν is dependent on the slit width + is smoothed out with $\Delta\nu$ provided by the spectrometer. But the fractional absorption, A, over the entire absorption band is const.:

$$\int_{\nu_1}^{\nu_2} A_\nu \, d\nu = K,$$

provided ν_1 + ν_2 are taken far enough apart, so there is negligible absorption outside the limits.

We have already mentioned that $3n-5=4$ modes of vibration are expected for CO_2. These 4 fundamental frequencies are usually represented as

Change of electric dipole moment	O C O	Mode
None	$O \rightarrow \quad O \quad \leftarrow O$	ν_1
$\perp$		ν_2
$\parallel$	$\leftarrow O \text{———} O \rightarrow \quad \leftarrow O$	ν_3

The ν_2 vibration is really the degenerate ref. of 2 = freq. In ν_1, the C atom is stationary + the dipole moment is 0 and unchanging ∴ no ~~pure rot. lines~~ ν_1 vib.-rot. lines except in comb. ∴ ∃ 2 fundamentals for CO_2,

ν_2	667 cm^{-1}	15 μ
ν_3	2349 cm^{-1}	4.3 μ.

The bending ($\perp$) vib. takes less energy than the $\parallel$ vib. The "inactive" ν_1 can be located by Raman spectra, + observed frequency is

ν_1	1388 cm^{-1}	7.2 μ.

$$\begin{array}{r} 2349 \\ 5 \\ \hline 11745 \\ 1388 \\ \hline 13133 \end{array}$$

This is close enough to $2\nu_2$ that $\exists$ a resonance interaction, the Ferm. resonance, between ν_1 and $2\nu_2$ & both are displaced from their normal frequencies in comb. bands. Neglecting comb. bands, the vib. energy of CO_2 can be written as

$$E = h(v_1 + \tfrac{1}{2})\nu_1 + h(v_2 + \tfrac{1}{2})\nu_2 + h(v_3 + \tfrac{1}{2})\nu_3 .$$

$\exists$ also of cons. rot. energies. The freq. of a transition can then be computed from the diff. between energy levels. The ~~state~~ is written

$$v_1 \quad v_2^{\,l} \quad v_3$$

where l is a subsidiary quantum no. which measures, in units of $\hbar$, the ang. mom. of the C atom about the internuclear axis.

E.g. bands at 7820 Å on ♀ are from

$$0 \quad 0^\circ \quad 0 \quad \text{to} \quad 1 \quad 0^\circ \quad 5 .$$

Very roughly,

$$\Delta E = \tfrac{3}{2} h\nu_1 + \tfrac{1}{2} h\nu_2 + \tfrac{11}{2} h\nu_3 - \tfrac{1}{2} h\nu_1 - \tfrac{1}{2} h\nu_2 - \tfrac{1}{2} h\nu_3$$

$$= h\nu_1 + 5\, h\nu_3$$

$\tilde{\nu} = 1388 + 5 \times 2349 = 1388 + 11745 = 13133.$

Observed $\tilde{\nu} = 12775$. Comb. ~~terms~~ account for the rem. diff.

$$y = k_\nu x = \frac{S}{\pi} \frac{\gamma}{(\nu-\nu_0)^2} x \; ; \; y^{3/2} = \frac{S^{3/2}}{\pi^{3/2}} \frac{\gamma^{3/2}}{(\nu-\nu_0)^3} x^{3/2}$$

$$dy = x\, dk_\nu = x \frac{S}{\pi} \gamma \, d(\nu-\nu_0)^{-2} = -2x \frac{S}{\pi} \gamma (\nu-\nu_0)^{-3} d\nu$$

$$= -2 y^{3/2}$$

$$\int_{-\infty}^{+\infty} (1 - e^{-y})\, d\nu$$

For a strong line, the center of the line is completely absorbed, + any further increase in absorption takes place in the wings only.

$$\therefore k_\nu \simeq \frac{S}{\pi} \frac{\gamma}{(\nu-\nu_0)^2}.$$

Let $u = k_\nu \rho x$.

$$\therefore \int_{-\infty}^{+\infty} A_\nu \, d\nu = \sqrt{2\pi}\,\gamma \int_0^\infty (1-e^{-u})\, u^{-3/2}\, du$$

$$\therefore A = \sqrt{8\pi\gamma^2 x} \quad \propto \sqrt{w}$$

Thus weak lines show linear curves of growth + strong lines show square-root absorption.

Now a band is composed of a superposition of such rot. lines. For lg. pressures or paths, the bands overlap + cannot be represented by the same law that the component lines are.

For σ pressures, start worrying about Doppler broadening — logarithmic part of curve of growth.

Weak line + strong line bands. If get both, get P + w. σ.

$$\int_0^\infty u^{-3/2}\, du = \left[\frac{u^{-1/2}}{-1/2}\right]_0^\infty \quad \therefore A = \sqrt{2\pi}\,\gamma\, 2\sqrt{k_\nu x}^{\,-1}$$

$$= \sqrt{2\pi}\,\gamma\, 2 \frac{1}{\sqrt{\frac{S}{2\pi\gamma} x}}$$

$$= \sqrt{4}$$

Spinrad & Adams + Dunham plates at 7820 Å.
Measured equiv widths:

$$A = \int_{\nu_1}^{\nu_2} A_\nu \, d\nu = \int_{\lambda_1}^{\lambda_2} A_\lambda \, d\lambda \equiv \bar{A}_\lambda \, \Delta\lambda$$

When $\bar{A}_\lambda$ is set $= 1$, we have $\Delta\lambda_J \equiv W(J)$. E.g.,

Plate	Phase	4	6	8	14	18	20	22	24	26	30	34
					W(J) in mA							
1745	65°	36	42	51	52	51	48	43	48	49	22	31
3028	67°	32	44	49	73	83	76	56	82	77	70	59
					mA							

Even J's because of nuclear spin selection rule.
Missing J's because of telluric overlapping.

Now 7820 Å is a band of weak lines ∴ for a standard clear atmosphere,

$$W(J) \propto \rho x(J) \propto N(J).$$

$$\frac{N_J}{\not{2}J+1} = N_0 \, e^{-BJ(J+1)/kT_{rot}}$$

$$\therefore \log_{10} \frac{W(J)}{(\not{2}J+1)} = \text{const.} - 0.434 \, \frac{J(J+1)}{T_{rot}}$$

putting in appropriate value of B.

γ is the half-width at half-maximum k_ν, or, simply, the half-width of the line.

$$\gamma = \gamma_0 \left(\frac{P}{P_0}\right)\left(\frac{T_0}{T}\right)^{1/2}$$

Comparing lab. w. planetary γ's gives P's. Weak T dependence.

where 0 refers to some ref. level. When $\nu = \nu_0$, k_ν is max, + $k_\nu = S/\pi\gamma$. When $\nu - \nu_0 = \pm\gamma$,

$$k_\nu = S/2\pi\gamma,$$

or $\frac{1}{2}$ the max value.

Assume that I_0 is const. within the line's $\Delta\nu$.

$$\therefore A_\nu = \frac{I_0 - I_\nu}{I_0} = 1 - \frac{I_\nu}{I_0} = 1 - e^{-k_\nu \rho x}.$$

$$\therefore A = \int_{\nu_1}^{\nu_2} A_\nu \, d\nu = \int_{\nu_1}^{\nu_2} (1 - e^{-k_\nu \rho x}) \, d\nu.$$

In the case of a weak line,

$$1 - e^{-k_\nu \rho x} \approx k_\nu \rho x,$$

and

$$A = S \rho x \propto P w$$

The total absorption is $\propto$ to the amount of gas.

Absorption by a single spectral line:

In the lower atmos. + troposphere, the shape of a spectral line is essentially determined by pressure broadening alone. Doppler, Stark, + Zeeman effects can generally be neglected except on other Doppler impurities - [illegible]

Consider rad. of intensity I_ν^0 incident upon a slab of thickness x. Some of radiation will be absorbed, and some transmitted. At any pt. within the slab, the loss of energy $\propto I_\nu$ and to ρ. ∴ in each thickness dx,

homog. ρ distrib.

$$dI_\nu = -k_\nu I_\nu \rho \, dx$$

where k_ν is a mass absorp. coeff. characteristic of the material.

$$\therefore \; I_\nu = I_\nu^0 e^{-k_\nu \rho x} = I_\nu^0 e^{-\tau}$$

The relation is true only for monochromatic radiation, since k_ν is func. of ν. For a pressure-broadened absorption line, the variation of k_ν w. ν is given by the Lorentz line-shape:

$$k_\nu = \frac{S}{\pi} \frac{\gamma}{(\nu - \nu_0)^2 + \gamma^2}$$

an eq. derived from consid. of broadening of energy levels by the field of impacting atoms. S is called the integrated absorption coeff., since

$$\int_{-\infty}^{+\infty} k_\nu \, d\nu = S$$

Tm between colls which determines line shape.

