

LIGHT FROM REACTIONS IN SOLUTION

with 2 separate pamphlets

A thesis presented for the
degree of Doctor of Philosophy in Chemistry

in the University of Canterbury,
Christchurch, New Zealand,

by

TERENCE IVAN QUICKENDEN

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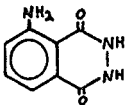
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ABSTRACT

A photon counter which measures weak light in the wavelength range 2,000 to 6,000 Å has been constructed. This device counts the amplified photo-electron pulses released in a photomultiplier tube. It was shown both experimentally and theoretically that the measured pulse rate r , is related to the light intensity I by $r \propto I^k$ where k is unity at low discriminator heights but increases as the discriminator height is raised. The photon counter was used to investigate the following light emitting reactions between liquids.

It was observed that solutions which generate di-imide cause alkaline solutions of luminol  to emit light even when hydrogen peroxide is not present. Kinetic measurements were consistent with the view that in the usual light emitting reactions of luminol, H_2O_2 first oxidises a molecule of luminol to di-imide which collides with and excites another luminol molecule.

Weak light was detected when aqueous solutions of inorganic acids and bases were reacted in a flow system at 0.066 moles/second. The intensity of the light from the reaction between analytical grade NaOH and H_2SO_4 was unaffected by displacing dissolved oxygen from the solutions but was reduced by $\frac{3}{4}$ when the acid and alkali were strongly heated to remove organic impurities. The quantum yield after purification was 1 photon per 10^{19} reacting molecules.

No light (mitogenetic radiation) could be detected from rapidly dividing cultures of various yeasts and bacteria. If light is emitted, it must be less than $\frac{1}{8}$ as intense as the light from a reference solution containing 0.01M $KMnO_4$ and 0.01M oxalic acid.

The literature relating to the above studies has been reviewed and computer programs for curve fitting, graph plotting, the generation of random numbers and the calculation of pulse overlap have been written.

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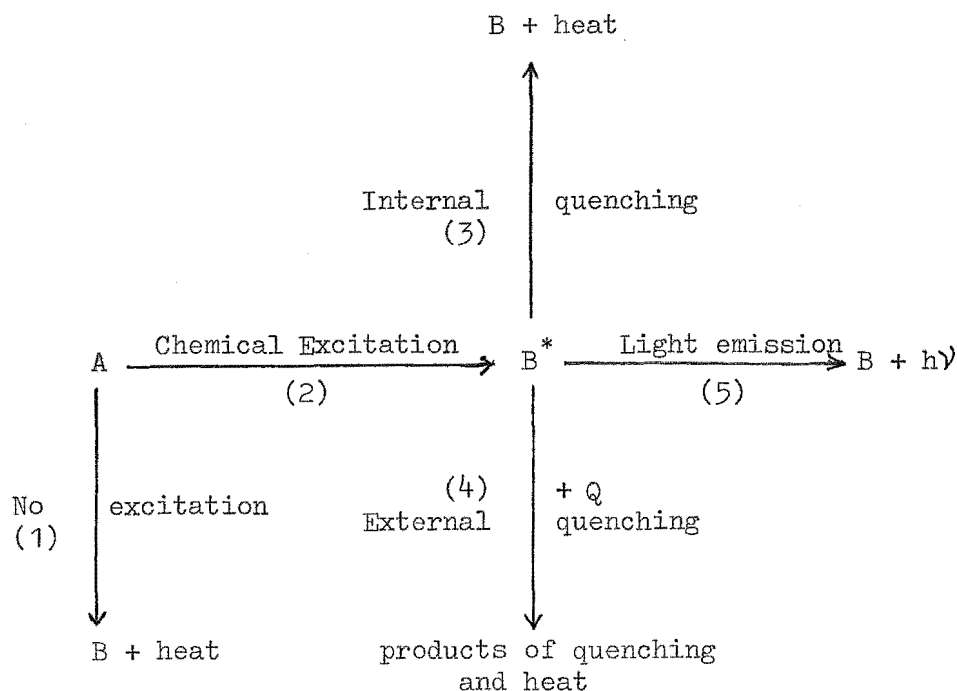
LIGHT FROM REACTIONS IN SOLUTION

PART I : A REVIEW OF THE LITERATURE

Myth and folklore¹ abound with references to unquenchable fires, perpetual lamps, ignes fatui, icy noctiluces and many other names which express primitive man's wonderment at finding light unaccompanied by heat. This same problem is the spur to modern investigations of chemiluminescent reactions, which emit light in excess of black-body radiation. In gas phase reactions the distinction between chemiluminescence and black-body radiation can be marginal, but in liquids at room temperature where Planck's law predicts fewer than 1 visible photon per year per square centimetre, any detectable light is certainly not black-body radiation.

The light emitting products of chemiluminescent reactions are electronically excited and in due course reach equilibrium with their environment by one or more of the various paths in figure 1 (overleaf) where A represents the reactants, B* the excited products and B the products in their usual ground state.

Figure 1. The Steps in a Chemiluminescent Reaction



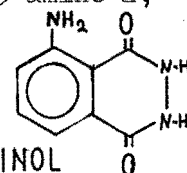
Reid² proposes that the activated complex of a reaction is always electronically excited in which case step (1) does not exist and presumably steps (3) and (4) account for the rarity of bright chemiluminescence.

Apart from the naturally occurring glows^{3,4} of fire-flies, glow-worms, luminous bacteria and other creatures, the first record of chemiluminescence in liquids is by Radzewski⁵ (1877) who observed light when lophine was oxidised by oxygen in alcoholic KOH.

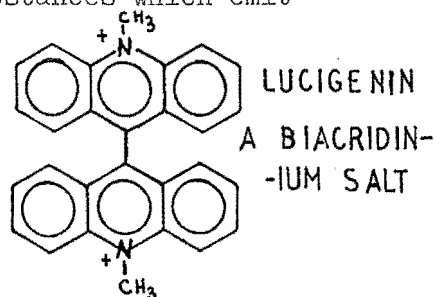
A few years later Lenard and Wolf⁶ (1888) observed a red light from photographic solutions of pyrogallol, and Trautz⁷ (1905) extended this observation to other polyphenols. Wedekind⁸ (1907) noted that phenylmagnesium bromide or iodide emitted light when dissolved with

chloropicrin in ether and later workers⁹ found that Grignard reagents with magnesium attached to an unsaturated carbon atom glowed when oxidised.

By 1917, Harvey¹⁰ was able to compile an extensive list of chemiluminescent reactions and in 1928 Albrecht¹¹ reported the discovery by Lommel of a reaction brighter by several orders of magnitude than any reaction then known. This reaction was between luminol (5-amino-2,3 dihydro-1,4-phthalazinedione) and H_2O_2 in the presence of catalytic ions (e.g. $Fe(CN)_6^{4-}$), in the alkaline solution. Despite intensive research for 29 years it is still unclear why this reaction is so efficient and the mechanism remains controversial. A review of the luminol reaction and an experimental study by the author are found in section IV of this thesis (page 24).



Apart from some substances^{12,13} closely related to luminol, the biacridinium salts¹⁴ are the only other substances which emit bright light when oxidised in solution.



The most significant development in recent years is the detection¹⁵⁻¹⁸ of very weak light (ca. 1 photon per $10^{14} - 10^{19}$ reacting molecules) from a wide range of chemical reactions using sensitive photon counters similar to the one described in Section II of this thesis. Similar observations¹⁹⁻²¹ were made in the 1930s but were treated with considerable scepticism as was the weak chemiluminescence or 'mitogenetic radiation' from dividing cells which could be detected by some^{21,22} but not other^{23,24} workers.

The cause of widespread weak chemiluminescence is unclear and in some cases^{18,25} the emission has been attributed to excitation of trace impurities. A study of the weak light from inorganic acid-base

reactions is commenced in section V of this thesis.

Studies of mitogenetic radiation - a radiation usually considered to be ultra-violet, which is emitted by dividing cells and which stimulates other cells to divide, largely ceased in England and America in the mid 1930s after workers^{23,24,27} in these countries were unable to reproduce the results of Gurwitsch²² and others^{21,27}. Research still continued in other countries and recent publications by workers^{28,29,30} in the U.S.S.R. indicate that the radiation can be detected with modern photon counters. This is attempted in section VI of this thesis which contains a review of mitogenetic radiation.

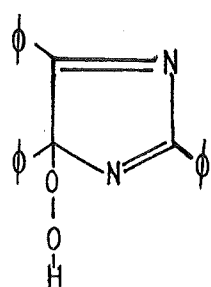
A very wide range of light emitting reactions is now known and a number of recent reviews^{13,31,32,33} including one by the author³⁴ (see the papers inside the back cover) give access to the relevant literature. Several points arise from a study of this literature.

Light emitting solutions of Iophine, Grignard reagents, the phthalic hydrazides (e.g. luminol), the biacridinium salts and anthracene contain the isolable peroxides in figure 2 (overleaf). As all these peroxides emit light when they decompose, they are frequently postulated as reaction intermediates³⁵⁻³⁹.

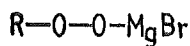
The structure of the luminol peroxide, originally proposed by Drew and Garwood³⁷ (1938) has recently been questioned by White³¹ (1961) who finds the carbonyl stretching frequencies are unaffected by the supposed peroxide bridge and suggests that this substance is only a salt of luminol solvated by H_2O_2 . The structures of all the peroxides in figure 2 would bear careful verification as many of the proposed mechanisms of chemiluminescence depend on them.

Figure 2.

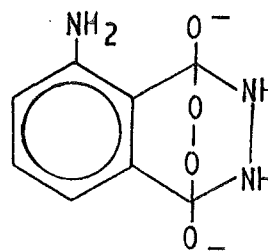
Light Emitting Peroxides



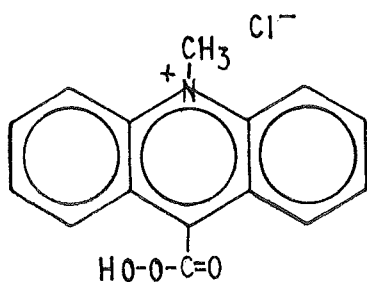
Lophine peroxide ³⁵



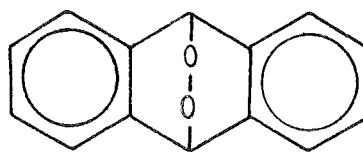
Peroxide of a Grignard reagent ³⁶



Luminol Peroxide ³⁷



Peroxide formed from Lucigenin ³⁸



Anthracene peroxide ³⁹

Another significant point is that quantum yields of chemiluminescence provide no information about the efficiency of chemical excitation (step 1, figure 1) unless corrected for the reabsorption of chemiluminescence and accompanied by the fluorescence efficiency, similarly corrected and measured under the conditions of the reaction. Most studies ^{12,13,40,41} of substituent effects on chemiluminescence are deficient in this respect.

One of the common aims in chemiluminescence studies is identification of the light emitting species and this is often done by comparing the chemiluminescence spectrum with various fluorescence spectra. This procedure is complicated for liquids by the reabsorption of chemiluminescence and its re-emission as fluorescence of longer wavelength, and the absorption of some wavelengths by coloured ions in the

solution. Unless corrections are recorded for these effects, published spectra are difficult to interpret reliably. The current disagreement^{43,44} about the light emitting species in the luminol reaction may be of this origin.

The most significant identification of a light emitting species has been the recent assignment of the red light from a variety of reactions^{45,46,47,53} including oxidation of aldehydes and polyphenols¹⁸ to transitions of molecular oxygen or complexes of oxygen. The almost universal role of oxygen in chemiluminescent reactions is well known and this development is therefore of considerable interest.

PART II : THE MEASUREMENT OF LIGHT

Table I summarises the various methods which can be used to measure light.

TABLE I

METHOD	APPLICATION	MAX. SENSITIVITY	COMMENTS
CHEMICAL	The Chemical Actinometer ⁴⁸	(Ca. 10^{10}) photons/sec.	To determine absolute quantum yields.
	The Photographic Plate ⁴⁹	∞ exposure time. 10^4 photons/sec. (10hrs)	To integrate light over long periods.
BIOLOGICAL	The Eye ⁵⁰	A pulse of ca. 100 photons.	Non-quantitative.
	'Mitogenetic' Detectors ²¹	A few photons per minute claimed	Existence controversial
THERMAL	Thermopile ⁵¹	ca. 10^8 photons/sec.	For absolute energy determination.
ELECTRICAL	Photocells and Light sensitive Semiconductors	A few photons per minute	The most versatile and widely used. (see Table II)

Photoelectric detectors are the most versatile and widely used and their characteristics are listed in Table II. The relative merits of photoelectric devices largely depend on three quantities. These are: the primary photoefficiency P , which is the mean number (0-1) of primary photoelectrons released per incident photon; the secondary efficiency G , which is the number of secondary electrons released by each primary photoelectron; and the dark current I_D .

Table II shows typical values of P, G and I_D for a number of photoelectric devices. For optimum performance, P and G should be large and I_D should be small.

Table II

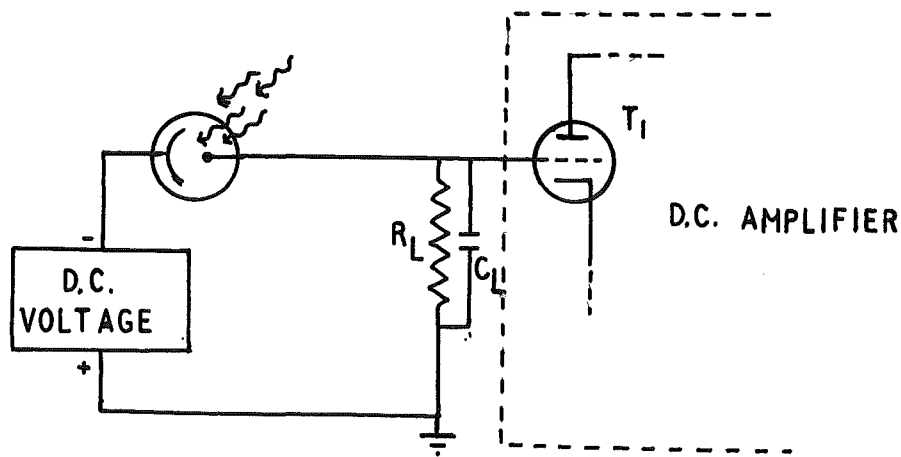
Detector	Comments	Primary Photo-efficiency	Secondary Efficiency	Overall Photo-efficiency	Typical Dark Current
Vacuum Photo-cells e.g. Philips 92 AV	Stable	0.1-0.3	1	0.1-0.3	10^{-7} amp
Semiconductor Photodiodes e.g. Philips OAP12	Robust	0.8-1.0	ca. 100	ca. 100	15×10^{-6} amp
Photomultiplier Tubes e.g. EMI 6256SA	14 dynode electron multipliers	0.1-0.3	$10^5 - 10^8$	$10^4 - 10^7$	$(3-10) \times 10^{-9}$ amp
Gas filled Photocells (Light sensitive Geiger Tubes) e.g. Philips 92AG	Stable low gain operation Unstable high gain operation	0.1-0.3 0.1-0.3	5 to 10 up to 100	0.5 to 3 up to 30	$ca. 10^{-7}$ amp
Photo-transistors e.g. Mullard OCP71	Temperature dependent and poor spectral range	0.8-1.0	ca. 400	ca. 400	3×10^{-4} amp
Photoconductors e.g. Cadmium sulphide prepared according to Frerichs. (ref.52)	Poor high frequency response	Very dependent on impurities (up to 0.82)	$ca. 10^4$	up to 10^4	$10^{-4} - 10^{-6}$ amp.
Photovoltaic cells e.g. Philips BPY10		0.8-1.0	ca. 500	ca. 500	$ca. 10^{-5}$ amp.

Photomultiplier tubes are the most sensitive and stable light measuring devices.

Photodetectors in D.C., A.C., or Pulse Counting Circuits

The Direct Current Circuit: In this method, the photocurrent i_p is either measured directly with a microammeter or is passed through a load resistor R_L attached to a D.C. amplifier (figure 1).

Figure 1.



The sensitivity of the method is increased by amplification but is limited by the Johnson noise in R_L . The R.M.S. noise voltage is $V_N = \sqrt{4kT\Delta F R_L}$ where T is the absolute temperature, k is Boltzmann's constant and the bandwidth $\Delta F = \frac{1}{2.2R_L C_L}$. It follows that $V_N = \sqrt{\frac{4kT}{2.2C_L}}$, which is independent of R_L and equals 8.7 microvolts when C_L has a typical value of 10pF. If a photocurrent i_p is to be detectable, the P.D. $i_p R_L$ must exceed V_N . To achieve this, R_L can be large, and providing T_1 is an electrometer tube with a low grid current, the only limitation to increasing R_L indefinitely, is that the response time ($R_L C_L$) becomes excessive. An ultimate sensitivity of 10^4 photoelectrons per second is possible if a response time of 0.1 seconds is acceptable.

Unfortunately, electrometer amplifiers are subject to gain fluctuations, pick up of stray signals and other⁵⁴ practical difficulties, particularly when R_L is large. These limitations are avoided if the photocurrent is amplified by nearly noiseless⁵⁶ electron multiplication, as is done in photomultiplier tubes.

The current gain in a photomultiplier tube of n dynodes is $G = p^n$, where p is the number of secondary electrons ejected at each dynode, per incident electron. p depends on the interdynode voltage and is typically 2-4. In modern 14 dynode photomultipliers, $G = 10^8$ and a current of 10^4 photoelectrons/second at the cathode releases an anode current of 0.16 microamps, which is readily measured with a microammeter or potentiometric recorder.

Dark current from the non-illuminated photomultiplier may limit the sensitivity unless it is bucked out by a D.C. voltage or is negligible (< 20 photoelectrons/sec.) as in some modern tubes. When the dark current is low, shot noise limits the sensitivity of a photomultiplier. The R.M.S. noise current in a photomultiplier of gain G is⁵⁶ $I_N = 2G^2 e^2 n \Delta F$ where n is the photocurrent in photoelectrons per second, $e = 1.6 \times 10^{-19}$ coulombs and ΔF is the bandwidth. I_N is typically 3.5×10^{-9} amps, when $n = 10^4 \text{ sec.}^{-1}$, $G = 10^8$ and the time constant $(\frac{2.2}{\Delta F})$ is 0.1 sec. Under these circumstances a light flux of $10^4 \text{ photons sec.}^{-1} \text{ cm.}^{-2}$ is measurable.

The Alternating Current Circuit

One method to avoid the difficulties of D.C. amplification, remove dark current and reduce photomultiplier shot noise is to modulate the incident light and pass the modulated photocurrent to an

A.C. amplifier and phase sensitive detector. The latter rejects all the noise except those components which have the same frequency and phase as the modulation signal. Signals 40 db below noise have been⁵⁷ extracted in this way. A mathematical treatment of the difficulties which arise when the pulse rate becomes low compared with the modulation frequency is not available.

'Photon Counting' for very weak light

Following a suggestion by Kron⁵⁸, Engstrom⁵⁹ in 1947 devised an ingenious method for measuring weak light below 10^5 photons $\text{sec.}^{-1} \text{ cm.}^{-2}$. Instead of trying to reduce photomultiplier noise, he arranged to count the photoelectron pulses.

If the photoelectron pulses proceed to an amplifier via a coupling capacitor C, the mean pulse height at the amplifier input is $h = \frac{Ge}{C}$ where G is the photomultiplier gain and e is the charge on the electron. Typically, C = 5,000 pF and $G = 10^7$ whence $h = 0.3 \text{ mV}$, which is about 300 times greater than the Johnson noise voltage $\frac{4kT}{2.2C}$ ₃ at the amplifier input. If the amplifier gain is 5,000, pulses of ca. 1.5 volts are produced and can be discriminated from small noise pulses and counted by orthodox counting equipment.

'Dark counts' from the non-illuminated photomultiplier limit the sensitivity of the method. If the photocathode is refrigerated to reduce thermionic emission and is shielded from ambient γ -rays and if cosmic ray pulses are rejected by an anticoincidence circuit⁵³, the dark count can be reduced to several counts/minute and light fluxes as

low as $10 \text{ photons min.}^{-1} \text{ cm.}^{-2}$ can be measured reliably. Without any of these precautions, light fluxes as low as $20 \text{ photons sec.}^{-1} \text{ cm.}^{-2}$ can be readily measured with tubes designed for low thermionic emission, (e.g. EMI 6256SA). The limitations placed on photon counting due to pulse overlap are fully investigated on page 17.

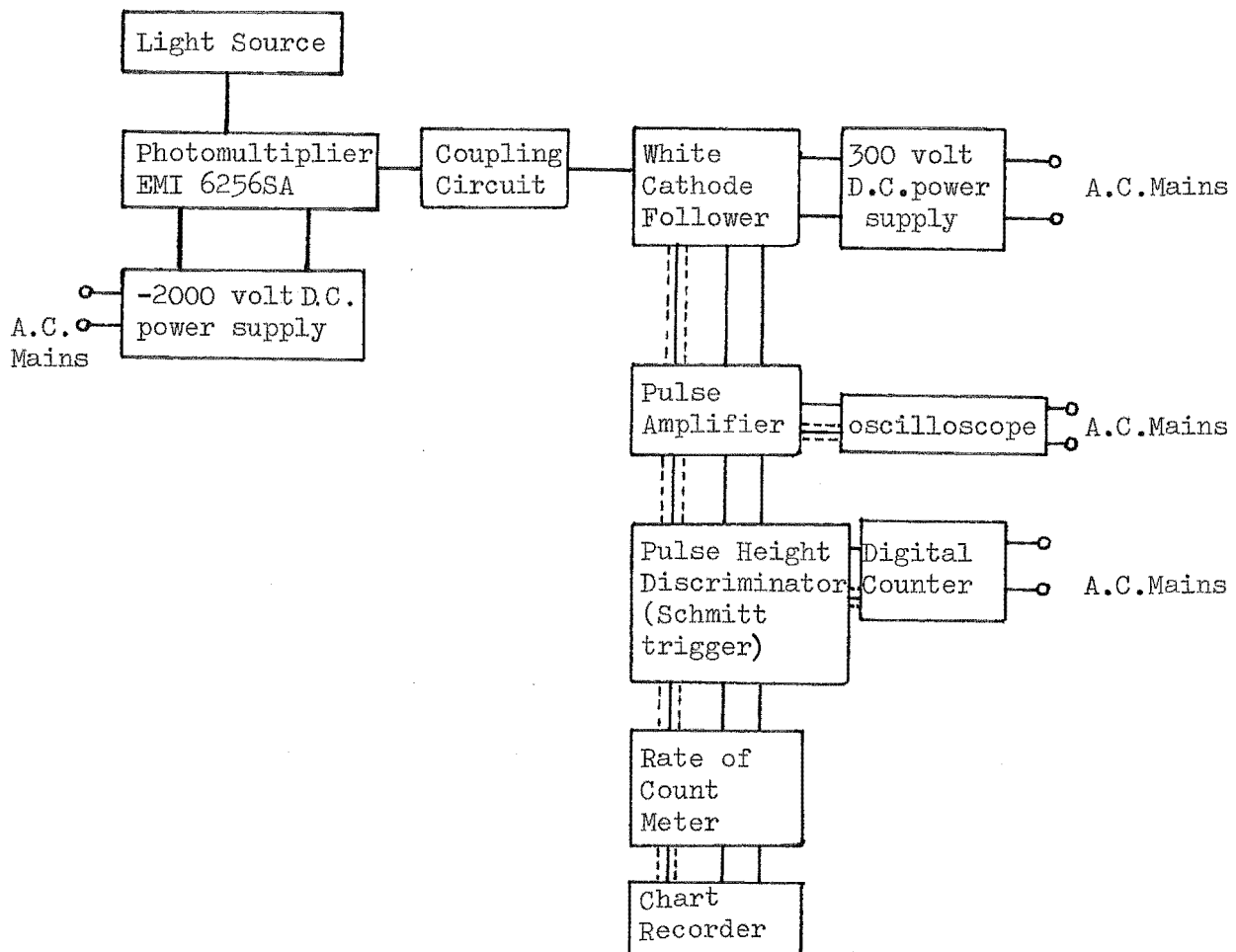
A description of the photon counter used in this thesis follows:

The Description of a Practical Photon Counter

Figure 2 is a block diagram of the photon counter and is followed by detailed descriptions of each part.

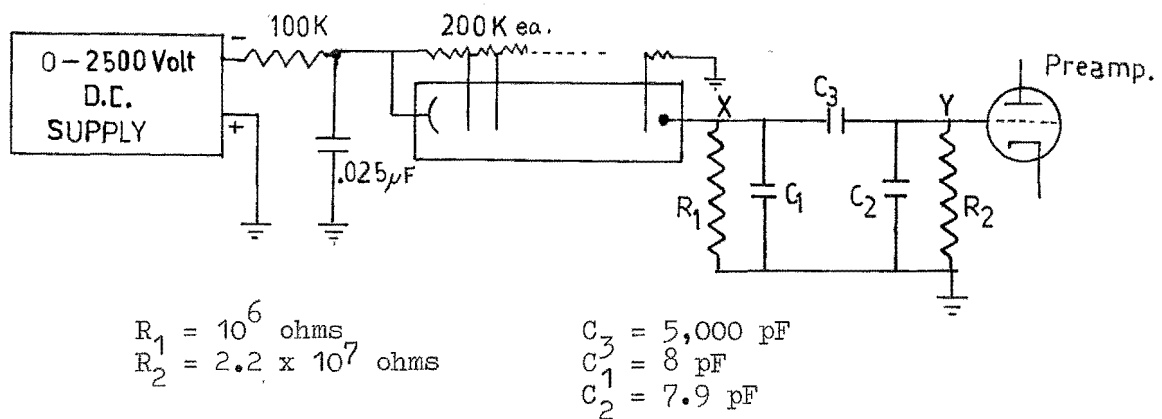
Figure 2

The Photon Counter



The photomultiplier dynode voltages were tapped off a resistor chain (figure 3) attached to a Philips 0-2,500 volt D.C. supply (PW 4024/01) stabilised to $\pm 0.05\%$ and freed from 30 kc ripple by an external RC filter of time constant 2.5×10^{-3} sec. The dynode chain carried at least twenty times the maximum photomultiplier current.

Figure 3. The Photomultiplier and Coupling Circuit



The coupling circuit XY in figure 3 comprises the photomultiplier load R_1 , the grid leak R_2 , the coupling capacitor C_3 and C_1 and C_2 which are parasitic capacitances.

A typical photoelectron pulse at the anode of the photomultiplier has a rise time of 7 nanoseconds and height of 3×10^{-4} volts (page 11). It is shown in Appendix 1 that the resultant voltage pulse at Y rises instantly and falls according to $h_t = h e^{-At}$ where h is the

maximum height, and $A = \frac{R_1 + R_2}{R_1 R_2 C_2 + R_1 R_2 C_1}$ providing $C_1, C_2 \ll C_3$.

The pulses at Y pass to a White⁶⁰ cathode follower (figure 4) of gain ≈ 1 and R.M.S. shot noise of 0.1 millivolts.

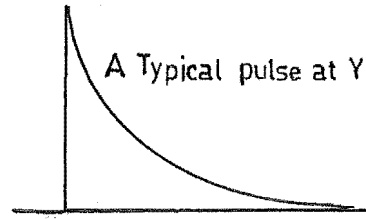
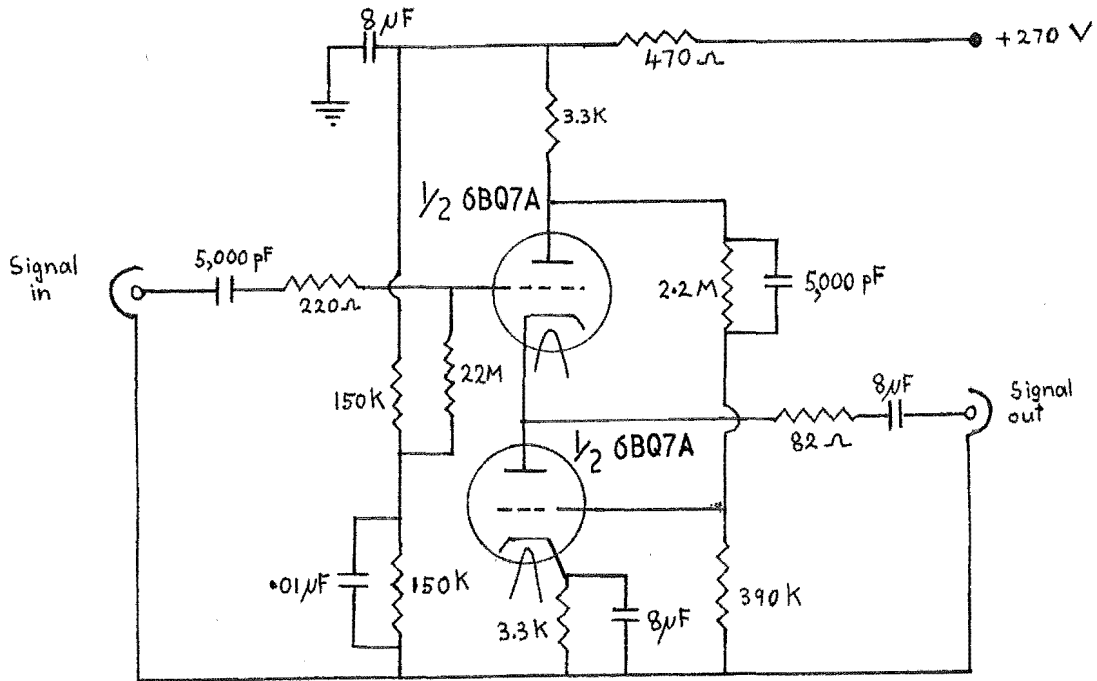


Figure 4. White Cathode Follower (Preamplifier)



The photoelectron pulses and the preamplifier noise then proceed to a pulse amplifier (figure 5) where the noise level is augmented to 0.12 millivolts by the input valve. When the amplifier gain is at its maximum of 7,000, a typical output pulse has a height of 2.2 volts and rise time of 10^{-6} seconds and is accompanied by 0.8 volt R.M.S. noise.

The photoelectron pulses are separated from most of the noise by a precision Schmitt Trigger designed by Fischmann-Arbel⁶¹ (figure 6).

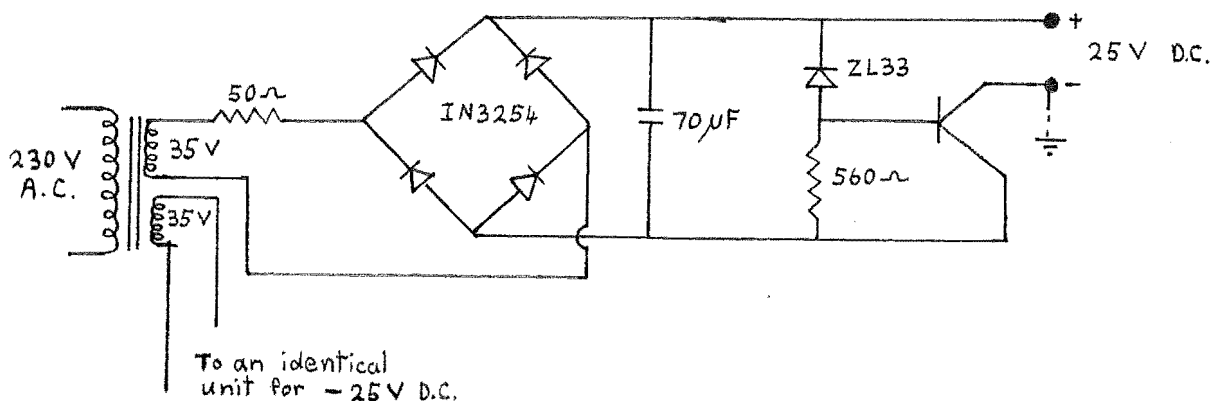
This circuit accepts pulses exceeding a set voltage which can be varied between 0.01 and 50 volts with a drift of less than 0.01 volts and a hysteresis smaller than 0.1 volts.