Sagan & Pollack (1967) JGR 72 469

Anisotropic nonconservative scattering ...

$$\frac{1}{\sqrt{3}}\frac{dI_+}{d\tau} = -I_+ + I_+\tilde{\omega}_0(1-\beta) + I_-\beta\tilde{\omega}_0$$

$$-\frac{1}{\sqrt{3}}\frac{dI_-}{d\tau} = -I_- + I_-\tilde{\omega}_0(1-\beta) + I_+\beta\tilde{\omega}_0$$

two stream average of μ

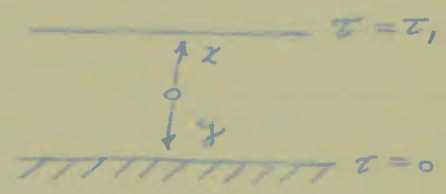
forward & back scatter

$$\frac{dx}{d\tau} = ax + by \qquad x = I_+ \qquad a = \sqrt{3}\left[\tilde{\omega}_0(1-\beta) - 1\right]$$

$$-\frac{dy}{d\tau} = ay - bx \qquad y = I_- \qquad b = \sqrt{3}\beta\tilde{\omega}_0$$

$$(D-a)x = by$$

$$-bx = (D+a)y$$



$$(D+a)(D-a)x = (D+a)by$$

$$(D^2 - a^2)x = -bx^2$$

$$\left[D^2 - (a^2 - b^2)\right]x = 0 \qquad (D^2 - c^2)x = 0 \qquad \frac{d^2x}{d\tau^2} = c^2 x$$

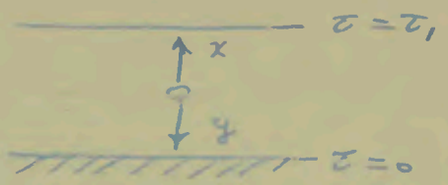
$$c^2 = a^2 - b^2 = 3\left[(1-\tilde{\omega}_0)(1-\tilde{\omega}_0 + 2\beta\tilde{\omega}_0)\right] = 3u^2(1-\tilde{\omega}_0)^2$$

$$1 - \tilde{\omega}_0 + 2\beta\tilde{\omega}_0 - \tilde{\omega}_0 + \tilde{\omega}_0^2 - 2\beta\tilde{\omega}_0^2$$

where $u^2 \equiv 1 + \dfrac{2\beta\tilde{\omega}_0}{1-\tilde{\omega}_0}$

$$x = fe^{c\tau} + ge^{-c\tau}$$

$$y = \frac{1}{b}(D-a)x = \frac{c-a}{b}fe^{c\tau} - \frac{c+a}{b}ge^{-c\tau}$$



$$x = f e^{c\tau} + g e^{-c\tau}$$

$$x_0 = 0 \qquad f + g = 0 \qquad g = -f$$

$$x = f\left(e^{c\tau} - e^{-c\tau}\right)$$

$$y = \frac{c-a}{b} f e^{c\tau} - \frac{c+a}{b} g e^{-c\tau}$$

Can show $\quad \dfrac{c-a}{b} = \dfrac{u+1}{u-1} \qquad \dfrac{c+a}{b} = -\dfrac{u-1}{u+1}$

$$y = \frac{u+1}{u-1} f e^{c\tau} - \frac{u-1}{u+1} f e^{-c\tau}$$

$$= f\left[\frac{(u+1)^2 e^{c\tau} - (u-1)^2 e^{-c\tau}}{u^2-1}\right]$$

$$R = \frac{x_{\tau_1}}{y_{\tau_1}} = \frac{\left(e^{c\tau_1} - e^{-c\tau_1}\right)(u^2-1)}{(u+1)^2 e^{c\tau_1} - (u-1)^2 e^{-c\tau_1}}$$

$$= \frac{(u+1)(u-1)\left(e^{\tau_{eff}} - e^{-\tau_{eff}}\right)}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 e^{-\tau_{eff}}}$$

$$J = \frac{y_0}{y_{\tau_1}} = \frac{(u+1)^2 - (u-1)^2}{(u+1)^2 e^{c\tau_1} - (u-1)^2 e^{-c\tau_1}}$$

$$= \frac{4u}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 e^{-\tau_{eff}}}$$

where $\tau_{eff} = c\tau_1$

$$x = fe^{c\tau} + ge^{-c\tau} \qquad y = \frac{u+1}{u-1} fe^{c\tau} + \frac{u-1}{u+1} ge^{-c\tau}$$

$$x_0 = Ay_0 \qquad f+g = A\left(\frac{u+1}{u-1} f + \frac{u-1}{u+1} g\right)$$

$$g = -Bf \qquad B = \frac{1 - A\frac{u+1}{u-1}}{1 - A\frac{u-1}{u+1}}$$

$$x = f\left(e^{c\tau} - Be^{-c\tau}\right) \qquad y = f\left(\frac{u+1}{u-1} e^{c\tau} - \frac{u-1}{u+1} Be^{-c\tau}\right)$$

$$R = \frac{x_{\tau_1}}{y_{\tau_1}} = \frac{\left(e^{c\tau_1} - Be^{-c\tau_1}\right)(u^2-1)}{(u+1)^2 e^{c\tau_1} - (u-1)^2 Be^{-c\tau_1}}$$

$$= \frac{(u+1)(u-1)\left(e^{\tau_{eff}} - Be^{-\tau_{eff}}\right)}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 Be^{-\tau_{eff}}}$$

$$J = \frac{y_0}{y_{\tau_1}} = \frac{(u+1)^2 - (u-1)^2 B}{(u+1)^2 e^{c\tau_1} - (u-1)^2 Be^{-c\tau_1}}$$

$$= \frac{(u^2+1)(1-B) + 2u(1+B)}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 Be^{-\tau_{eff}}}$$

Conservative scattering $\varpi_0 \to 1$

$$\tau_{eff} = 3^{1/2} u(1-\varpi_0)\tau_1 = \sqrt{3(1-\varpi_0+2\beta\varpi_0)(1-\varpi_0)}\,\tau_1$$

$$\approx \sqrt{3(2\beta)(1-\varpi_0)}\,\tau_1 \to 0$$

so can write $e^{\pm\tau_{eff}} \approx 1 \pm \tau_{eff}$. Then

$$J = \frac{4u}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 e^{-\tau_{eff}}} \approx \frac{4u}{(u+1)^2(1+\tau_{eff}) - (u-1)^2(1-\tau_{eff})}$$

$$= \frac{4u}{[(u+1)^2-(u-1)^2] + \tau_{eff}[(u+1)^2+(u-1)^2]}$$

$$= \frac{4u}{4u + 2\tau_{eff}(u^2+1)} = \frac{4u}{4u + 2\sqrt{3}\,u(1-\varpi_0)\tau_1(u^2+1)}$$

$$= \frac{1}{1 + \frac{\sqrt{3}}{2}(1-\varpi_0)\tau_1(u^2+1)}$$

Now $u^2 = 1 + \dfrac{2\beta\varpi_0}{1-\varpi_0}$, so $u^2+1 = 2\left(\dfrac{1-\varpi_0+\beta\varpi_0}{1-\varpi_0}\right)$.
thus

$$J \approx \frac{1}{1+\sqrt{3}\,\tau_1(1-\varpi_0+\beta\varpi_0)} \approx \frac{1}{1+\sqrt{3}\,\beta\tau_1}$$

$$\approx \frac{1.16}{2\beta\tau_1 + 1.16}$$

Nonconservative isotropic scattering with $\tau_1 = \infty$

$$\left.\begin{array}{l} \varpi_0 \neq 1 \\ \beta = \frac{1}{2} \\ \tau_1 = \infty \end{array}\right\} \quad u^2 = \frac{1 - \varpi_0 + 2\beta\varpi_0}{1 - \varpi_0} = \frac{1}{1 - \varpi_0}$$

$$\tau_{eff} = 3^{1/2} u (1 - \varpi_0) \tau_1 = 3^{1/2} (1 - \varpi_0)^{1/2} \tau_1 = \infty$$

$$\mathcal{R} = \frac{(u+1)(u-1)(e^{\tau_{eff}} - \cancelto{0}{e^{-\tau_{eff}}})}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 \cancelto{0}{e^{-\tau_{eff}}}} = \frac{u-1}{u+1}$$

$$= \frac{1 - 1/u}{1 + 1/u} = \frac{1 - \sqrt{1 - \varpi_0}}{1 + \sqrt{1 - \varpi_0}}$$

$$\mathcal{T} = \frac{4u}{(u+1)^2 e^{\tau_{eff}} - (u-1)^2 \cancelto{0}{e^{-\tau_{eff}}}} = \frac{4u}{(u+1)^2 e^{\tau_{eff}}} = 0$$

$$\mathcal{A} = 1 - \mathcal{R} - \mathcal{T} = 1 - \mathcal{R}$$

$\varpi_0 = 0.1 \qquad \mathcal{R} = \dfrac{1 - \sqrt{0.9}}{1 + \sqrt{0.9}} = \dfrac{0.051}{1.949} = 0.026$