The pulses from the Schmitt Trigger are led to a transistorised rate of count meter (figure 7) similar to a design by Gilland⁶² or are counted with a commercial Ekco scaler (type N530G). The ratemeter output was recorded on a Philips potentiometric recorder type PR2210A/21 and the pulses from the amplifier were conveniently displayed on a Cossor oscilloscope, model 1058.

The pre-amplifier, amplifier and Schmitt trigger were supplied with 300 volts D.C. stabilised by a circuit (figure 8) similar to one by Elmore and Sands⁵⁴. The Schmitt trigger filaments were provided with 7.2 volts (below which the pulses from the Schmitt trigger show some dependence on the filament voltage) from lead accumulators, to minimise feedback to the pulse amplifier through the filament leads.

The ratemeter was supplied with 25 volts D.C. (figure 9) stabilised by a Zener diode which had its impedance reduced to 4 ohms by a transistor in the emitter-follower configuration.

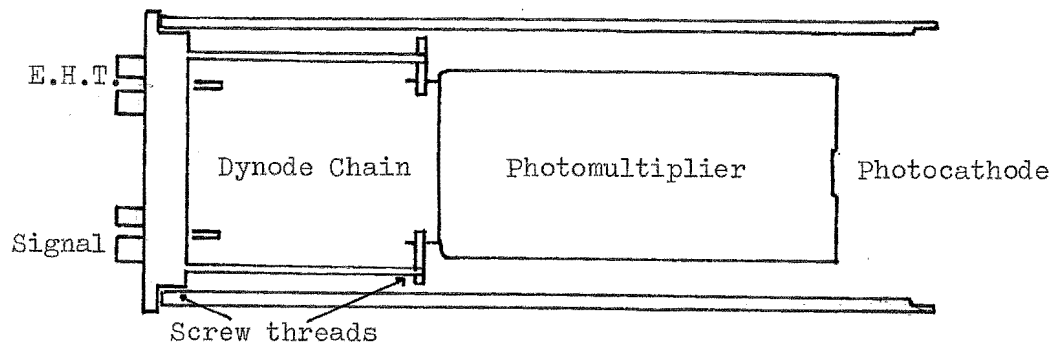
Figure 9. Stabilised 25 Volt Power Supply



Construction Details

Each circuit (figures 4-9) was constructed on a separate chassis, well shielded from electrical interference and mounted on a 19 inch rack. Coaxial cable was used to conduct the signal between chassis, care being taken to avoid earth loops. The photomultiplier and dynode chain were shielded from light and electrical interference by the casing in figure 10. This case could be attached to a Hilger monochromator or the various cell compartments described in the different sections of this thesis.

Figure 10. The Photomultiplier Casing



Scale: $\frac{1}{2}$ actual size

PART III : THE RELATIONSHIP BETWEEN LIGHT INTENSITY
AND COUNT RATE IN PHOTON COUNTERS

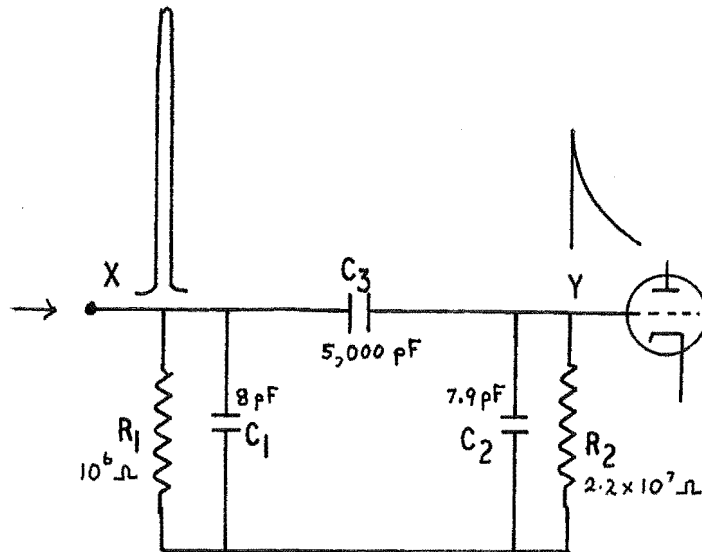
As described on page 11, light intensities I , in the range 10^{-10} photons per second are usually measured by counting the $n = I\phi$ amplified photoelectron pulses they release per second in a photomultiplier tube of cathode photoefficiency ϕ . If a pulse height discriminator which accepts pulses above h volts allows r of the n pulses to be counted, then it will be shown that r is proportional to I only when I and h are small. In only a few^{63,64} of the published measurements made with photon counters, has a check on the proportionality of the measured pulse rate r to light intensity I been recorded. If I and h are not small, many pulses overlap, their heights are augmented and $r \neq I$. This effect is not to be confused with the loss of pulses due to the resolving time of the counter which can be allowed for by the usual correction⁶⁶.

It is shown in Appendix 2 that when pulses overlap, the total pulse height depends on n/A where n is the rate of single photoelectron pulses and A is the reciprocal time constant for the decay of pulses due to the photomultiplier coupling circuit. The calculations which follow relate r to n/A and through this, to the light intensity I .

Theory

In a typical photon counter, the pulses from the photomultiplier proceed to an amplifier through the coupling circuit (figure 11) already described on page 13.

Figure 11 The Photomultiplier Coupling Circuit



The time constant of the coupling circuit XY is usually much larger than the transit time (10^{-9} to 10^{-8} sec.) of the photomultiplier, so that effectively rectangular, narrow pulses arrive at X. It is shown in Appendix 2 that h_t , the output voltage at Y rises sharply to a maximum h and falls according to $h_t = he^{At}$

where
$$A = \frac{R_1 + R_2}{R_1 R_2 C_2 + R_1 R_2 C_1}$$

providing $C_1, C_2 \ll C_3$. In the present equipment $A = 6.3 \times 10^4 \text{sec.}^{-1}$.

When the pulses are far apart, the probability $P(1,h)dh$ that a pulse reaches a height between h and $h+dh$ volts, depends on the photomultiplier but not on A or the light intensity I . In the absence⁶⁵ of a satisfactory calculation, $P(1,h)dh$ can be measured and most

workers⁶⁵, including the author, find that

$$F(1,h)dh = Be^{-Bh}.dh$$

where B is a constant which depends on the interdynode voltage.

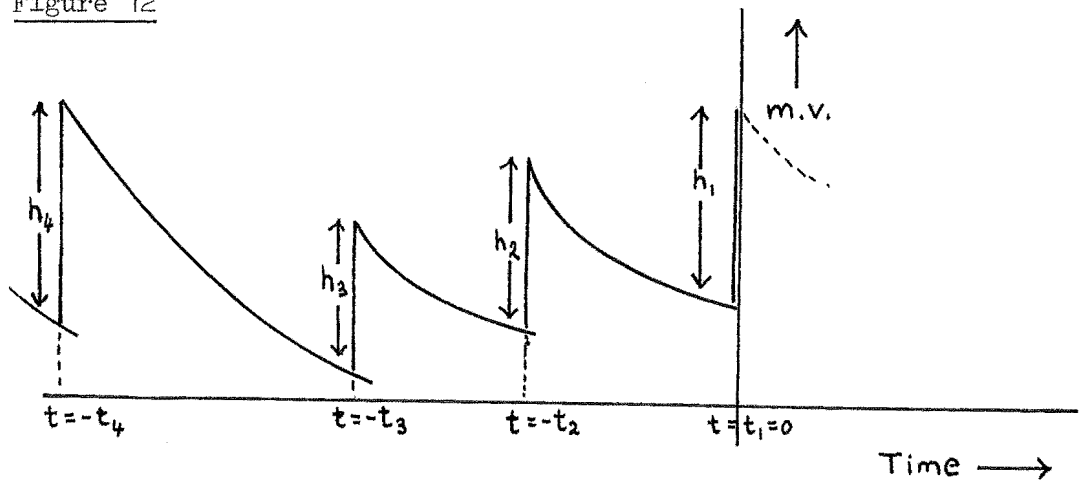
If $\frac{n}{A} < 0.05$ (figure 13), appreciable pulse overlap occurs and the height of a pulse is augmented by the residual heights he^{-At} of all the previous pulses. Consider a sequence of x random pulses (figure 12), each starting with heights

$h_x, h_{x-1} \dots \dots \dots h_2, h_1$ at times $t_x, t_{x-1} \dots \dots \dots t_2, t_1$ before the last pulse for which $t_1 = 0$. The augmented height of the last pulse will be

$$h = h_1 + h_2e^{-At_2} + h_3e^{-At_3} + \dots \dots \dots h_xe^{-At_x}$$

which should converge to a limit when x is made large.

Figure 12



Thus the height of the last pulse depends on the height of each preceding pulse and on the probability $Q(x,t)dt$ of finding x random pulses in a sequence of duration t.

To evaluate $Q(x,t)dt$, consider a pulse at zero time and divide the preceding t seconds into m equal parts Δt such that $\Delta t = \frac{t}{m}$. If the pulse rate is n , the probability of a pulse in the interval Δt at $-t$ seconds is $n\Delta t$. For this to be the first of x pulses, there must be $x-2$ pulses distributed in ${}^{m-1}C_{x-2}$ ways among the remaining $m-1$ parts, and $m-x+1$ parts must be empty.

Hence

$$Q(x,t)\Delta t = {}^{m-1}C_{x-2} \cdot (n\Delta t)^{x-1} \cdot (1-n\Delta t)^{m-x+1}$$

$$= \frac{(1-\frac{1}{m})(1-\frac{2}{m}) \dots (1+\frac{2-x}{m}) \cdot (nt)^{x-1} \cdot (1-\frac{nt}{m})}{\frac{1}{x-2} \cdot \left(\frac{1-nt}{m}\right)^{x-1}} \cdot \frac{\Delta t}{t}$$

To ensure that there is not more than one pulse in any one part,

let $m \rightarrow \infty$ then $t \rightarrow dt$

$$\text{and } Q(x,t)dt = \frac{(nt)^{x-1} \cdot e^{-nt}}{\frac{1}{x-2}} \cdot \frac{dt}{t}$$

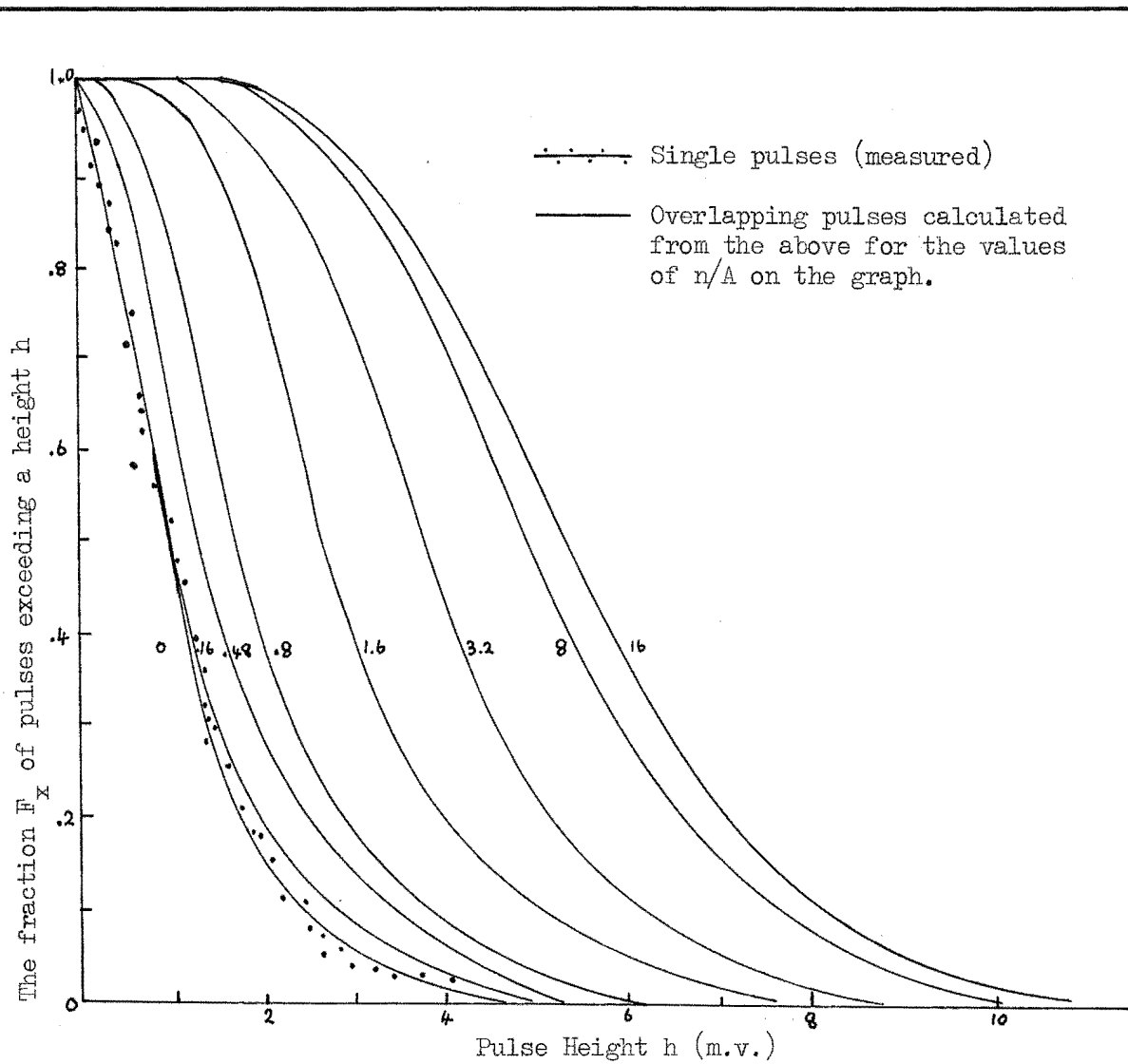
The height distribution of overlapping pulses can be calculated from this result by an analytical method (Appendix 3) requiring repetitive numeric integration or by the quicker Monte Carlo method which follows.

MONTE CARLO CALCULATIONS

The method described applies only to pulses with a sharp rise followed by an exponential fall, but is not specific to the present expressions for $P(1,h)dh$ or $Q(x,t)dt$. These expressions were digitised and stored as 1-dimensional arrays of heights and times in a computer memory. The pulse heights $h_1, h_2 \dots h_x$ were then selected randomly from

Figure 13

The Distribution of Pulse Heights



The above lines are described by the equation $F_x = \text{EXP}(C+Dh+Eh^2+Fh^3+Gh^4)$ where C,D,E,F,G are constant for a given line but depend on n/A as follows.

n/A	C	D	E	F	G
0	0.00	0.751	0	0	0
0.16	0.0471	-0.550	-0.181	0.0284	0.00257
0.48	0.00213	-0.00691	-0.650	0.221	-0.0257
0.80	-0.0313	0.239	-0.536	0.120	-0.0092
1.6	-0.0105	0.586	-0.0416	-0.0339	0.00382
3.2	0.00310	-0.0157	0.0416	-0.0303	0.00204
8.0	-0.00672	0.0286	0.0333	-0.0152	0.000809
16	-0.0317	0.0223	0.00171	-0.00688	0.000271

the height array. The quantity

$$h = h_1 + h_2 e^{-At_2} + h_3 e^{-At_3} \dots h_x e^{-At_x}$$

was computed from the times t_2, t_3, \dots, t_x which were also selected randomly from the respective arrays for $Q(2,t), Q(3,t) \dots Q(x,t)$. To obtain a distribution $P(x,h)dh$ of composite pulses, h was re-calculated many times. The fraction $F_x = \int_h^\infty P(x,h)dh$ of the composite pulses which exceed a height of h volts was found by integration and the results are plotted in figure 13 for various values of n/A . F_x was not changed appreciably by increasing x beyond 5.

From the values of F_x in figure 13, the measured rates of count r were calculated by $r = n \cdot F_x$ for various discriminator heights and are plotted against n on the logarithmic graph in figure 14. The dashed lines indicate the range $10 - 10^5$ c.p.s. usually accessible to measurement. Within this range, the slopes $d(\log r)/d(\log n)$ have a constant value which depends on the discriminator height.

Experimental values for these slopes were found (as described overleaf) by measuring the count rate r from a photomultiplier exposed to various light intensities I . The measured values of r and I are plotted in Appendix 5 on logarithmic graphs whose slopes are $d(\log r)/d(\log n)$ since $I \propto n$. The measured slopes are compared with the calculated slopes on the graph in figure 15 which shows that r is proportional to n only at low discriminator heights.

Figure 14

Calculated Rates of Count

(at various discriminator heights, in millivolts)

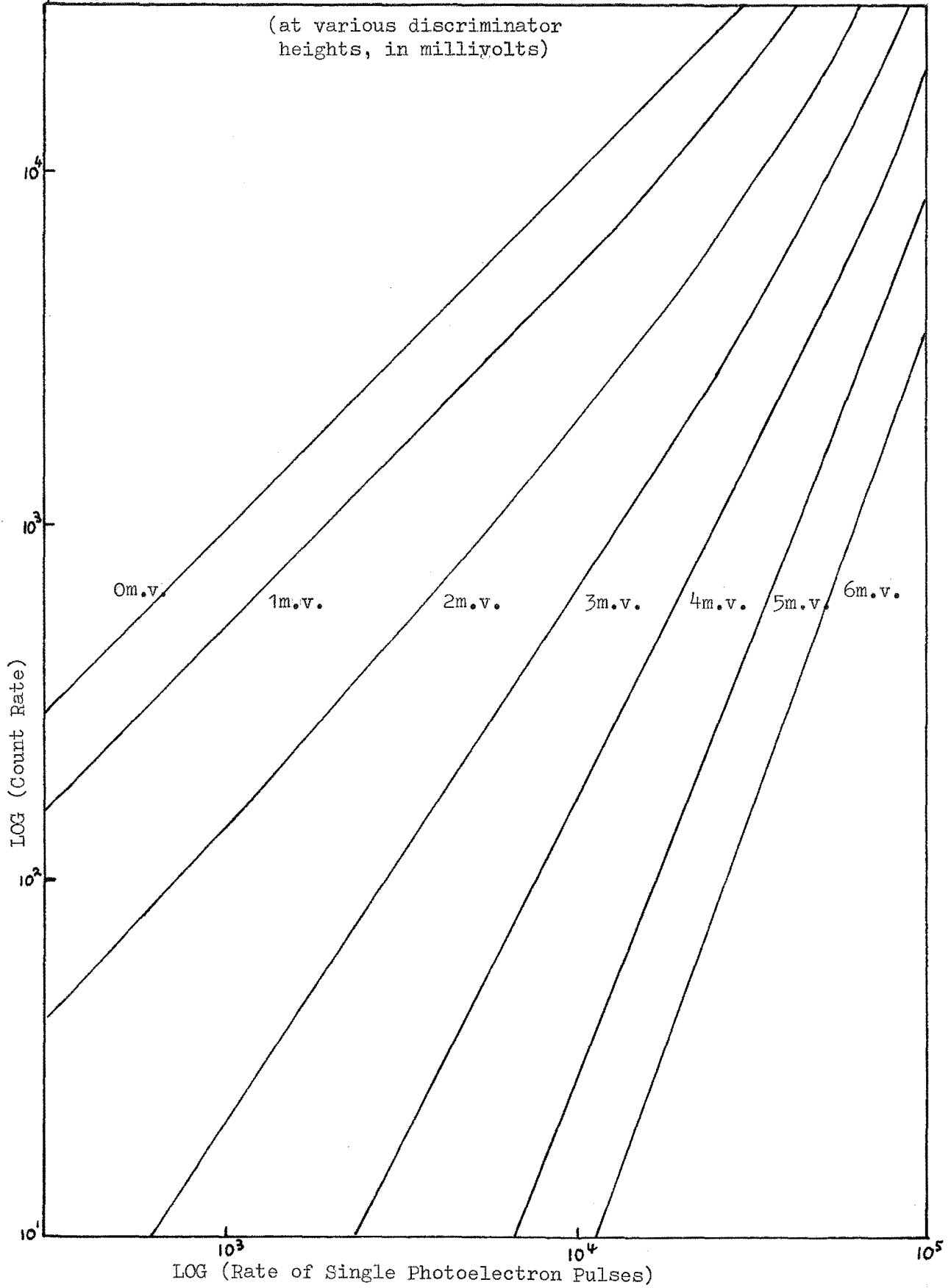


Figure 15 A comparison between calculated and measured values of the slope $d(\log r)/d(\log n)$

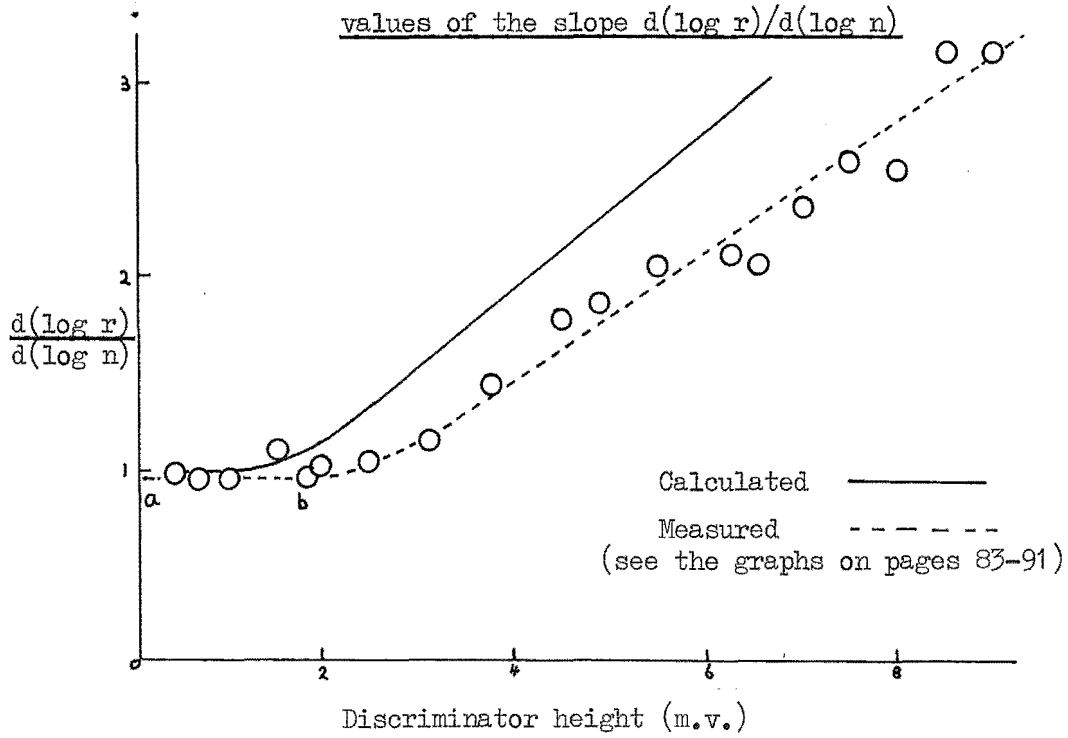
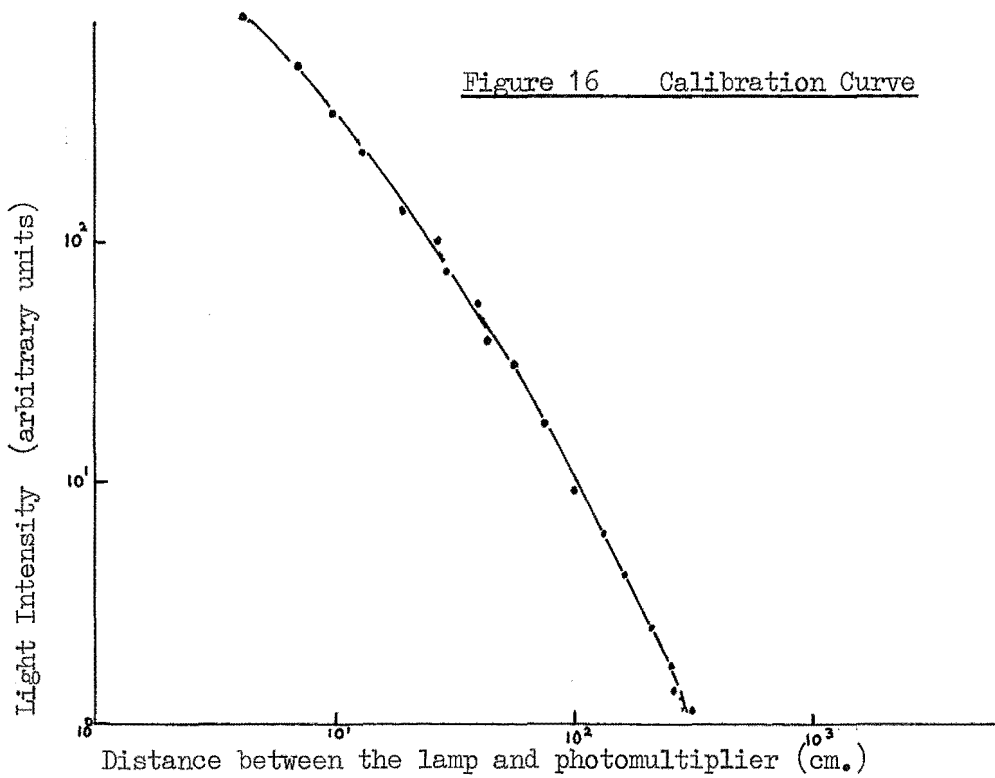


Figure 16 Calibration Curve



Experimental Details

The photon counter was described in part II of this thesis. The effect of light intensity on count rate was determined by varying the distance between the photomultiplier and a tungsten lamp which emitted light through a pinhole in its blackened glass envelope. The lamp and photomultiplier were enclosed in a long light tight tube (figure 15), painted inside with non-reflecting black paint. The relationship between the light intensity and the distance between the lamp and photomultiplier was determined (figure 16) by measuring the photocurrent when a sufficiently bright lamp was moved along the tube.

When the photomultiplier was weakly illuminated, ($\frac{n}{A} < 0.05$) the distribution of pulse heights $P(1,h)dh$ was found to be $Be^{-Bh}dh$ where $B = 0.751 \text{ volt}^{-1}$ as determined by regression analysis on the pulse heights which were measured at an interdynode potential of 73 volts and plotted in figure 13. In all counting measurements the usual⁶⁶ correction was made for the resolving time (5 microseconds) of the digital counter.

All calculations were carried out on an I.B.M.1620 computer equipped with 40,000 units of storage. The distributions were digitised according to the formulae $\Delta n_h = n_h P(1,h) \Delta h$ and $\Delta n_t = n_t \cdot Q(x,t) \Delta t$ where n_h, n_t are the number of elements in the height array and in each time array respectively, and $\Delta n_h, \Delta n_t$ are the number of elements of magnitudes h and t . In view of the usual criteria⁶⁷ for digitising data and the limitations imposed by computer storage, $n_t = n_h = 400$, $x = 2$ to 5 ; h and t were such that Δn_h and Δn_t changed by less than 4

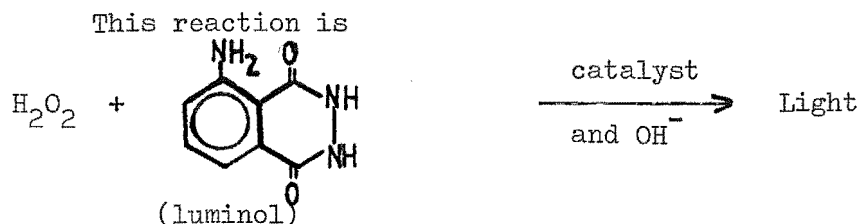
when h and t respectively were incremented. Increasing x beyond 5 made negligible difference to the results. All random selections were made with a random number program based on one by Gruenberger and McCracken⁶⁸.

The Fortran II listing of the Monte Carlo program, the operating instructions, input data and arrays generated by it are found in Appendix 4.

Conclusions

Both theory and experiment indicate that the count rate r in a photon counter is proportional to the light intensity I only at low discriminator heights (a - b in figure 15). At higher settings of the pulse height discriminator, r depends on increasingly higher powers of the light intensity. The range of proportionality a - b can be extended by decreasing $A = (R_1 + R_2) / (R_1 R_2 C_2 + R_1 R_2 C_1)$. Measurements with photon counters should be accompanied by a check that r is proportional to I .

PART IV : AN INVESTIGATION OF THE LUMINOL REACTION

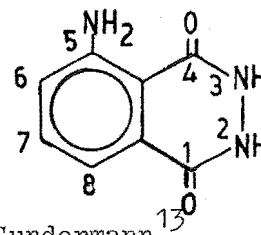


Alkaline solutions of luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione) emit visible light in the presence of hydrogen peroxide¹¹, sodium hypochlorite^{11,40} or ozone⁷³. The light is brighter but fades more rapidly if any of the substances $\text{K}_3\text{Fe}(\text{CN})_6$ ¹¹, MnO_2 ⁶⁹, some metal colloids⁶⁹, many copper and cobalt complexes^{70,71}, RuCl_3 ⁷², VOSO_4 ⁷² or hemin¹¹ are also present. Other substances KCN ⁷⁴, Na_2S ⁷⁴, hydroquinone and phenol⁴⁰ which remove free radicals, reduce the light intensity. It is unclear whether these substances affect the reactions which lead to excitation or merely quench the excited species or do both. Measurements of the reduction of fluorescence by the added substances would disclose any quenching process and a technique developed by Bersis⁷⁵ allows simultaneous measurement of fluorescence and chemiluminescence.

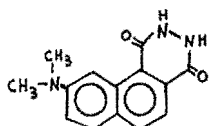
The quantum yield of the luminol reaction depends markedly on the conditions and is usually about 0.005 or less, although yields as high as 0.1 and 0.33 have been reported^{76-79, 80, 81}. As mentioned on page 5 these figures do not indicate the efficiency of chemical excitation unless the fluorescence efficiency is also measured and corrections are made for reabsorbed light.

Many workers^{12,13,40,41} have examined substances related to luminol for similar light emitting reactions. A few derivatives of

phthalic hydrazide, e.g. the 5-OH⁴¹, the 5-NHCH₃⁴¹,
 the 6-N(R)₂¹³ where R = CH₃, C₂H₅ or n-C₃H₇, and the
 5,6,7 tri(OCH₃)⁴⁴ derivative give a light comparable
 with the glow of luminol (the 5-NH₂ derivatives) and Gundermann



finds that



is about three times as bright.