$\varpi_0 = 0.5 \qquad \mathcal{R} = \dfrac{1 - \sqrt{0.5}}{1 + \sqrt{0.5}} = \dfrac{0.293}{1.707} = 0.172$

$\varpi_0 = 0.9 \qquad \mathcal{R} = \dfrac{1 - \sqrt{0.1}}{1 + \sqrt{0.1}} = \dfrac{0.684}{1.316} = 0.520$

$\varpi_0 = 0.975 \qquad \mathcal{R} = \dfrac{1 - \sqrt{0.025}}{1 + \sqrt{0.025}} = \dfrac{0.842}{1.158} = 0.727$

$$p = \varpi_0 (1 + \cos\Theta) \quad ; \quad \tau_1 = \infty$$

$$2\beta = 1 - \int p \cos\Theta \frac{d\Omega'}{4\pi} = 1 - \varpi_0 \int (1 + \cos\Theta) \cos\Theta \frac{d\Omega'}{4\pi}$$

$$= 1 - \frac{\varpi_0}{2} \int_0^\pi (1 + \cos\Theta) \cos\Theta \sin\Theta \, d\Theta$$

$$= 1 - \frac{\varpi_0}{2} \int_0^\pi \cos^2\Theta \sin\Theta \, d\Theta = 1 - \frac{\varpi_0}{3}$$

$$u^2 = \frac{1 - \varpi_0 + 2\beta\varpi_0}{1 - \varpi_0} = \frac{1 - \varpi_0 + \left(1 - \frac{\varpi_0}{3}\right)\varpi_0}{1 - \varpi_0}$$

$$= \frac{1 - \frac{1}{3}\varpi_0^2}{1 - \varpi_0}$$

With $\tau_1 = \infty$, $\quad R = \frac{u-1}{u+1}$, $\quad T = 0$, $\quad A = 1 - R$

ϖ_0	R
0.1	0.025
0.5	0.150
0.9	0.460
0.975	0.680

For $\varpi_0 = 1$ and $\tau_1 = 20$

$$\mathcal{T} = \frac{1.16}{2\beta\tau_1 + 1.16} \qquad 2\beta \approx 1 - \tfrac{1}{3}\varpi_1$$

$$\therefore \mathcal{T} \approx \frac{1.16}{(1-\frac{1}{3}\varpi_1)\tau_1 + 1.16} = \frac{1.16}{20(1-\frac{1}{3}\varpi_1) + 1.16}$$

Agrees with the table for various ϖ_1.

β, $\varpi_0 \Rightarrow u$. Trade anisotropy for many scatterings. Effect of surface s

Critical Review

A. Intro
B. Origin of Life. ♃
C. Environmental specification: ♀, ☾, ♂, ♃
D. Direct evidence
  a. Chondrites
  b. ♂ Expt's: seasonal ir changes; uv correlation
E. Intelligence

A. Introduction

Ladies + gentlemen, it is my pleasant ~~duty~~ task to ~~review~~ discuss with you some aspects of extraterrestrial life, that never-never land between astronomy and biology, on whose borders we now ~~stand~~ find ourselves ~~standing~~ perhaps a little breathless. Dr. Rasool has given an excellent summary of the pertinent physical + chemical information, + evolutionary hypotheses, which we have about the planets, and I will rely on his presentation. There are three general approaches to the problem of extraterrestrial life. First, we ~~can~~ may ask how general were the processes leading to the origin of life on earth. ~~Second, we If they were very general, we~~ Second we may investigate the planetary environments, and inquire what the likelihood is that familiar lifeforms can there survive and grow. Thirdly, we may ~~search for~~ seek direct evidence for extraterrestrial life. I would like to discuss these approaches in this sequence. I will ~~try~~ my best to separate fact from speculation, and distinguish the enthusiasts from the pessimists. Almost every issue of consequence, it turns out, has two sides.

B. Origin of Life

To talk of e.t. life, we must have some idea of terr. life, The ⊕ is the only ~~one~~ plant, after all, which we are certain ~~[illegible]~~ is inhabited

1. Contemporary terrestrial organisms all have a common biochemical ground plan.

DNA, RNA, ~~adapter~~ messenger+, proteins, enzymes.
DNA synthesis from triphosphates, ATP, + ADP

2. These common substances, are they rare mols. selected for their efficiency, or are they easily produced?

Miller - pim. environ., hydrides ⟹ amino + hydroxy acids. Corona discharg., uv. Intermediate steps involve cyanides + aldehydes.

Adenine produced by electron beam irradiation of CH_4, NH_3, H_2O + H_2 + by uv irradiation of dilute sol. HCN. Guanine also formed in latter exp't: Orō, Pommerenne, + co-workers.

Uv irrad. of dilute aldehyde sols produces ribose + 2-deoxyribose. Condensation of several aldehydes.

Some expectation of phosphates in pim. seas. ∴ all building blocks of nucleic acids around. P.P + Syn makes nucleoside triphosphates, in high yield, e.g., ATP. We have not yet done w. 2-deoxyribose. If that succeeds, we are up to Kornberg w. enzyme replaced by time. ∴ from pim atmos. to self-replicating polynucleotide understood, +, it is sometimes said, nat. selec. then takes over, + we understand all. Subsequent evol. to preserve initial conds. Butler's aphorism. This may be an important part of the story, but not all of it.

3. Criticisms

a. All these mols. may be used later because they are efficient. Earliest life-forms may have been very diff. Pirie

b. Pure sols. instead of gunk. Horowitz, Miller

c. Ethyl metaphosphate not most common P cmpd. in prim. environ.

d. No environmental control. Elaborate contemporary apparatus. Rich + Stout.

Much still to be performed on origin of life. But results so far — only 10 yrs. since Miller's classic exp't — are extremely encouraging, + give us some confidence that protein + nucleic acids may be dominant elsewhere, + that life arises easily. Morphology elsewhere, diff., of course. This makes chem. life-detection exp'ts easier, but it also makes the risk of biolog. contam. much higher. [Insert from Denver p. 4]

4. Panspermia

C. Environments

There are locales in our solar system where the above events leading to origin of life may today be occurring. 4. Depending on NH_3 abundance, Gallet has a H_2O cloud layer at

$$10^{-3}\ gm\ cm^{-3} \le \rho \le 10^{-1}\ gm\ cm^{-3}$$

and

$$200°K \le T \le 400°K$$

From afar best poss. for life in solar system.

x lot clouds?

☿, ♀, ☾ · subsurface – ∃ H_2O, org. matter, heat suggestions based on various evidence. Direct obs. needed

♂: Conds. no doubt severe. Alfred Russel Wallace (1907). Expt's performed using known organism + selective culture techniques. Reported this symposium. Both survival + growth reported.

Growth especially difficult to assay because of starvation replication w. no organism mass increase.

H_2O content of soil similar to the Martian? What's the Martian soil?

But vast literature in soil science + food tech. ⇒ survival + growth. Relevant for contam., not for ♂ biology – except to suggest that mechanism exist.

D. Direct evidence

1. Carbonaceous chondrites which we get free.
   a. Org. matter: Berthelot, Berzelius, Wöhler. ~few % by mass + not contam. Somewhere a lot of org. matter was made. Asteroid? ☾?

   b. Organized elements. Claus + Nagy. Andrus + Fitch

   c. Viable organisms. Sisler + Newton. Facultative anaerobe.

2. ♂

2. Life on ⊕.

a. Green

b. Canals

c. Wave of darkening + color Δs. — Organisms — Deliquescent salts

c'. Secular Δ's.

d. Polarimetric evidence — growth — size change due to H_2O adsorption. (Not enough H_2O, prob.)

e. Ir spectrum. If absorption, at 3.45, 3.58, + 3.69 μ. But refl. complicates. If absorp., only identifier which has been proposed, + which explains the features are HC + CH_3CHO. On ♂, CH_3CHO will be a gas when $T \gtrsim 240°K$. Thus, gen. a gas in day, + condensate at night. Two approaches: gas restricted to dk. area is absurd, so its something else which we don't understand. Other approach is to ask how the gas makes sense. Aldehyde polymerization $\xrightarrow{uv}$ sugars. Might we here have a limit of a ♂ biochem, utilizing uv? Need glass envelope, opaque in vis., transp. in ir + uv. No H-C's

If we take optimistic side of all the above, what do we have? We don't even know if the data all refer to the same organism. Like parable of blind men + elephant, but with ~~many~~ a lot of other log. animals added.