In general¹³, sterically unhindered substituents capable
 of resonant donation of electrons usually cause bright light when
 attached to the aromatic ring, particularly at the 5-position. Without
 a parallel study of fluorescence efficiencies, it is not clear whether
 the substituents affect the rate of chemical excitation or merely
 stabilise the molecules against radiationless loss of excitation energy.

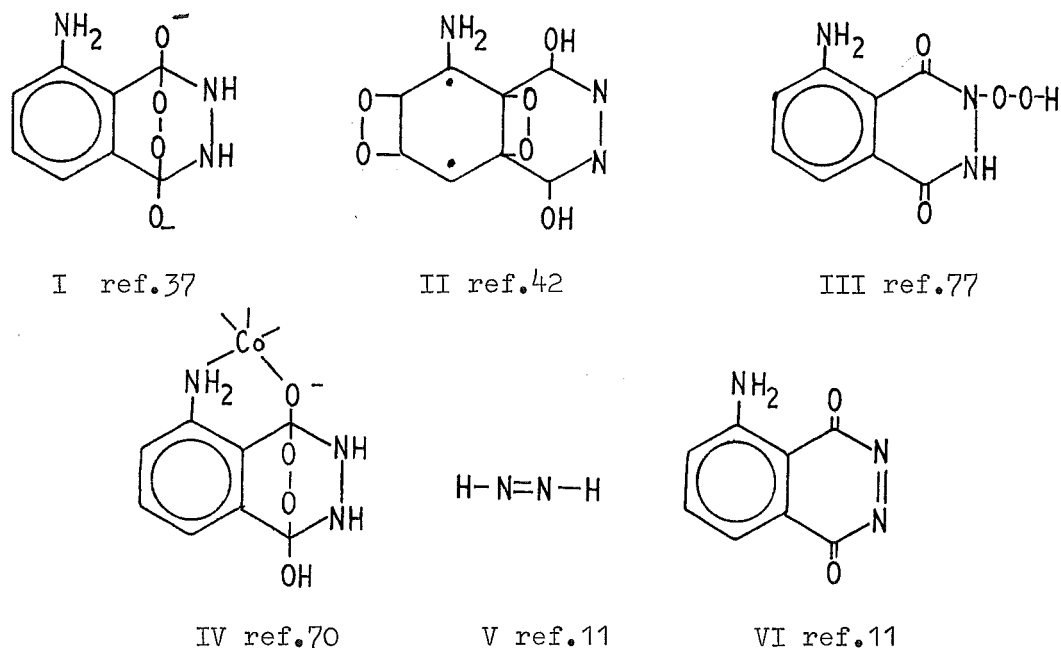
The chemiluminescence spectrum of luminol is similar to
 the fluorescence spectrum of either luminol^{11,82,83} or aminophthalate
 ion^{44,84} (which is a decomposition product of luminol), depending on
 the conditions and the solvent. These differences which may result
 (page 5) from the absorption and re-emission of light by substances
 in the solution, make identification of the light emitting molecule
 uncertain.

A number of hypothetical substances (figure 18) formed
 either by free radical or other reactions from luminol and H₂O₂, have
 been proposed as alternative intermediates in the luminol reaction.

Each intermediate forms the basis of a mechanism described
 in the references to figure 18. Only two of these intermediates (I and V)
 have been isolated and White⁴⁴ proposes that because the C-O stretching

Figure 18

Hypothetical Intermediates in the Luminol Reaction



frequencies in I are unaltered by the suggested³⁷ peroxide bridge, I is only a salt of luminol solvated by the hydrogen peroxide.

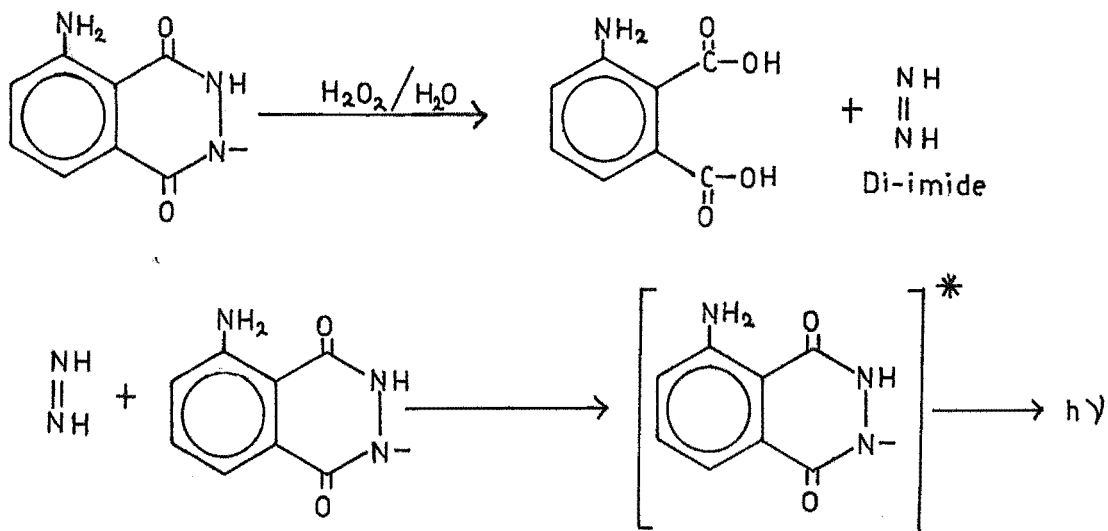
Albrecht¹¹ (1928) proposed a simple mechanism which attracted the attention of several subsequent workers^{74,81,83}, but has been neglected in recent years. According to Albrecht, the reaction proceeds through the steps in figure 19, a molecule of hydrogen peroxide oxidising luminol to release di-imide which collides with and excites another luminol molecule.

Because di-imide was a purely hypothetical intermediate, this mechanism received little attention. Recently (1961) however, di-imide has been isolated⁸⁵ as a solid at low temperatures and detected in gases by mass spectrometry⁸⁶. A growing body of evidence indicates that it is

Figure 19

The Di-imide Mechanism of Albrecht

(neglecting hypothetical intermediates)



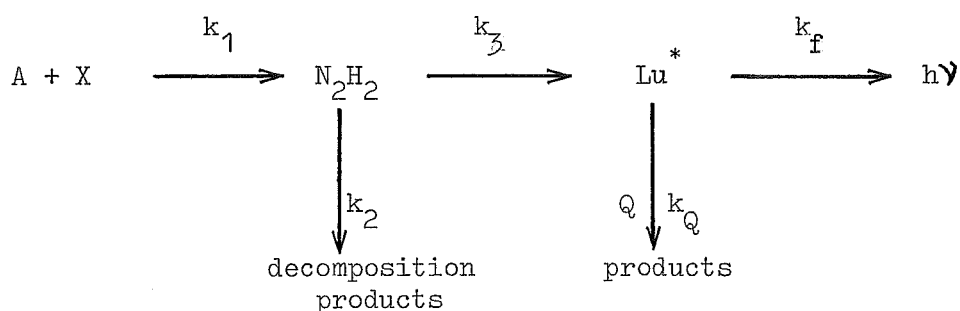
the reducing agent in solutions containing either hydroxylamine-O-sulphonic acid⁸⁷, or hydrazine⁸⁸ or p-toluene-sulphonyl hydrazine⁸⁹, which are used to hydrogenate unsaturated compounds.

We find⁹⁰ (see the papers inside the back cover) that luminol in aqueous solutions of these reagents emits visible light even in the absence of hydrogen peroxide, providing the solutions are alkaline and all the reactants are in the concentration range 10^{-2} - 10^{-4} M. The rate of formation of di-imide in these solutions is reported⁸⁷⁻⁸⁹ to increase on warming. So also does the brightness of the light from luminol dissolved in them. The luminescence is comparable with that obtained from luminol in solutions of either hydrogen peroxide or of potassium ferricyanide, but it is less than that emitted when both these substances are present. These observations, which are consistent with Albrecht's mechanism were extended by the following kinetic study.

Kinetics of the Reactions of Di-imide with Luminol

For the production of di-imide from hydrazine, catalytic ions

and/or oxygen are required while p-toluene sulphonyl hydrazine and hydroxylamine-O-sulphonic acid require an alkaline solution. If di-imide is produced by a substance A in the presence of a substance X, the excitation of luminol (Lu) by di-imide can be described by the reaction scheme



where k_1 , k_2 , k_3 , k_Q and k_f are respectively the rate constants for the formation of di-imide, loss of di-imide by side reactions, excitation of luminol by di-imide, quenching of the excited luminol molecules and emission of light by excited luminol molecules Lu^* .

Once a steady state is set up,

$$\frac{d [N_2H_2]}{dt} = 0 \quad \text{and} \quad \frac{d [Lu^*]}{dt} = 0$$

whence
$$[N_2H_2] = \frac{k_1 [A][X]}{k_2 + k_3 [Lu]} \quad \text{and} \quad [Lu^*] = \frac{k_3 [N_2H_2] [Lu]}{k_f + k_Q [Q]}$$

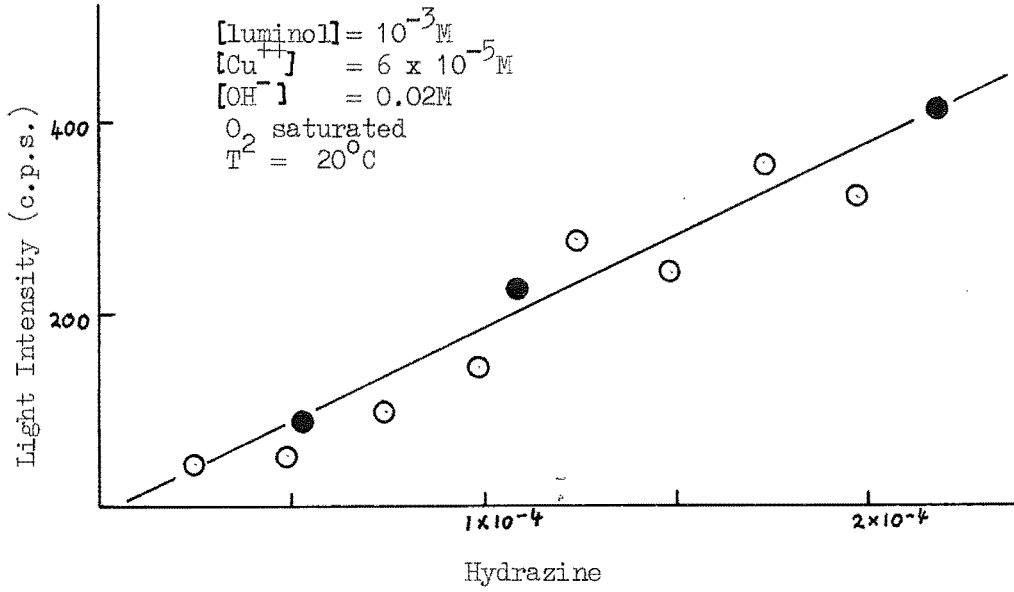
Eliminating the di-imide concentration, the light intensity

$I (= k_f [Lu^*])$ is given

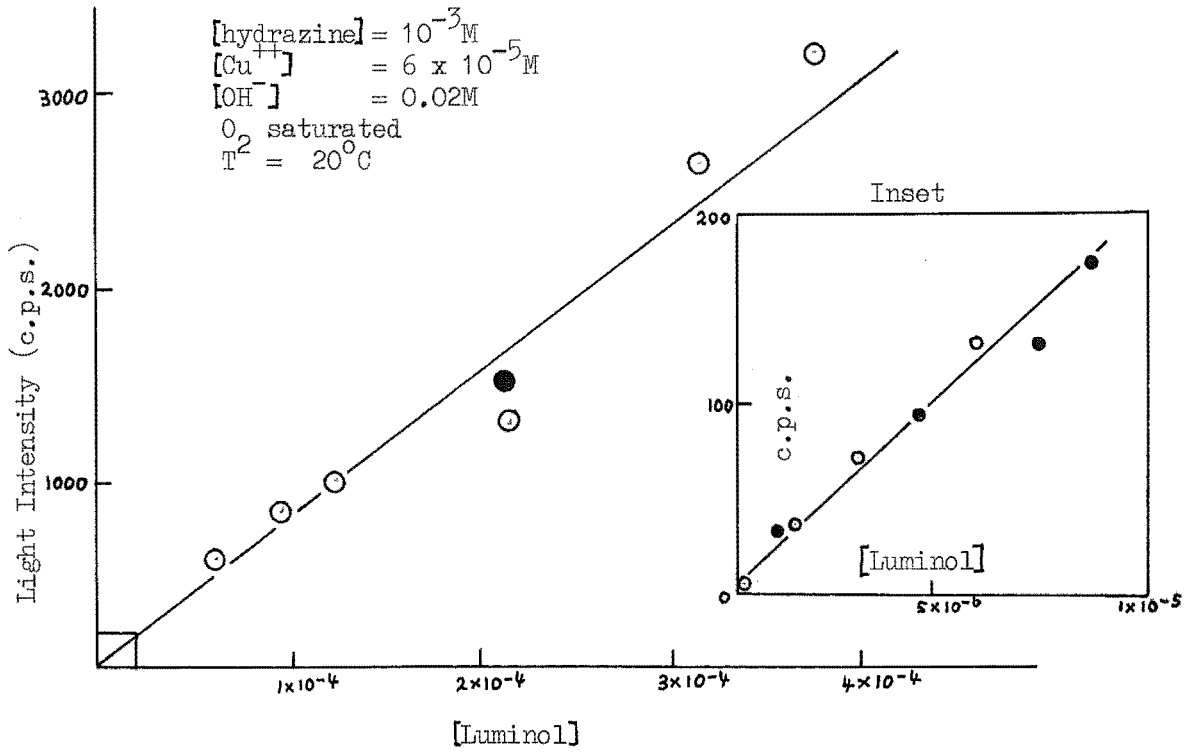
by
$$I = \frac{k_3 k_1 k_f [A][X][Lu]}{(k_f + k_Q [Q])(k_2 + k_3 [Lu])} \dots\dots\dots (1)$$

Figure 20

The variation of light intensity with hydrazine concentration



The variation of light intensity with luminol concentration



If the luminol concentration is low and if there is negligible quenching of the excited luminol

$$I = \frac{k_2 k_1 [A][X][Lu]}{k_2} \dots\dots\dots (2)$$

The predicted proportionality between the light intensity and the concentrations of luminol and di-imide producer A was checked using the equipment and methods described at the end of this section.

RESULTS

Because hydrazine hydrolyses slowly in water at 20°C, it was the most convenient source of di-imide for quantitative study. As shown in figure 20, the light from luminol in an oxygenated solution of hydrazine and copper ions is proportional to hydrazine concentrations up to 2×10^{-4} M and to luminol concentrations up to 6×10^{-4} M when these are varied separately in the solution. Equation (2) predicts this result.

The change of light intensity I, with time t, was studied for a solution containing hydrazine and excess luminol (figure 21). As X is a catalyst and the luminol is in excess, equation (2) becomes

$$t = \frac{1}{I} - \frac{1}{C[A_0]}$$

where C is a constant and A_0 is the initial concentration of hydrazine. When the data of figure 21 are plotted on figure 22 according to this equation, a straight line of the predicted slope is obtained.