If we nevertheless assume a common origin, the ff picture emerges:

An organism, 0.1 to 1 mm in size, with a glassy cover, transp. in ir + uv, + opaque in visible. Color predominantly gray, sometimes browns, greens, blues, etc. Contains CH_3CHO inside envelope. Cover a few tens of % of the dk. areas. Changes color, size, + reflectivity in spring when H_2O + content greatest.

Of course this description may be totally wrong. But it does emphasize that if ∃ life on ♂, it will ~~very~~ likely be very unfamiliar.

No. exp'ts poss. to improve our understanding of poss. life on ♂, without actually landing there.

(1) High topograph. resol. ir

(2) Seasonal Δ ir bands

(3) Correlation blue clearing w. polarimetric or ir spectrograph or vis. obs.

~~Eventually~~ Soon we will have ~~sophisticated~~ the capability for landing instruments ~~biology expts~~ for ~~vehicles for~~ ♂ biology exploration. We must prepare for that unique opportunity. It will only come once. ~~Our scientific descend.~~ Scientists of future generations will judge how well we do.

M31: Other opportunities may exist, but they will be inaccessible for a long time

Murray calls "conductive" theoret. temp. when heating on πR^2, conduction results in ~~cooling~~ heating of from $2\pi R^2$, both hemispheres.

If ♀ microwave limb-darkening cannot be explained by atmos. absorption, then might it not — as is probably the case for the ir limb-darkening — be explicable by absorption + scattering by a cloud layer, here possibly a H-C layer in the lower atmosphere? Plot HC v.p. curves vs. atmos. v.p. curves. Likely H-C's from high pressure N_2, H_2O, CO_2 interaction. Note v.p. curves not isothermal, but subadiabatic.

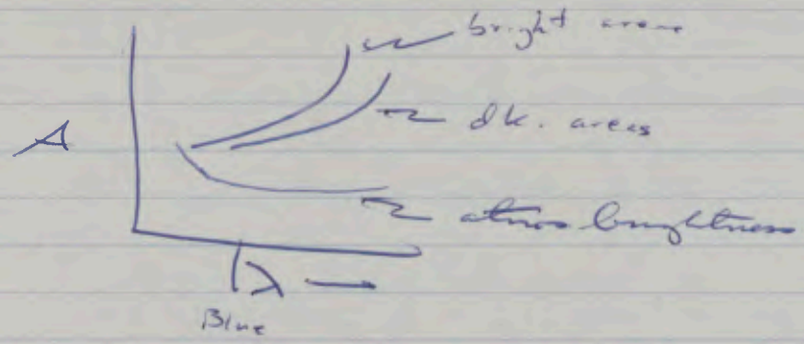
- Dollfus' $A = A(\lambda)$ permits an integration,

$$A_{bol} = \frac{\int_0^\infty A(\lambda) B_\lambda \, d\lambda}{\int_0^\infty B_\lambda \, d\lambda} \quad ; \quad B_\lambda \text{ for } T \simeq 5550\,°K.$$

+ will give an $A_{bol} \gtrsim 0.20$ for both bright + dark areas. Extension beyond $\lambda \sim 8000$ Å can be made (1) by assuming $A_{\lambda > 8000\,Å} \simeq A_{\lambda = 8000\,Å}$, or by (2) extension of existing slope. This gives "min" + max values of A_{bol}. ∴ computed T for airless ♂ will be $\ll 219\,°K$. ∴ greenhouse effect very important there. I suspect that even with $w_{CO_2} = 100$ m-atm + $w_{H_2O} = 10^{-2}$ gm cm^{-2}, greenhouse effect will not be sufficient to explain observed temps. w. emissivity correction [Note Rea thinks $T \propto \varepsilon^{-1/4}$ for 10μ window. Actually less steep.] ∴ another absorber on ♂. CH_3CHO, e.g.? Scattering by dust?

- Seems to exist a ⊙ argument regarding CO_2 + P_s on ♂. For photometric + polarimetric determination of P_s, need w_{CO_2}, because by Rayleigh coefficient of CO_2. But to determine w_{CO_2}, need P_s for the pressure broadening.

Dollfus pointed out blue haze may be mostly illusory.



I.e., contrast actually declines towards UV. Blue clearing may simply represent the tendency of blue clouds to form in bright areas, thereby enhancing the contrast mentally. ∃ some evidence bright areas are higher + ∴ are more likely to have clouds above them. Residual frost near polar caps and more frequently left in bright areas than in dark. Also Dollfus detected polarimetrically one frost deposit on Nix Olympica, which is in a bright area.

Chromatography Notes

Aniline Hydrogen Phthalate spray
for Sugars

0.93 gm aniline
1.66 gm phthalic acid – 8.30
100 ml H_2O ~~saturated~~ w. N. Butanol

Nature 164: 443, 1949

5 min at 105°C develops.

Chromatog. Systems

Amino acids

Lutidine : Ethanol : Water

55 : 25 : 20

n-Propanol - Ammonia - Water
40 : 30 : 10

Acetone : 25% Trichloracetic Acid
75 : 25

25 gm Trichloracetic Acid + enough H_2O
to make 100 cc.
100 gm to 400 cc.

Beaker weighs 112.5 gm.
add 50 gm solid trichloracetic acid
so total weighs 162.5 gm.
Add 200 cc. water

n-Propanol : Ethyl acetate : water
7 : 1 : 2

To make 1500 ml,

n Propanol : 700 + 350 = 1050
Ethyl acetate : 100 + 50 = 150
Water 200 + 100 = 300
1500 ml ✓

Actually mixed 500 ml

n Butanol : Acetic acid : water
4 : 1 : 5

To make 1500 ml,

n Butanol : 400 + 200 = 600
Acetic acid : 100 + 50 = 150
Water : 500 + 250 = 750
1500 ml ✓

$\lambda = 1\,\mu$liter

①

Whatman #4 washed w. 1% solution Oxalic acid + Folded
Rinse w. distilled H_2O
Dry overnight

Whatman #1 is a slow paper.

Sample evacuate until bubbling stops.
Freeze in N_2 ↓ dewar
Evacuate to ~150 μ pp air.
Then, if required, add H_2.

Irradiate

Then access of air is given to the samples
Add unlabled carrier.

Mark paper for origin
Turn hairdryer to hot + on.
For simultaneous spotting of several papers,
back origins w. glass to insure reabsorbtion
+ prevent spotting of underlying papers.

Spot 1 drop on origin w. dispo pipette.
~~Burn~~ Dry w. hair dryer.
Add 2nd drop. Dry.
Continue until sol. depletion. > 10.
(Do not touch glass pipette to paper)

If too much sample + carrier, first evaporate w. evaporator. Be careful to first wash all parts of evaporator which will have contact w. sol.

Label papers below origins, at lower rt. crnr.
Do not heat w. dryer longer than necessary.
∃ some poss. of thermal degradation of sample.

Wash trays.

Put paper on tray; hold w. weight.

Put first solvent system at bottom of chromatography cabinet, below, but not touching paper.

Seal cabinet, + let solvent permeate cabinet (at least 30 min.; sometimes overnight).

Drop ~ 100 ml of the <u>same</u> solvent system through funnel into tray.

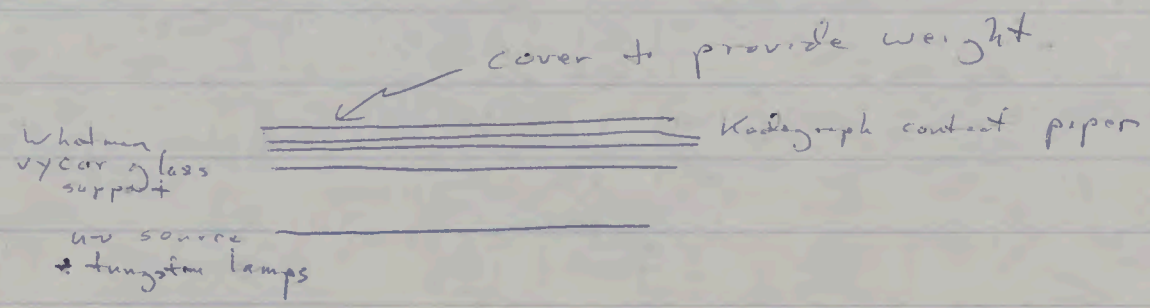
Let for ~ 5 or 6 hrs for most solvent systems; or enough for spots to move $\frac{2}{3}$ to $\frac{3}{4}$ of paper, but not off it. (Not, usually for overnight.).
or it moves off the paper altogether

Then open to exhaust system + open ~~a~~ holes.
Leave overnight to dry.

Next A.M. remove paper, remove dish at bottom.
Cut fold w/ paper cutter.
Turn so origin moves from upper left to upper right. Fold again.
Put new solvent system in bottom of cabinet + repeat everything as before.