The classical 'luminol reaction' was studied by carrying out the previous measurements with hydrogen peroxide instead of the

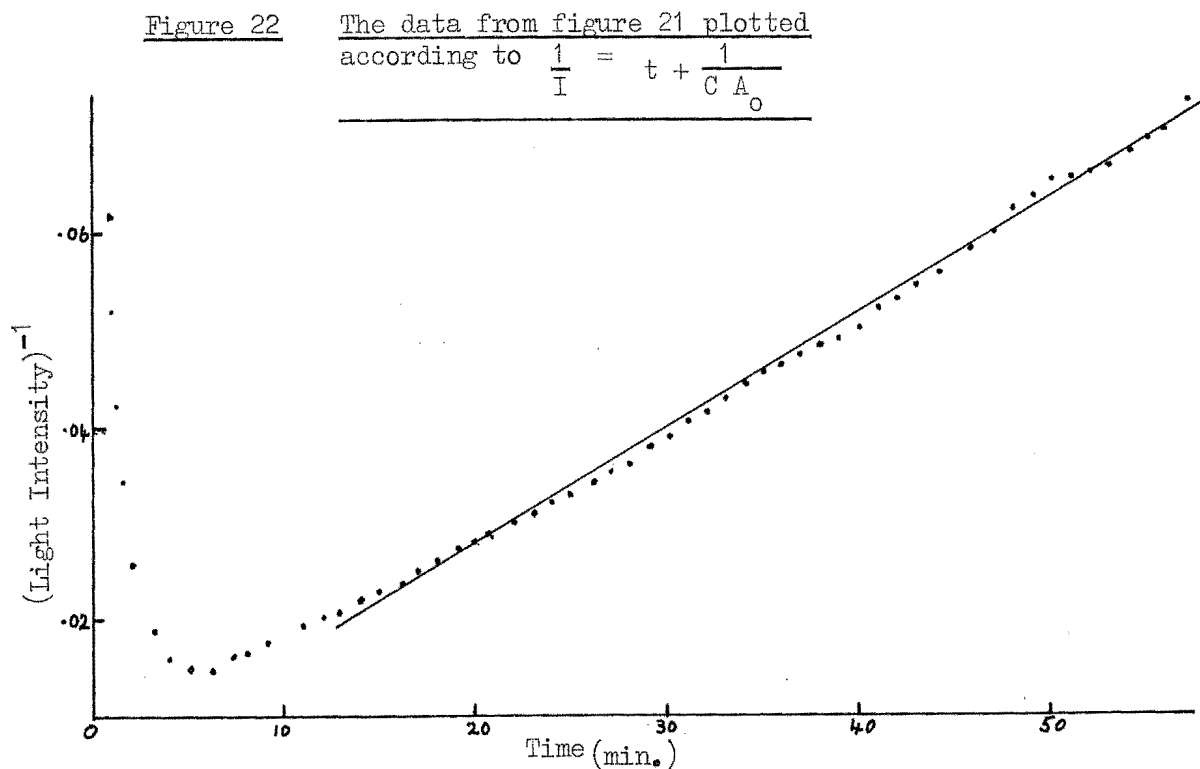
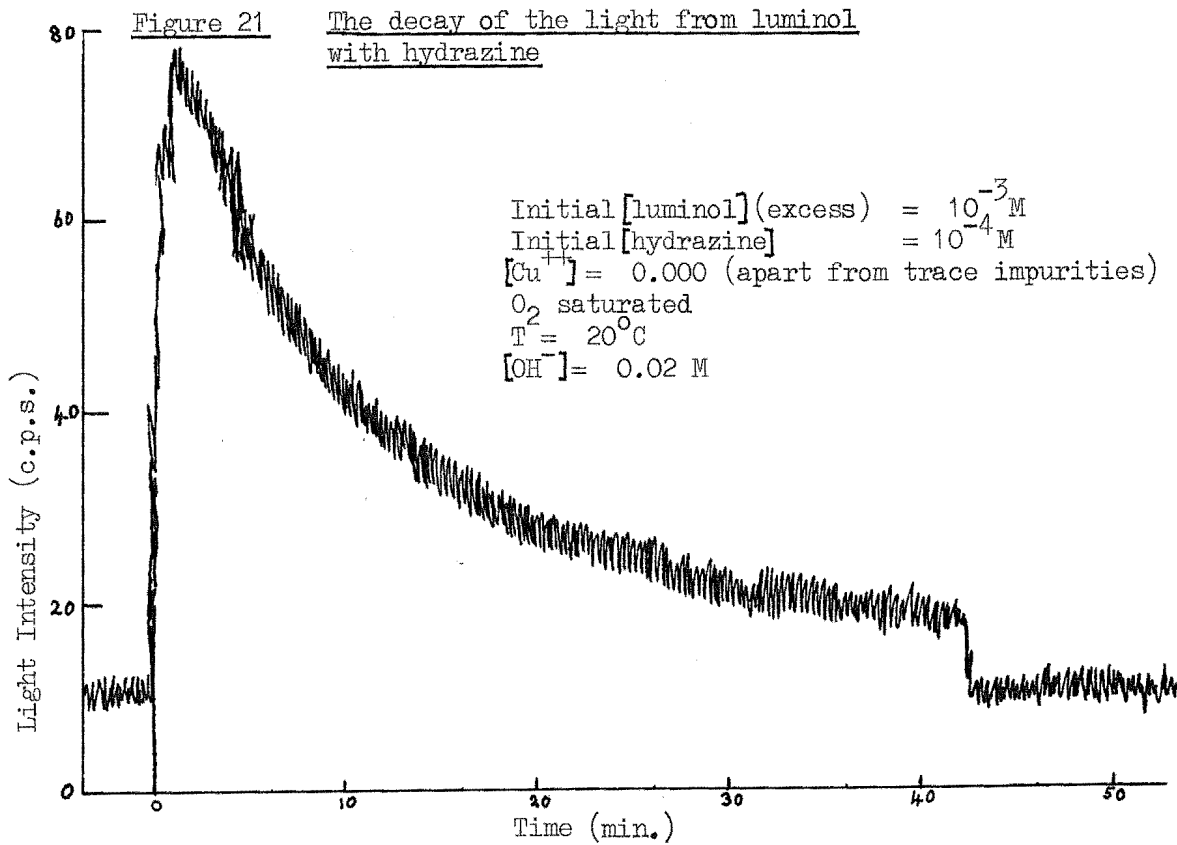


Figure 23 The variation of light intensity with luminol concentration

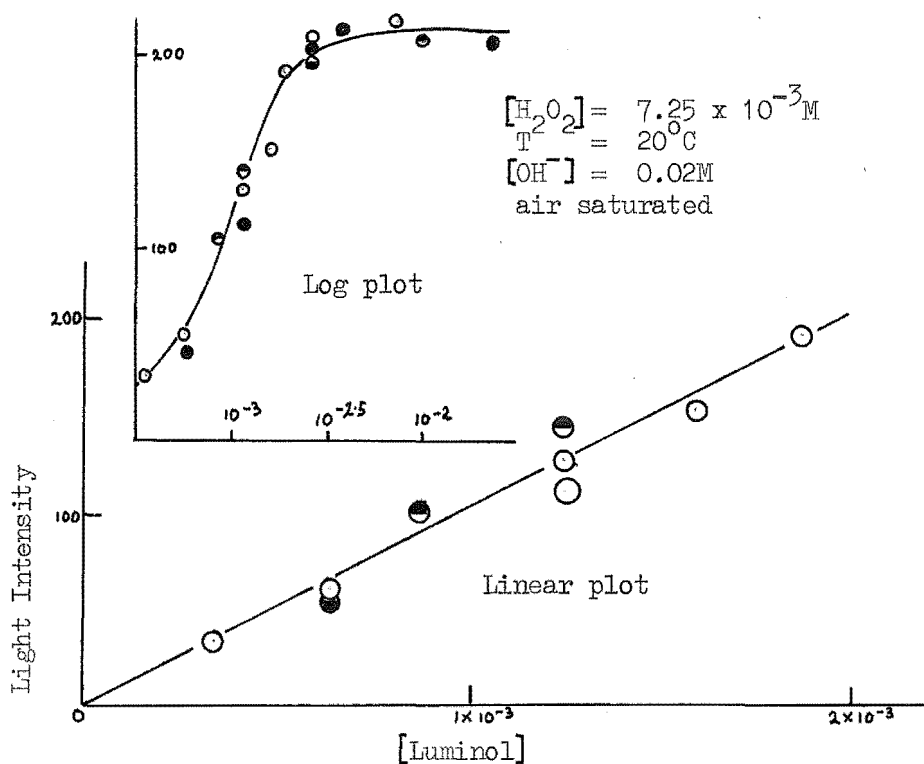
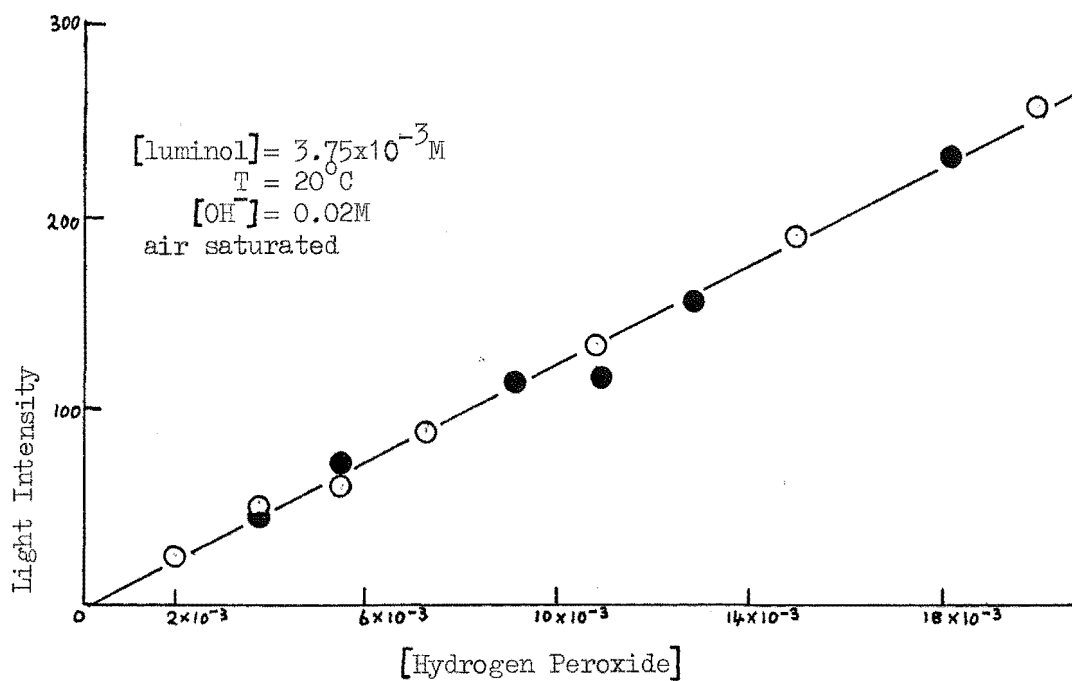


Figure 24 The variation of light intensity with hydrogen peroxide concentration



hydrazine/Cu⁺⁺/O₂ mixture. According to Albrecht's mechanism (page 27) di-imide is now produced by the reaction of luminol with H₂O₂. The kinetic scheme and derived equations 1 and 2 still hold if A is replaced by Lu and X by H₂O₂.

The experimental results in figures 23 and 24 show that the light intensity is proportional to the luminol and H₂O₂ concentrations when these are varied separately. This is as predicted by equation (1) which becomes

$$I = k_1 k_3 [H_2O_2] [Lu] \dots\dots\dots (4)$$

providing quenching is negligible and $k_3 [Lu] \gg k_2$. The luminol concentration was about 100 times greater for the measurements made with H₂O₂ than when hydrazine was used. Under these conditions the light intensities were similar.

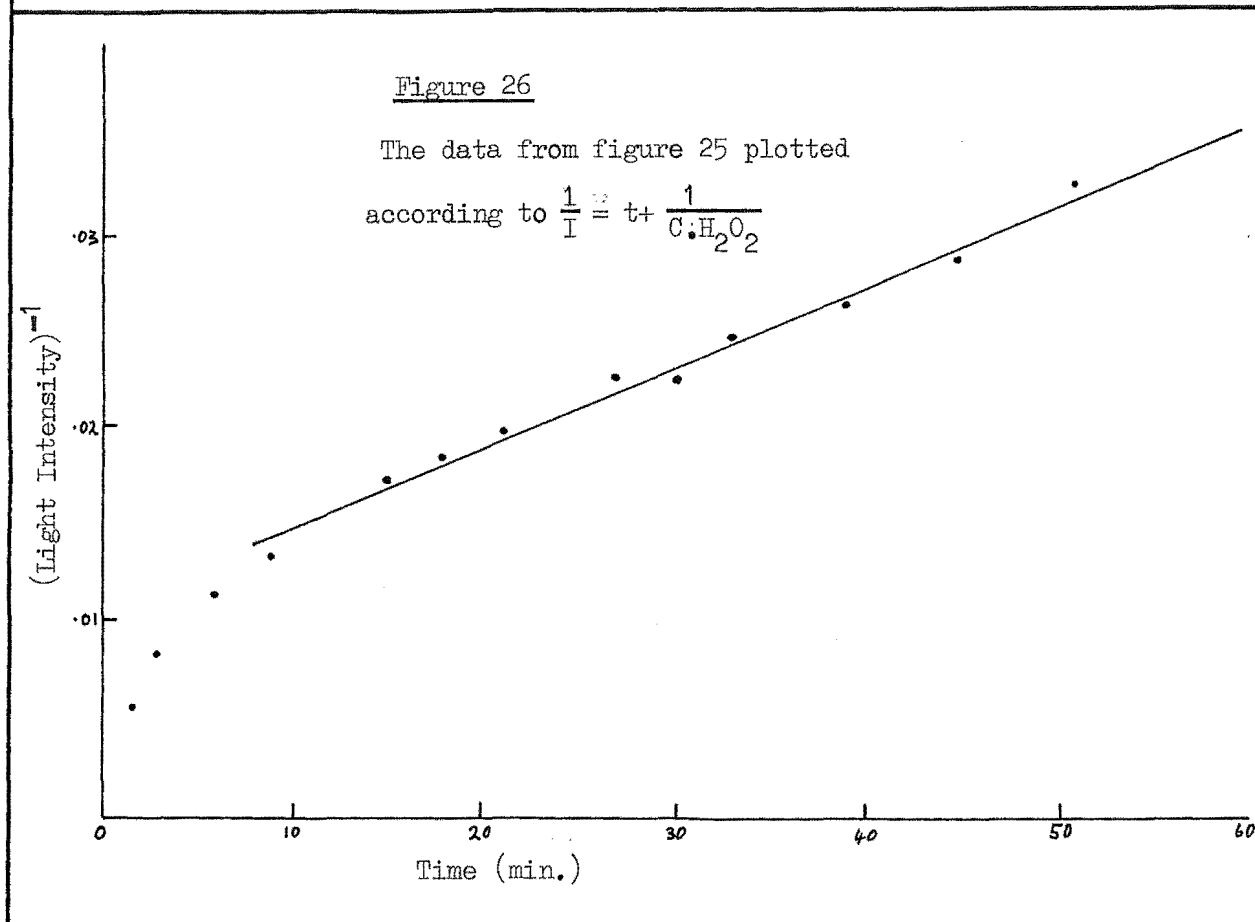
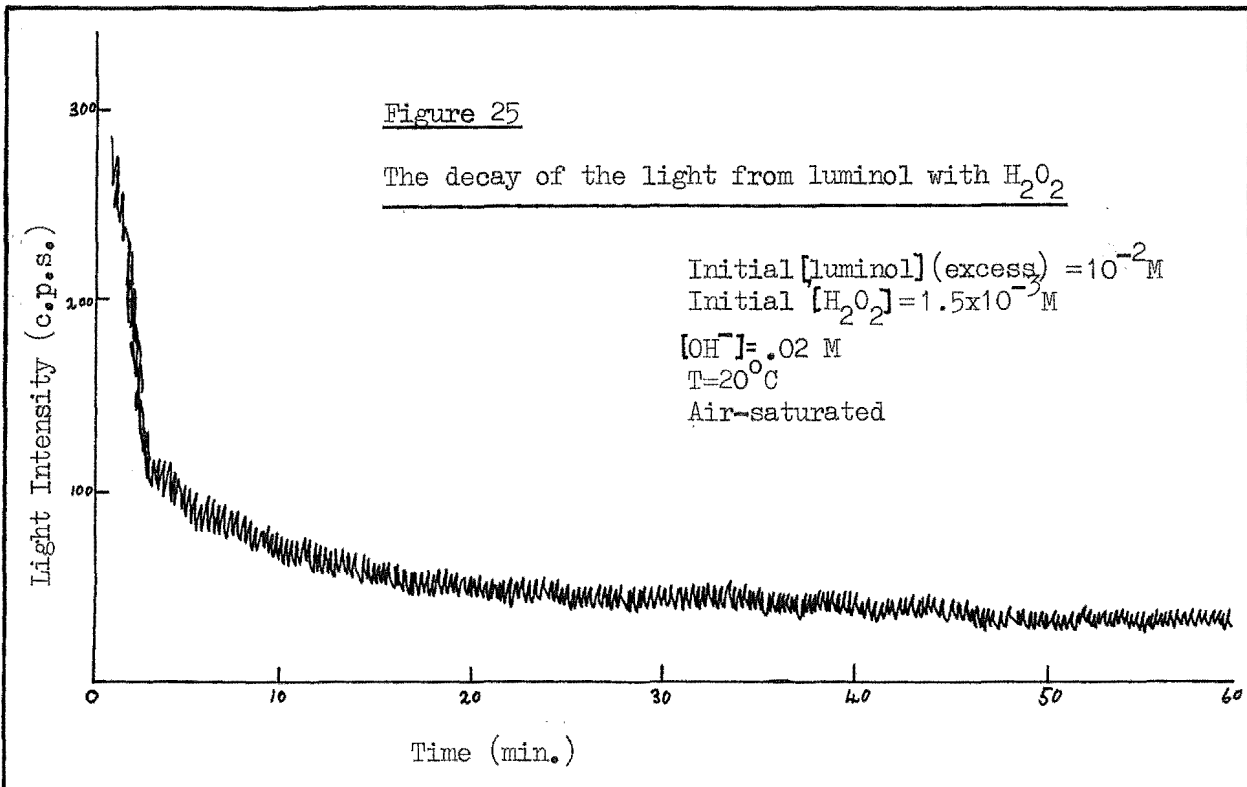
As expected from equation (4), the light intensity I, should decay with time t, according to

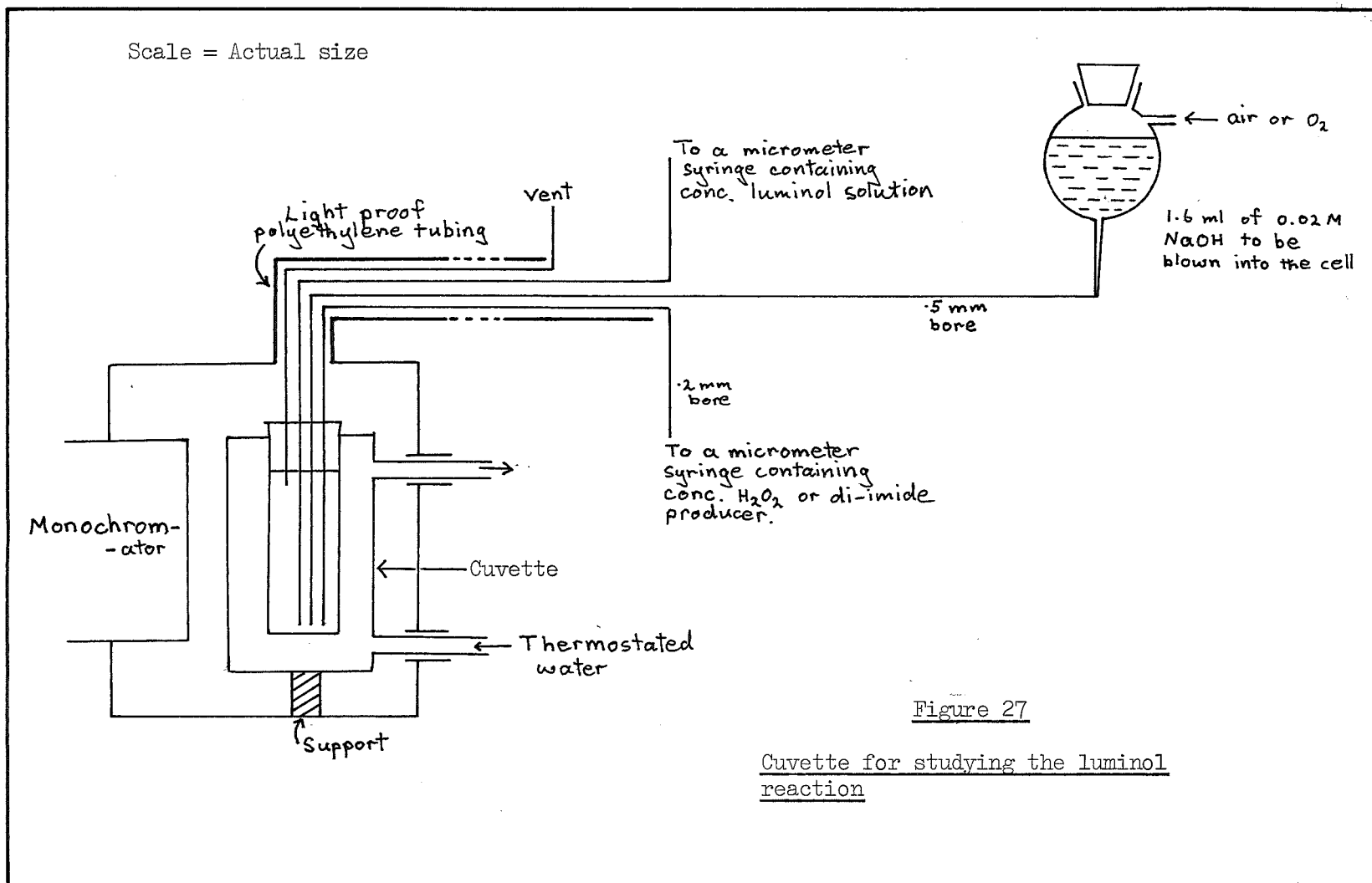
$$t = \frac{1}{I} - \frac{1}{C [H_2O_2]}$$

providing luminol is in excess. The measurements in figures 25 and 26 indicate that this is so after a brief induction period.

Experimental Details

Although the light from the luminol reaction is visibly bright, it was conveniently measured with the photon counter described in part II of this thesis. The light was attenuated by selecting a narrow wavelength range at the spectral maximum of 4,600 Å (uncorrected for absorption of light) with a Hilger monochromator (D285) interposed between the reaction vessel and the 6256SA photomultiplier.





The reaction vessel (figure 27) was a jacketed silica cuvette placed against the monochromator entrance slit inside a light-tight compartment. The cuvette was thermostatted by water circulating through a refrigerator and a coil of nichrome wire heated by current from a variable transformer which was occasionally adjusted to keep the temperature within $\pm 0.15^{\circ}\text{C}$.

1.6 samples of 0.02 M NaOH were placed in the mixing chamber with a stopped syringe accurate to $\pm 0.5\%$ and blown into the cuvette with a stream of air or oxygen which was also used to mix the solutions in the cuvette. Small quantities of strong solutions of luminol stored under N_2 and the di-imide producer entered the cuvette from separate micrometer syringes ($\pm 5 \times 10^{-5}$ ml) through 0.2 mm bore blackened polythene tubing. Reproducible results were obtained only when the same containers and syringes were used consistently for each solution.

Luminol was prepared by nitrating phthalic anhydride (Moser and Gompf⁹¹) and by converting the nitrophthalic acid formed to luminol by the method of Huntress et al⁹². The luminol was purified by dissolving it in aqueous NaOH and re-precipitating it with glacial acetic acid. The dried precipitate (M.P. = 324°C u.c.) was dissolved again in alkali and crystallised as the mono-sodium salt.

The sources of di-imide were p-toluene sulphonyl hydrazine purchased from Aldrich and recrystallised quickly from water (M.P. = 110°C u.c.), hydroxylamine-O-sulphonic acid prepared from bis-hydroxylamine sulphate by the method of Matsuguma and Audrieth⁹⁴, and May and Baker hydrazine containing less than 0.002% of iron.

Sodium hydroxide, copper sulphate and hydrogen peroxide were of ANALAR grade and oxygen was the commercial gas freed from CO_2 by

soda-lime and presaturated with water. All the solutions were made with water redistilled once after distillation from alkaline permanganate. Care was taken to keep metal ions which often⁸⁸ catalyse the formation of di-imide away from the solution.

All kinetic studies were carried out in 0.02 M NaOH in which luminol ($K_{a_1} = 10^{-6}$, $K_{a_2} = 10^{-13}$) should exist almost entirely as the mono-negative ion.

Conclusions

Three different solutions which produce di-imide caused alkaline solutions of luminol to emit light. Kinetic measurements of the light from one such mixture (luminol/hydrazine/ $\text{Cu}^{++}/\text{O}_2$) and of the light from the usual reaction of luminol with H_2O_2 , were consistent with the predictions of a kinetic scheme based on the di-imide mechanism of Albrecht¹¹.

PART V : LOW INTENSITY CHEMILUMINESCENCE

When the human eye is dark adapted it becomes very sensitive and can detect⁵⁰ a pulse of light containing as few as 100 photons. Harvey¹ records a number of weak chemiluminescent reactions which were discovered in this way during the 19th century. These reactions include electrolysis, the dilution of acids, the reactions between inorganic acids and bases and the decomposition of H_2O_2 . Some of these observations, particularly those made by Reichenbach⁹⁵ may have been purely subjective.

Interest in very weak chemiluminescence revived when Rajewsky⁹⁶ (1929) followed by other workers^{20,97} detected weak ultra violet light from dividing cells and various chemical reactions using u.v. sensitive Geiger tubes. As described in Part VI of this thesis, these observations were not^{23,24} always repeatable and received little attention in English speaking countries.

In 1939, Audubert¹⁹ stimulated passing interest at an international symposium on chemiluminescence when he presented evidence for the emission of weak u.v. light from the oxidations of sulphites, pyrogallol and ethanol; the reactions between inorganic acids and bases; electrode reactions and cell division.

Interest in the field again revived¹⁵ in 1960 when the sensitivity of modern photon counters became apparent. Publications^{16,17,18,25,98} on weak chemiluminescence are now frequent and indicate that most reactions in liquids emit very weak light in yields

of 1 photon per 10^{12} - 10^{18} reacting molecules. The steps leading to excitation are usually obscure although the moderately weak light from the electron transfer reactions⁹⁹ of organic radical ions is partly understood.

Light from inorganic acid-base reactions has been observed by several^{25,100} recent workers and some¹⁰⁰ attribute it to excitation of impurities, as occurs when traces of luminol¹⁰⁰ or fluorescent dyes²⁵ are added to the reaction. However, according to Stauff et al.²⁵ who have measured the spectrum of the reaction, the light comes from the excitation of dissolved oxygen.

The aim of the present study was to test these proposals. For this purpose, the photon counter described in Part II of this thesis was used to measure the light emitted when acid and alkali were mixed in a quartz mixing vessel (figure 30) designed for rapid flow rates (20 ml/second each), optimum light collection and avoidance of photo-cathode heating.

Results

In a series of preliminary measurements, the following pairs of substances gave detectable light (>10 photons/sec.) when 2N aqueous solutions made from analytical grade reagents were mixed; NaOH with H_2SO_4 , HCl or HNO_3 ; KOH with H_2SO_4 , HCl or HNO_3 . No light was detected under similar conditions when NH_4OH was mixed with acetic acid or H_2SO_4 nor when KOH was mixed with acetic acid.

The reaction between NaOH and H_2SO_4 was selected for a quantitative study which gave the detailed results in figure 28. These results were found by drawing lines of best fit through recorder traces

Figure 28 The light emitted when 3.30N H₂SO₄ and NaOH are mixed at a total flow rate of 40 ml./sec. under various conditions.

<u>Conditions</u>	Light intensity c.p.s.	Std. dev.	Back-ground c.p.s.	std. dev.
Solutions of 'Analar' reagents.	146	5	19	.5
	165	5	30	.5
	245	5	45	1
	250	5	45	1
	230	5	80	1
	240	5	100	1
	240	5	100	1
	257	5	100	1
Solutions of Riedel de Haen analytical reagents	210	5	55	1
	255	5	65	1
	240	5	65	1
	230	5	70	1
	225	5	70	1
Solutions of R.D.H. reagents in which dissolved oxygen was displaced by H ₂ (< 1ppmO ₂).	232	5	75	1
	250	5	85	1
	255	5	80	1
	245	5	80	1
Solutions of R.D.H. reagents which have been stored in polyethylene containers	450	7	75	1
	490	7	80	1
	500	7	80	1
After the R.D.H. NaOH is boiled with polyethylene	>full scale		60	1
Solutions made from R.D.H. NaOH which was baked for 12 hours in a silver crucible at 450°C and from R.D.H. H ₂ SO ₄ which was refluxed at 330°C for 6 hours and distilled.	60	2	70	1
	65	2	80	1
	70	2	80	1
After a repetition of the above purification on unused samples.	50	2	40	1
	60	2	45	1
	60	2	50	1
After 10 ⁻⁴ moles of AgNO ₃ were added to 1 litre of unpurified R.D.H. NaOH solution and 0.1 ml of chromic acid were added to 1 litre of unpurified R.D.H. H ₂ SO ₄ solution	240	5	50	1
	230	5	55	1
	245	5	60	1
When water at 90°C flows through the system	2	2	50	1
	3	2	60	1
	4	2	75	1

(see figure 29 for a typical example) of the rates of count r , measured with the photon counter. The standard deviations in figure 28 were calculated by $\sigma = \sqrt{\frac{r}{t}}$ ref.¹⁰¹. t is the duration of the recorder trace and is much greater than the time constant of the rate-meter.

It is clear from the measurements in figure 28 that the light from the reaction of NaOH with H₂SO₄ can be measured with a reproducibility of +5%, that polyethylene containers impart a chemiluminescent impurity to the acid or base, that dissolved oxygen is not important and that disruption of possible organic impurities by strongly heating the acid and base reduces but does not remove the light. Traces of chromate and Ag⁺ ions in quantities which might have entered the acid and base during heating have no effect. As the data in figure 28 show, neither the high temperature of the solution nor the dilution of acid or base are responsible for the observed counts.

If the fraction of the emitted light reaching the photomultiplier is of the order of 5%, the quantum yield of the reaction is 1 photon per 10¹⁹ reacting molecules, which is lower than that measured by Audubert¹⁹.

Experimental

The photon counter described in Part II of this thesis was used to measure the light produced in the quartz mixing chamber in figure 30. The solutions of acid and alkali entered this chamber from containers three feet above it, at individual rates of 20 ml/sec. through glass tubes, butt-jointed to the mixing chamber. The present mixing chamber transferred more light to the photomultiplier than did a simple T joint or the cyclone arrangement described by Hartridge and Roughton¹⁰².