Now autoradiography, shadowgrams, + end-window counting

~~Autoradiography~~
Shadowgrams:
In dark room.



Stamp w. radioactive ink.

~½ sec uv dose (uv lamp has timer)
developing sol. until can see developing (red lt. on).
dilute acetic acid bath quick rinse. (use rubber gloves).
>5 min in fixer (hypo).
hypo clearing agent (reduces required washing time)
rinse in water for ~ 10 min in special trays.
About ½ hr in all.
Dry contact paper in drying machine.

While developing above, make contact of paper with X-ray film.
2 types " " : blue-sensitive, & no-screen.
No screen has emulsion on both sides.
Fold Whatman paper around X-ray film.
Put in X-ray folder.

Carl Sagan

A CONCIDENCE TECHNIQUE FOR PAPER CHROMATOGRAPHY OF SOME BIOLOGICALLY IMPORTANT COMPOUNDS

The almost explosive growth of chromatographic methods during recent years has provided us with powerful tools for the separation of a wide variety of chemical substances. Such diverse materials as complex macromolecules of biological systems or the fission products from atomic piles have been effectively separated by these new methods.

The impetus given to the use of paper chromatography by the Cambridge group of workers under A.J.P. Martin has resulted in the great popularity of this technique. The isolation and identification of trace quantities of a host of natural and synthetic substances has thus been made possible.

The Rf value or ratio of distance traveled by substance to the distance traveled by solvent front, is generally used as the criterion of identity. If two substances have the same Rf values in two or more solvent systems, they are assumed to be identical. But although one often sees in the literature, Rf values quoted to an astonishing degree of precision, experience shows that Rf values are at best only a rough guide, and should not be used as the sole means of identifying a substance.

Rf values are dependent on a large number of variables, among which may be mentioned:

1. Composition of development phase
2. Kind of paper
3. Direction of paper
4. Manner of development (descending, ascending, ascending-descending, radial).

5. Length of paper used for development

6. Distance of starting line from solvent

7. Concentration of solute

8. Presence of other substrates

9. Temperature of development

The uncertainty accompanying the identification by Rf values alone is illustrated very pertinently from the following case study from some of our own work on the radiolysis of adenine. The first slide is an autoradiograph of an irradiated solution of adenine. Two major products are indicated, A and B. We identified A as 4-6·diamino 5-formamido pyrimidine and B as 8-hydroxyadenine. The chromatographic identification was further confirmed by chemical test and ultraviolet spectrophotometry.

However, at the same time another group of workers reported that compound B was Adenine 1-N-oxide, basing their identification on Rf values. The following Rf values for 8-hydroxyadenine and Adenine 1-N-oxide indicated that no differentiation could have been made on this criterion alone:

	ADENINE-8-OH	ADENINE-1-N-OXIDE
Propanol-Ammonia-Water	.47	.51
Butanol-Propionic Acid	.61	.66
Isobutric Acid-Ammonia	.54	.58
Butanol-Water	.62	.64

To establish the identity of compound B the chromatography of the original sample was repeated, adding adenine-1-N-oxide as a carrier. (The

solvent systems were composed of Propanol-Ammonia-Water and Butanol-Propionic Acid-Water.) The next slide demonstrates the results obtained. The bright areas on the shadowgram are the Adenine-1-N-oxide and the residual adenine. The dark spot B on the autoradiograph appears to be coincident with the bright area on the shadowgram.

The experiment was then repeated in two other solvent systems. Isobutyric acid-ammonia and Butanol-Water. The results are shown in the next slide. The spot produced by the unlabeled Adenine-1-N-oxide has shifted further away from the radioactive spot B, as shown in slide #3. If B was indeed Adenine 1-N-oxide, the radioactivity and the absorption would have coincided in both systems as in the case of the residual Adenine.

In our experimental work we have developed a technique which gives unambiguous results. Ultraviolet absorption photography has been used as early as 1949 by Markham and his co-workers for the location of purines and pyrimidines. Autoradiography has been extensively used since the pioneering work of Calvin and his associates with C^{14}. In the work we are presenting, the two techniques have been combined as one operation. This method has been successfully used for purines, pyrimidines, amino acids and sugars. C^{14} labeled compounds are used as starting materials in all reactions studied. Non-radioactive carriers of the assumed resultant products of the experiment are added to the original material before spotting. The material is co-chromatographed in two dimensions on Whatman No. 4 paper, previously washed with oxalic acid.

Autoradiographs are prepared by placing an x-ray film in close contact with the chromatogram for a period determined by the amount of radioactive material present. This time may range from one day to six months. We have found that 10,000 dpm or five micromicrocuries produces a spot on the x-ray film in approximately a week. Radioactive compounds appear as dark spots on the transparent x-ray film. If the radioactive products are identical with the carriers, the dark spots on the autoradiograph will correspond precisely to the brightenings of the shadowgram

When working with purines, pyrimidines, and other ultraviolet absorbing products, the shadowgrams are prepared by shining an ultraviolet light through the chromatogram on to Kodagraph contact paper. A G.E. germicidal lamp was used as the uv source from a distance of ten inches. The time of exposure is 1-2 seconds. To insure close contact of the papers and therefore sharper prints, a sheet of vycor glass which transmits light of the wavelength at which purines and pyrimidines absorb is placed between the light and the paper. The ultraviolet absorbing areas on the chromatogram appear as well-defined white spots on a dark background.

The next slide illustrates an experiment confirming the formation of adenine by the electron irradiation of a mixture of CH_4, NH_3 + H_2O. Radioactive compounds appear as dark spots. The non-radioactive carriers appear as bright spots on a dark background.

For greater certainty, the area corresponding to the matching dark and bright spots is cut out from the paper chromatogram, eluted with a suitable solvent, and rerun in two different solvent systems. This is

the equivalent of analysis in four single systems by any other means. If coincidence is established with this repeated procedure, the positive identification of the material can be assumed.

With amino acids or sugars, the carriers are located on the chromatogram with the aid of a color reaction. The shadowgram is prepared in the same manner as for the purines and pyrimidines, but a visible light source is used instead of an ultraviolet light. The colored areas absorb the light and a well-defined bright spot appears on a dark background. The following three slides demonstrate the results obtained with purines and pyrimidines, with amino acids, and with sugars. In all these cases, it is understood that the amount of radioactive material is insufficient to be detected by a shadowgram alone.

This identification technique can be performed in reverse order also. When starting with unlabeled material, a radioactive carrier is added before spotting. The amount of radioactive product should be below the limit of detectability for the shadowgram but large enough to produce a dark spot on the x-ray film. (The limit of detectability for purines and pyrimidines is about 0.003 ug of material. For amino acids, the limit is .04 ug).

The next slide illustrates this reverse procedure. The identification of a trace amount Guanine which was synthesized during the thermal polymerization of amino acids.

Our work has been primarily concerned with purines, pyrimidines, amino acids and sugars. This coincidence technique, however, can be applied to

many other compounds which will give a color reaction such as fatty acids, peptides, steroids, etc., in fact, to any compound which can absorb ultra-violet or visible light. The application of this coincidence technique provides an unambiguous identification of compounds without the uncertainty involved in using Rf values and often eliminating the need for tedious chemical analysis.

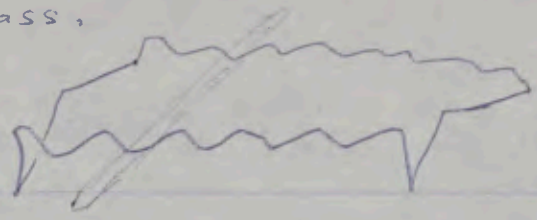
Chromatography
Protocol and
Equipment Notes

"Spectrometric Identification of Organic Compounds" — Silverstein Bassler, Wiley, 1963

Vycor tubes + glass stopcocks.

New England Nuclear Corp.
575 Albany Street
Boston 18, Mass.

Freezer for unstable chems.



Pipette tray

2.4 ml H_2O to Adenine-8-C^{14} → 3×10^{-3} M sol. adenine

500 μl of this " sol.

Add 22 μl ~25 μl 2-deoxyribose + 90 μl

For stoichiometric proportion add H_3PO_4 45 μl.

of 0.01 M sol. H_3PO_4 + 180 μl

500 μl

20 μl 2 do

36 μl H_3PO_4

1 ml

0.56 ml of sol + 0.44 ml of H_2O

= 1 ml solution

Deoxyadenosine H_2O ~10 mg/ml

Paper: 0.32182 gm.

With powder: 0.34382

$$\begin{array}{r} -0.32182 \\ \hline 0.02200 \text{ gm} \end{array}$$

add 2.2 ml.

2-deoxy-D-ribose ~10 mg/ml.

Paper: 0.33946

Add ~ 0.02 gm.

Neon ribose: 0.35807
−0.33946
0.01861 gm

Add ~1.9 ml.

Add ~ 10 mg → 0.35164 gm

Actual amount 0.35836
−0.34164
0.01672 gm.

∴ ~ 1.67 ml H_2O for 10 mg/ml.

DL-glyceraldehyde: $CH_2OH\ CHOH\ CHO$
25 μg. Very soluble

Powder paper: 0.32332 gm.

[Add 0.00003 gm – scale too inaccurate]

W. glyceraldehyde 0.32353
−0.32332
0.00021 = 210 μg.

in 2.1 ml water.

Solubility: β-D-glucose, M.W. 180.2

β has 154 at 15°C in H_2O

i.e., 154 gm/100 ml = 1.54 gm/ml.

∴ 10^{-3} gm requires $\frac{10^{-3}}{1.54} \simeq 0.6 \times 10^{-3} = 6 \times 10^{-4}$ ml

for saturated sol.

Ruth Mariner says 10 mg/ml for glucose standards.

& that 100 μg gives good color definition on paper.

Powder Paper: 0.34164 gm.

~~Add ~10 mg ~0.01 gm → 0.34[illegible] gm~~

~~Actual amt: 0.34548 gm~~

~~− 0.34164~~

~~0.00384~~

~~3.84 mg β-D glucose~~

Small Hood

Büchi Evaporators (2), Glasapparatefabrik Flawil
+ assorted evaporation flasks + ground glass stoppers + heavy ringstand bases.

Blotting Paper

Radioactive waste disposal bin; ordinarily for
sub millicurie range.

Disposable pipettes + rubber bulbs

Masslinn Non-woven towels

Ultrasonic cleaner: Acoustica Associates, Inc.,
Mineola, N.Y. Model DR 252

Sterilmaster small autoclave

Sinks

Lakeside Mfg. Inc., Milwaukee 7, Wisc.
Wheeled push cart, Model No. 526.

2 ~~2000~~ ml cylindrical graduates, stoppered; 2/500 ml.

Assorted 1.5 to 2 l ~~stoppered bottles~~

Marking crayons

Electric sockets on benches for hairdryers + evaporators.

N Butanol : Formic Acid : Water, 77 : 10 : 13

adenine deoxyadenosine standard

+ a adenine-deoxyribose-phosphoric acid mix

⇒ ~ 1500 ml in stoppered graduate.

N Butanol : 770 + 385 = 1155 ml
Formic Acid : 100 + 50 = 150 ml
Water : 130 + 65 = 195 ml
1500 ml ✓

Solvent solution must be made up fresh.
∃ a tendency to decay, if used > a few days after being mixed.

Micropipettes + syringes from Research Specialties Co, Model 1850

1000 ml Erlenmeyer
100 ml Narrow-necked Florence

Pipette Syringe: Instrumentation Associates, N.Y.

Kenmore Hair Dryers, Model 559-8710.

Khorana "Some recent developments in the chemistry of phosphate esters of biological interest," Wiley, 1961

Sudan black is a solvent front marker.

Acetic acid used as shadowgram development stop.
Kodagraph developer, Kodak fixer.
Kodagraph Contact Standard.

A Manual of Paper Chromatography
+ Paper Electrophoresis
– R. Block, E. L. Durrum, + G. Zweig
2nd ed., 1958
Academic Press

Uv exposure box:
front side
19" x 16" x 12" (depth)
Vycor glass sheet at top

Askay's Photo-Dry Model A-20A
Electric Print Dryer

Timers
Wratten Series 6B filters for darkroom lighting.

Drymaster X-ray Film Dryer, R-P Corporation

Picker X-ray
RK.

Universal Aerosol Spray Kit
Nutritional Biochem. Corp, Cleveland

Analine - hydrogen phthalate spray for sugars.

Nat. Appliance Co., Portland, Ore.
Chromatography oven

a) Y
Y

1) Y
Y Y

2)

Y

Standards.

a) 10 λ Adenine + 10 λ Deoxyadenosine

b) 10 λ β-D Glucose, 20 λ DL glyceraldehyde,
20 λ 2-Deoxy-D-ribose

Refrigerate overnight

For uv

(1)

Stoichiometric proportions
H_3PO_4
0.56 ml sol. +
0.44 ml H_2O

Tall
V_y cor

(2)

Min Ac. Dic proportions
H_3PO_4
0.56 ml sol. +
0.44 ml 0.01 M H_3PO_4.

Short
V_y cor

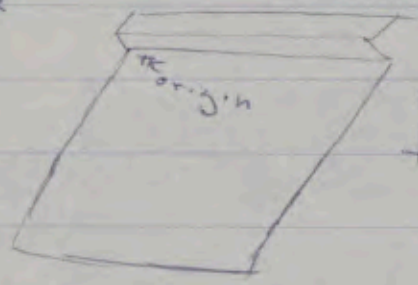
Irradiation Began at 3:55 P.M.

We've diluted so ∃ enough volume (~1 ml) to efficiently irradiate. Tomorrow we must evaporate so sol. is concentrated enough to make a few tens of λ spotting efficient.

~ 35 mL / trough of solvent permits
chromatographs to be run overnight

No. ① chromatogram, w. 2 x'a on
No. ② " , w. 2 holes in

Glass square 3" + 3" (approx.)
Rubber gloves
Fold paper



opposite folds to this.

End Window Counter - Nuclear Chicago Model 186 A.
with Model T1 Dual Timer.
Gas handling manifold and compressed gases

~~Chromatography~~ chambers cost ~$600.00 to build; sell at Richmond Co. Building for ~$1,000.00. They are, in this form, portable.

Glass still

"Data for Biochemical Research" – R.M.C. Dawson, D.C. Elliott, W.H. Elliott, K.M. Jones Oxford U.P., 1959.

~~to~~

– 1000 sheet boxes of Powder paper (glassine) $3\frac{1}{2}'' \times 4\frac{1}{2}''$ No. 4, El. Lilly + Co., Indianapolis, AX-29814-A.

– Mettler Instrument Corp., Highstown, N.J. balance.

– Distilled water siphon, cork-surrounded, in Florence flask.

– Laboratory benches w. lg. amt to drawers, stools.

– 5, 10, 20 ml beakers.

– Chromic trioxide for cleaning spatulas, etc.

Acetaldehyde note: redistilled CH_3CHO keeps indefinitely in 2 M- aqueous solution at −20°. Dilute solns keep in cold at least a week. Polymerizes readily at ord. temp (Dawson et al. p. 29) $\mu_{CH_3CHO} = 44 = \mu_{CO_2}$ ∴ it ∃ a physical reason for CH_3CHO restricted to dk. ones, also must ∃ CO_2

" " " " ,

Note ∃ glycoaldehyde CH_2OHCHO

1. Recipes for chromatography solvent systems
2. " " elution " "
~~3. Reactions by heating spot in air to dry?~~
4. Function of oxalic acid

Picker X-ray developing tank, 3 chambers + water bath; 6 min developer; wash; 5 min fixer; 5 min hypo clearing agent; wash.

Dry in oven ~ 15 min.

Shadowgram: diluted Kodagraph developer (powder) ~ few secs

~10% sol. Acetic acid stop bath ~ few secs

diluted Kodak fixer (powder) ~ 5 min

4:1 dilution Hypo clearing agent ~ 5 min

Wash ~ 5 min

Dry in

Arkay Photo-Dry

Model A-20 A

Electric print dryer

Paper ③ : 0.35013 gm.

added adenin makes 0.35167 gm.

$$\therefore \text{ an't adenin} = \begin{array}{r} 0.35167 \\ 0.35013 \\ \hline 0.00154 \end{array} \text{ gm} \simeq 1.54 \text{ mg.}$$

$$1.54 \text{ mgm requires } \begin{array}{r} 1.54 \\ 15 \\ \hline 1.69 \end{array} = 1.69 \text{ ml}$$

Standards preparation protocol

We desire 1 mg standard / mL solvent (usually water). If standard is entirely soluble in water we mix 1 mg w. 1 mL H_2O. Otherwise we add more water, weighted as the solubility, a quantity found in Dawson et. al.

The standard is weighed on a microbalance by difference methods. First weigh a piece of powder paper; then add the 1 mg or so of standard on the paper inside the balance chamber. Balance is sensitive to hands on table, the walking of passers-by etc. Powder is removed by spatulas; mixed w. water in glass vials. Water can't is measured by pipettes w. graduated scales. Vials have screw tops.

Solubility in gm solute dissolving in 100 ml solvent.

adenine : 0.09 at 25 °C.

$$0.09 \text{ gm} / 100 \text{ ml} = \frac{9\times10^{-2} \text{ gm}}{10^{2} \text{ ml}} = 9\times10^{-4} \text{ gm/ml.}$$

$$\therefore 10^{-3} \text{ gm requires } \frac{10^{-3}}{9\times10^{-4}} = 0.11\times10 = 1.1 \text{ ml.}$$

Just as liquid soap, ~ 1 mg / ml.

Paper weighing:

Paper ① 0.3615 gm

Paper ② 0.3423~~8~~ gm

We use paper ②. We want 1 mg = 0.001 gm adenine
∴ paper will weigh 0.34<u>34</u> gm w. adenine

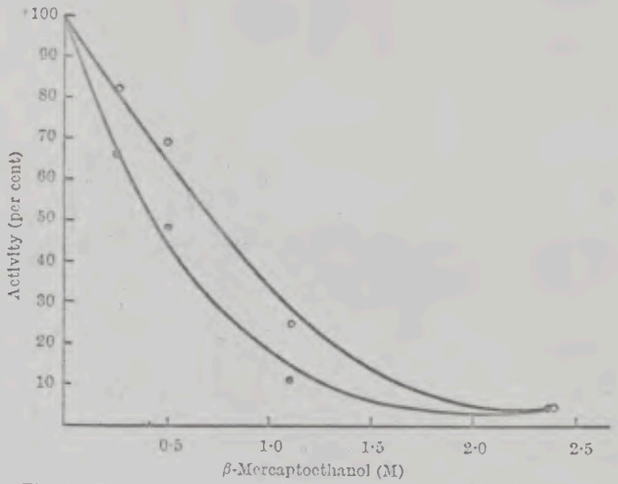


Fig. 4. Percentage enzymatic activity of crystalline pig heart LDH_1 (○) and rabbit muscle LDH_5 (●) isozymes after inhibition with β-mercaptoethanol at the concentrations indicated. Pyruvate was used as substrate

homogenates in normal human serum in order to simulate more closely the situation which arises in myocardial infarction. In these experiments it was verified, by calculation on the base of data observed with homogenates alone, that the degree of denaturation by urea in the serum corresponded with that received. Our observations of denaturation with 1·5 M urea in the conditions described indicate that this technique would be useful for separating the monomers occurring preponderantly in the heart tissue from others for clinical purposes; certainly the test described is simple to carry out.

After we had prepared our communication, Hardy (*Nature*, 206, 933; 1965) published work concerning the denaturation of LDH of human and liver homogenates by urea. The concentration used by him was higher (2·6 M). At this concentration our curves show nearly complete denaturation of all isozymes studied. This difference might well be due to the shorter incubation period used by him. Also the fact that the co-enzyme was added to the incubation solution may contribute to differences in the results.

This work was supported by the Sigrid Jusélius Foundation, Helsinki, Finland.

AARNE KONTTINEN
SEPPO LINDY

Wihuri Research Institute,
Helsinki, Finland.

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Ultra-violet Spectroscopy in the Analysis of Chromatograms

THE sensitivity of the normal human eye to light does not extend far below 400 mμ. Nevertheless, ultra-violet-absorbing compounds appear as dark spots on paper chromatograms illuminated with ultra-violet light. This is due to absorption of the light before it can excite the fluorescence of the paper, and constitutes one variety of quenching. Compounds which fluoresce in ultra-violet light will be detected on paper chromatograms if their fluorescence exceeds that of the surrounding paper. The light sources commonly employed emit the mercury lines at 254 mμ or 366 mμ. In chromatograms of biological extracts the short-wave lamp detects principally the absorbing compounds and the long-wave lamp fluorescing compounds. Beyond this the method possesses little inherent specificity. Thus compounds such as cortisone, nicotinic acid, caffeine and uric acid (λ_{max} 240, 260, 273 and 292 mμ respectively) all appear indistinguishable as dark spots under the short-wave lamp. Furthermore, for substances such as oestrogens, the absorption maximum (280 mμ) of which is very different from the 254 mμ emission of the handlamp, the method is relatively insensitive. A need therefore exists for an instrument with which absorption and activation maxima can be rapidly determined on the paper. I have accordingly designed an ultra-violet spectroscope in which the ultra-violet light is shifted to the visible region by the fluorescence of either the paper itself or a superimposed screen.

The light source, an 'Osram' 450-W xenon arc, provides an ultra-violet continuum. After passing through a quartz prism monochromator (Schoeffel Instrument Co., Westwood, N.J.) with variable exit slit, the light emerges through a $1\frac{3}{16} \times \frac{3}{4}$ in. rectangular aperture as a slightly divergent beam which illuminates the chromatogram. The latter is attached by spring clips to a frame, which can be moved manually in three planes, or by a motor in the direction of solvent flow only. A screen coated with a suitable phosphor can be placed in position over the illuminated portion of the chromatogram if desired. Among several phosphors examined, $ZnSiO_4$: Mn and $(Sr, Mg)_3(PO_4)_2$: Sn were found to be the most generally useful. The former is slightly phosphorescent, but not excessively so. It provides a bright illumination over the range 230–295 mμ and 330–400 mμ. The latter exhibits a continuous though less intense fluorescence over the range 230–370 mμ, and is therefore suitable for rapid, routine searching. To obtain linear wave-length drive the prism is rotated by means of a cam, which is attached to a digital counter. The optical arrangement is shown in Fig. 1, and the assembled apparatus in Fig. 2.

In order to make a visual estimate of the absorption maximum of a compound on a chromatogram, the wave-length knob is rotated until the spot is darkest. The fluorescent screen may be needed to provide adequate background illumination. With certain compounds the maximum is best approached from higher wave-lengths, as the absorption does not always fall off as sharply below the maximum. It is desirable that the brightness of the paper or screen should remain constant while the wave-length of the incident light is varied. To accomplish this a second cam can be used to vary the slit width at different wave-lengths, so as to compensate for changes in the output of the xenon arc, the dispersion of the monochromator, and the spectral response of the paper or screen phosphor.

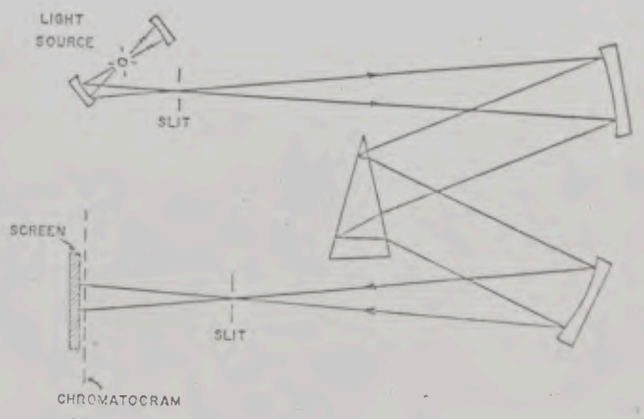

Fig. 1. Optical arrangement of the ultra-violet spectroscope

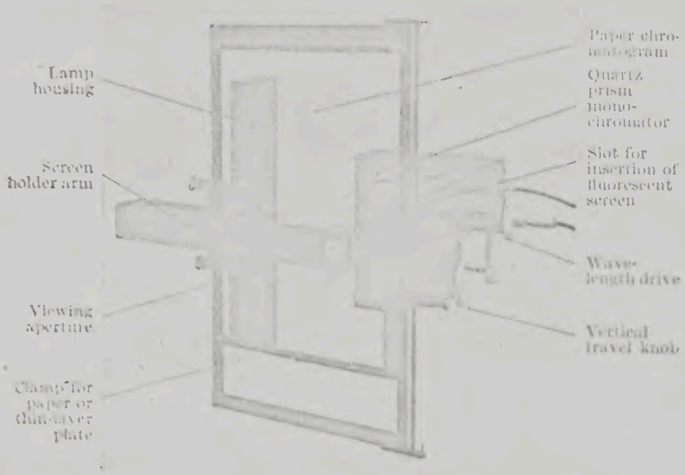

Fig. 2. The assembled spectroscope

If 'Kodabromide *F*5' or other suitable photographic paper[1] is placed in contact with the chromatogram, a record of absorbing or fluorescing[2] compounds can be obtained at any desired wave-length within the operating range of the instrument. If the entire strip is to be photographed the rectangular aperture is replaced by a narrow slit, past which the paper is drawn either by hand or by means of a motor. Arnold *et al.*[3] described a similar arrangement for photographing chromatograms, but they used a mercury source, which would only be suitable for observations at certain wave-lengths.

For the determination of the activation spectrum of a fluorescent compound the screen is best avoided, as its fluorescence may mask that of the compound under study. The wave-length knob is rotated until maximal fluorescence is produced. The point at which this occurs will differ slightly from the true activation maximum of the compound, because the intensity of the light issuing from the monochromator increases with wave-length (unless the slit is automatically adjusted by means of a cam). However, a reasonable approximation can be made.

Another application lies in the analysis of thin-layer chromatograms. For this purpose the addition of 10 per cent $ZnSiO_4$: Mn to the solid phase converts the plate itself into a fluorescent screen. Better contrast is obtained with this technique than with the screen superimposed on a paper chromatogram, which absorbs a significant proportion of the incident light. It was found that quantities of oestrogens which were barely detectable under the 254 mμ handlamp could easily be seen with the spectroscope set at 280 mμ.

The foregoing description has chiefly been concerned with the analysis of compounds on chromatograms. Analogous results can be obtained with compounds in solution in quartz cuvettes. To facilitate the observation of small changes in optical density a reference cell containing the solvent alone is placed alongside the unknown solution. With the aid of the fluorescent screen, absorption maxima can be estimated in exactly the same way as on paper. For example, as little as 2 μg cortisol in 1 ml. absolute ethanol could be spectrally analysed; and the end-absorption of absolute ethanol was discernible below 220 mμ in comparison with a reference cell containing water.

The apparatus can be adapted for automatic scanning of chromatographic strips at a given wave-length, or for recording the absorption spectra of compounds on chromatograms. Modifications of existing spectrophotometers for such purposes have been described by others[4–6]. The principal advantage of the present technique is that it provides a tool of high sensitivity, permitting the spectral characteristics of compounds to be studied visually. Furthermore, areas which appear only as streaks on conventional ultra-violet illumination can often be shown by spectroscopy to contain several discrete spots with different absorption or activation maxima. In this way, full advantage can be taken of the high resolving power of the paper or thin-layer chromatogram. These developments have been made possible by the availability of sufficiently intense sources emitting a continuous spectrum in the ultra-violet region.

This work was supported by grants *AM*–5912 and 2*A*–5370 from the National Institutes of Health.

DENIS M. ABELSON

Department of Medicine,
The Graduate Hospital,
University of Pennsylvania.

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Accumulation and Incorporation of Amino-acid in Rat Intestine *in vitro*

INVESTIGATIONS of the absorption of amino-acids from the intestinal tract have usually been concerned with a single amino-acid or, in some cases, simple mixtures of a few amino-acids[1–3]. Under physiological conditions, on the other hand, an initial stage in the assimilation of dietary proteins is the enzymatic liberation during digestion of a mixture composed of peptides and 18–20 free amino-acids[4]. Because of the interactions between the amino-acids in competing for several relatively specific transfer mechanisms, the total rate of absorption of amino-acids from a mixture need not be closely related to the absorption rates of the same amino-acids when measured singly, but will depend on the composition of the mixture present in the intestinal lumen[4]. There is also evidence that during absorption for 1 h of glycine *in vitro*[5] and of protein hydrolysate *in vivo*[6] there is an incorporation of the absorbed amino-acids into the protein of rat intestinal mucosal cells. This communication records an investigation of amino-acid transport and protein synthesis in rat intestine during absorption of an amino-acid mixture *in vitro*.

Amino-acid transport was measured over 8 min as the entry of ^{14}C-labelled amino-acids into the tissue water and proteins of rings of jejunum prepared from male albino rats ranging in weight from 260 to 300 g. The rings[7] (2–5 mg dry weight) were incubated in 2·5 ml. of medium containing a mixture of 18 amino-acids giving a total amino-acid concentration of 1·7 mM, and containing 0·4 μc. of a ^{14}C-labelled protein hydrolysate ('*CFB* 25', Radiochemical Centre, Amersham). The incubations were terminated by the addition of an excess of an unlabelled casein hydrolysate and the rings rapidly removed from the medium, blotted, and transferred to 3 ml. 0·5 M perchloric acid at 0·5° C. The tissue was dispersed with a glass rod and the precipitated protein removed by centrifugation. An aliquot of the supernatant was counted to give a measure of the amount of labelled material in the perchloric acid extract. A correction was made for the carry-over of label from the medium by counting similar aliquots of perchloric acid extracts of rings incubated for 15 sec in a medium identical except for the presence of the excess of unlabelled casein hydrolysate. Apart from this correction no attempt was made to allow for the extracellular space or for any non-absorbing tissue present in the rings. If such corrections were made, the ratio of the concentration of amino-acids in the tissue fluid to that in the medium (T/M) would tend to increase when the ratio was greater than 1.

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Consider a small scintillation counter

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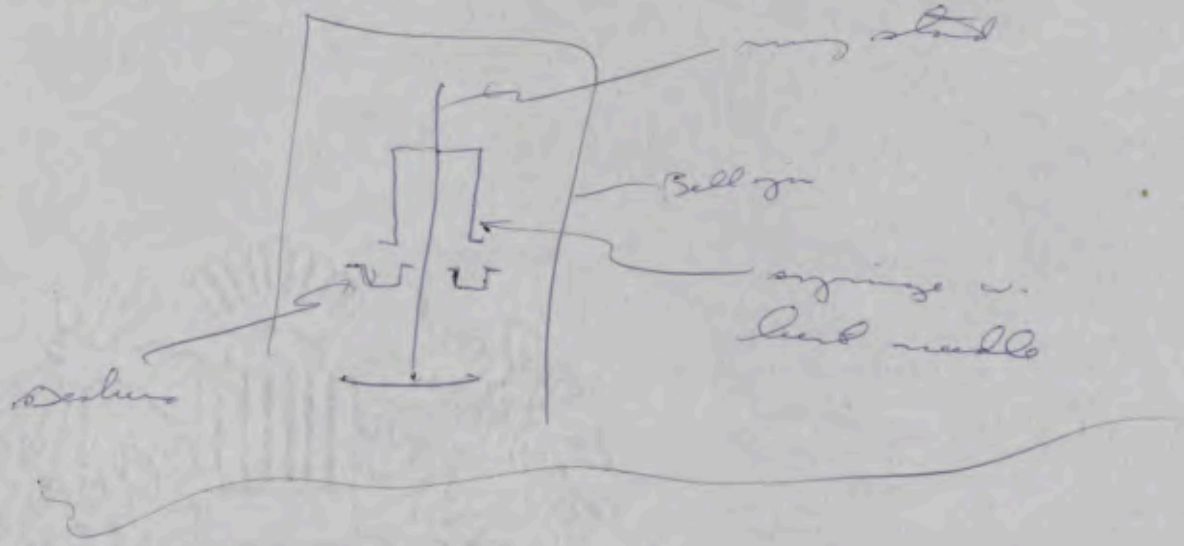

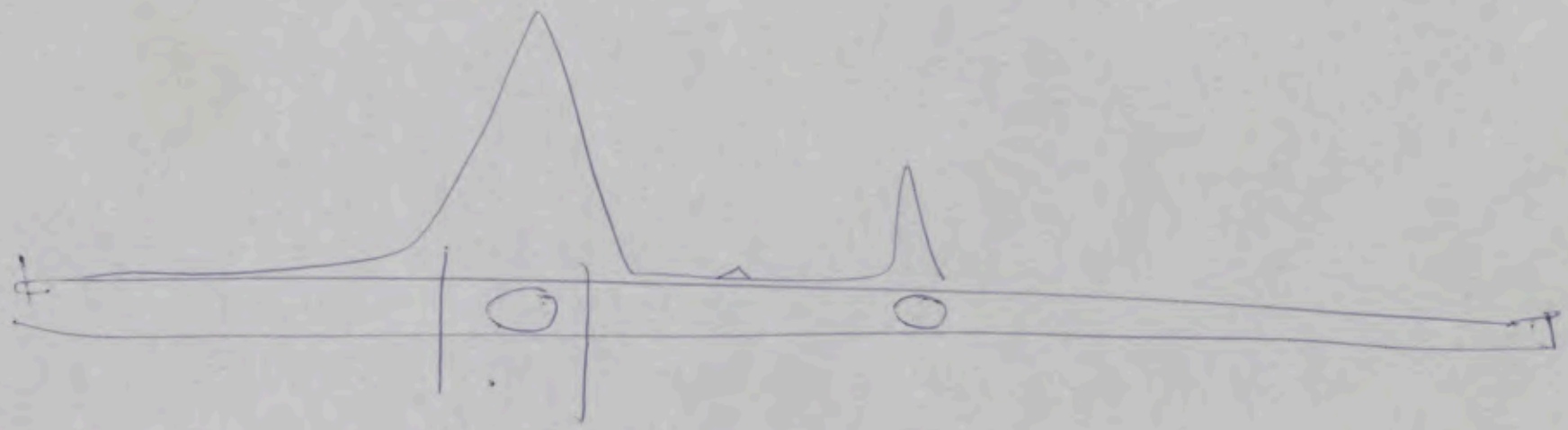

Res. Specialties Co., Richmond, Calif
[illegible]

X-ray development: GE, Milwaukee.

Cyril will provide window for end-window counter.
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blueprints for the cabs.