Figure 29 A typical run with NaOH + H₂SO₄

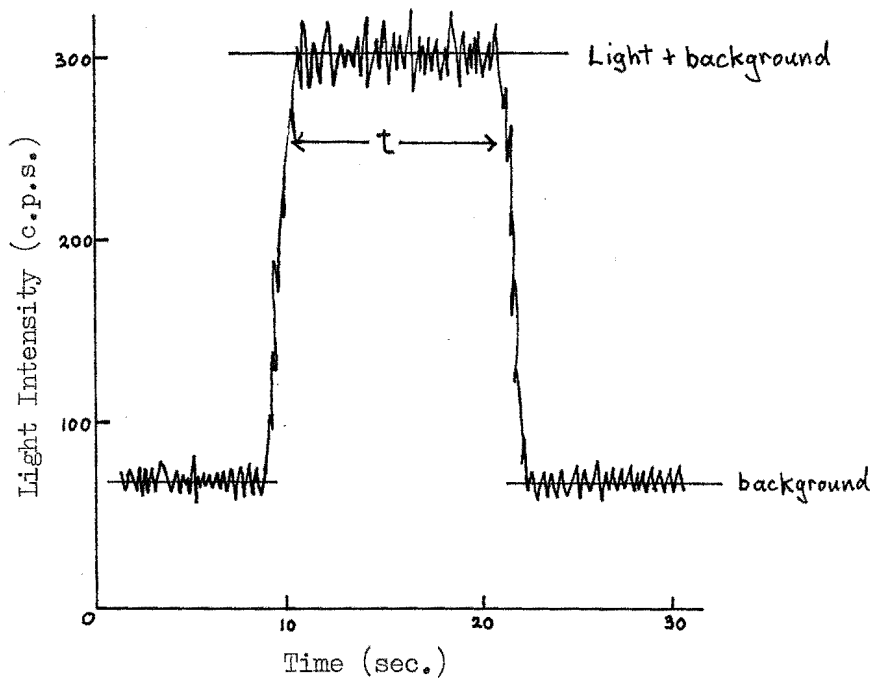
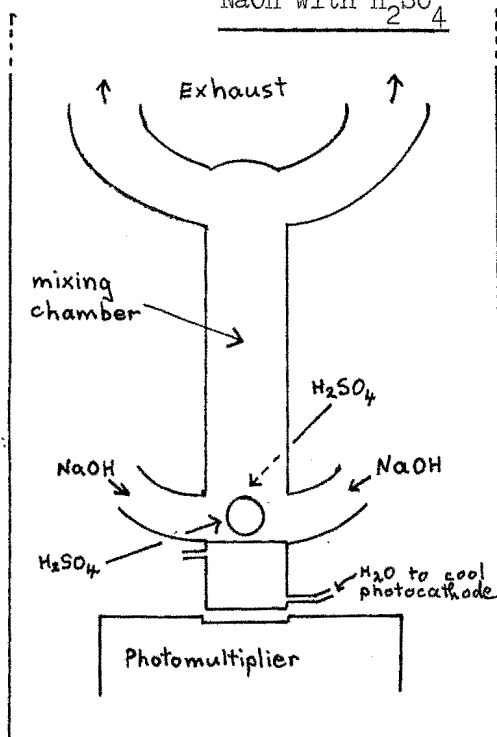
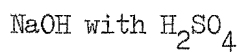


Figure 30 The mixing chamber for the reaction of



Scale: Actual size
(all dimensions
internal)

The count rate was increased ca. 5 times when the mixing chamber was coated externally with magnesium oxide from a burning magnesium ribbon. A water filled filter compartment prevented heat transfer from the reaction mixture to the photomultiplier cathode which was separated from the compartment by a 1 mm air gap which was found to be necessary to avoid spurious pulses.

Conclusions

The light from inorganic acid base reactions has been attributed to oxygen²⁵ and impurities¹⁰⁰. The present measurements show that neither of these is the whole explanation. Polythene containers are a potent source of light emitting impurities and should be avoided.

PART VI : A SEARCH FOR MITOGENETIC RADIATION

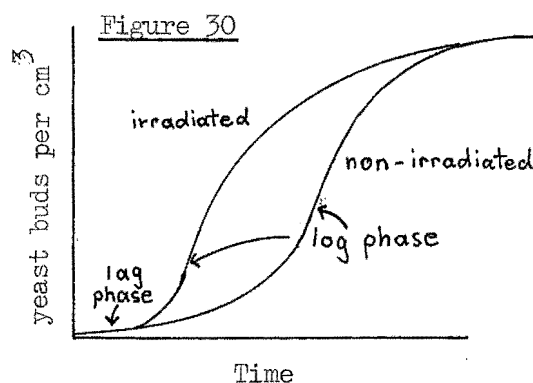
(Chemiluminescence from Dividing Cells)

Over 500 papers^{21,22,103}, mostly in the Russian and German literature support the observations of Gurwitsch²² (1923) who found that dividing cells emit radiation which can stimulate other cells to divide.

In a typical experiment, a detector culture such as yeast or bacteria was exposed to radiation from rapidly dividing yeast, bacteria, or plant or animal tissue. The detector culture showed more cell divisions than an unexposed control culture providing air or quartz separated the specimens, but not when glass was interposed. Such observations, if real, could indicate some biologically active radiation of wavelength below ca. $3,500\text{\AA}$.

Later workers²¹ found that a yeast or bacterial culture radiated most strongly when in its rapidly dividing logarithmic phase of growth (figure 30) and that a detector culture moved more rapidly from its lag to its log phase when irradiated.

All the forgoing views were strongly disputed by a number of workers^{23,24,26} in Britain and the U.S.A., who were unable to detect radiation from cells or to stimulate cell growth with weak ultraviolet light. The controversial nature of the field has been discussed by Rahn²¹ who has obtained positive results, and by Hollaender and Claus¹⁰⁴ whose painstaking study produced no evidence for mitogenetic radiation.



Positive results are currently reported by various Russian workers²⁸⁻³⁰, and the present ambiguous situation has been appraised by Hollaender¹⁰⁵ in a review of Gurwitsch's latest book¹⁰³.

Attempts to detect mitogenetic radiation by physical means give conflicting results. A number of early workers^{20,27,96} claimed significant increases in count rate when u.v. sensitive Geiger tubes were exposed to rapidly dividing yeast, bacteria and other living materials, whereas Lorenz²³, Gray and Ouellet²⁶ and Hollaender and Claus²⁴ observed no increase after all sources of spurious counts were removed. These negative results by this group of careful workers largely discredited the phenomenon, although many Russian biophysicists still support¹⁰³ the work of Gurwitsch and his associates.

Recently, various Russian workers²⁸⁻³⁰ have examined rapidly dividing yeast and tissue cultures with photon counters similar to the one built for this thesis. Such equipment has about 200 times the sensitivity of the equipment used by Hollaender and other early workers. The recent results by Troitskii et al.²⁸ are shown in figure 31.

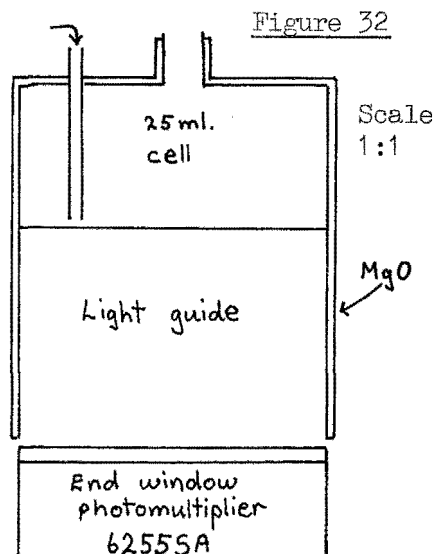
Figure 31		Radiation from Yeasts (Data of Troitskii et al. ²⁸)			
		(c.p.m. for 15 min.)			
	Liquid culture	Solid cultures			
		Expt. No.1	Expt. No.2	Expt. No.3	Expt. No.4
	16.9	7.2	5.0	11.0	8.9
	9.0	7.0	6.6	12.2	6.1
	20.0	7.4	7.1	11.4	7.1
	10.8	7.5	5.7	10.7	
	9.8	7.3	6.9		
	9.3				
	9.5				
Arith. mean	12.2 ^{+1.7}	7.4 ^{+0.1}	6.2 ^{+0.4}	11.3 ^{+0.3}	7.4
Background	8.3 ^{-0.7}	5.2 ^{+0.5}	5.2 ^{+0.2}	8.6 ^{+0.4}	6.4
Excess over Background %	47 ⁺²²	42 ⁻¹⁰	19 ⁺⁸	31 ⁺⁶	16

There is no record in English or American Journals of similar high sensitivity measurements, which are the purpose of this investigation. The emphasis is on optimum light collection, large samples of cell culture and large photocathodes, rather than on the ultra-low 'dark counts' of previous workers. Very low dark counts are easily falsified by external interference of a temporary and unpredictable nature.

EXPERIMENTAL

The detector was the photon counter described on page 12, equipped with a large 4 cm diameter photocathode sensitive to wavelengths between $2,000\text{\AA}$ and $6,000\text{\AA}$. Mitogenetic radiation is usually assigned²¹ to the range 1900\AA - $2,500\text{\AA}$ and occasionally to the range $3,200\text{\AA}$ - $3,500\text{\AA}$.

The suspensions of yeast or bacteria were contained in a 25 ml silica cell (figure 32), connected optically to the photo-

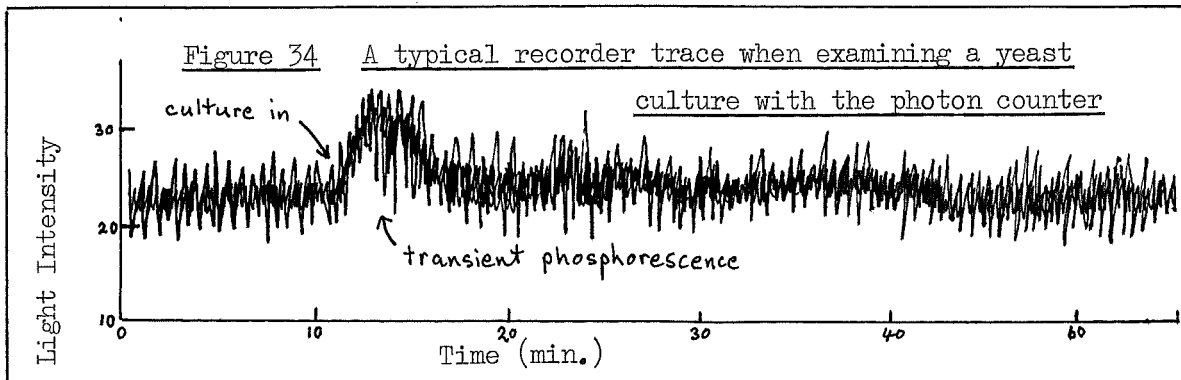


multiplier by a hollow silica tube and 1 mm air-gap. If the cell was close to the photocathode or if the air-gap was omitted, spurious pulses were observed when liquid filled the cell. The light reaching the cell was increased about 10 times by coating the light guide and cell externally

with magnesium oxide from a burning magnesium ribbon.

Figure 33

LIGHT EMITTED IN THE RANGE 2,000-6,000 Å BY SUSPENSIONS OF YEAST OR BACTERIA										
Content	Opacity		Light plus background		Background				Light Intensity	
	measured with Welcome Opacity tubes	Makers as millions per ml. of E.Coli	c.p.s.	σ	Before c.p.s.	σ	After c.p.s.	σ	c.p.s.	σ
E. Coli	6.5	2460	24.3	.3	24.5	.3	25.0	.3	0	.6
	6.5	2460	24.6	.3	24.8	.3	24.3	.3	0	.6
S. Pombe	4.0	1515	24.5	.2	24.0	.3	24.5	.3	0	.5
	4.0	1515	24.7	.2	24.5	.2	24.2	.3	0	.5
Candida	3.5	1330	24.0	.3	24.5	.3	24.0	.3	0	.6
	3.5	1330	24.2	.3	24.0	.3	24.7	.2	0	.6
S. Cerevis	2.5	950	26.0	.4	25.0	.3	25.5	.2	0	.5
	2.5	950	26.2	.1	25.5	.2	27.0	.3	0	.5
	2.5	950	24.2	.1	24.5	.2	24.0	.1	0	.3
(new sample)	6.0	2270	17.5	.2	17.8	.2	18.0	.2	0	.4
	6.0	2270	18.0	.3	18.0	.2	19.0	.2	0	.5
	6.0	2270	18.0	.2	19.0	.2	18.5	.3	0	.5
(new sample)	4.0	1515	31.0	.2	31.5	.2	32.0	.2	0	.4
Light from a reference solution ²⁸ of 0.01M KMnO ₄ and 0.01M oxalic acid ⁴ in water. (Passed through a filter transmitting 2,000-4,000Å.)			35.5	.3	28.2	.3	28.5	.3	7.0	.6
			35.0	.3	28.0	.3	26.5	.3	6.5	.6
			31.5	.3	24.5	.2	24.8	.2	7.0	.5
			30.0	.3	24.0	.2	24.3	.2	6.0	.5



RESULTS

Suspensions of bacterial and yeast cultures in the rapidly dividing logarithmic phase of growth were introduced into the cell from a syringe through a long black plastic tube. The table in figure 33 shows results for these and for a solution containing 0.01 M KMnO_4 and sodium oxalate which is reported²⁸ to emit light of similar intensity to mitogenetic radiation.

The count rates r in figure 33, were obtained by drawing lines of best fit through the chart recorder traces (see the typical example in figure 34) of the ratemeter output. For a recorder trace lasting t seconds, the standard deviation in r is $\sigma = \left(\frac{r}{t}\right)^{\frac{1}{2}}$ providing the time constant τ of the ratemeter is $\ll t$. Furthermore, by keeping τ small, occasional groups of pulses due to external interference have only a transient effect on the recorder trace and can be ignored when estimating the line of best fit. A brief phosphorescence from the yeast and bacterial cultures when they first entered the cell was also ignored as it was similarly observed from the pure nutrient media (Trypticase soy broth) and in all cases decayed (figure 34) to the background after ca. 5 minutes.

Opacity measurements on the suspensions before and after optical measurements, verified that the cultures were in their rapidly dividing log phase following incubation for 24 hours at 35°C and removal to an environment at $20 \pm 1^{\circ}\text{C}$, two hours before optical measurements were made.

Conclusion

No mitogenetic radiation was detected when the photon counter was exposed to rapidly dividing cultures of the yeasts *Candida Albicans*,

Saccharomyces Cerevisiae, Schizosaccharomyces Pombe or the bacterium Escherichia Coli, any increases in the background count rate being less than two standard deviations. If mitogenetic radiation exists and lies in the range 2000\AA - 3500\AA , it must be less than $1/10$ as intense as the light in this same range from an aqueous solution 0.01 M in KMnO_4 and sodium oxalate. According to results from the laboratory of Gurwitsch quoted by Troitskii²⁸, the light from this reaction is of similar intensity to mitogenetic radiation.

APPENDIX 1

GENERAL CURVE FITTING AND GRAPH PLOTTING PROGRAM

(Requiring no programming experience and minimal familiarity with a computer).

Description

Part I: This Fortran I program fits the equation

$$y = a_0 + a_1x + a_2x^2 + \dots + a_nx^n \text{ or the equation}$$

$$y = e^{(a_0 + a_1x + a_2x^2 + \dots + a_nx^n)} \text{ to up to 500 pairs of data } x, y$$

of weight w , for any chosen value of n between 1 and 15 inclusive.

The output, which may be typed or punched on cards includes the regression coefficients a_0, a_1, \dots, a_n , the standard deviation from the regression line, actual and fitted y values, their difference and x .

The methods of regression and calculation of standard deviation are from a program by Graves¹⁰⁶ and the calculation of weight factors and standard deviation in the exponential section have been described by Deming¹⁰⁷.

Part II: The punch output from Part I can, if desired, be input to Part II which plots original data x and y , the fitted equation, axes, grid and scale as a 5" x 5" or 10" x 10" graph.

Machine Requirements

Part I requires an I.B.M. 1620 computer with 40,000 units of storage and a 1622 card read/punch, or similar units. Part II requires in addition, an I.B.M. 870 unit for autoplotting.

Preparation of Data

Only one set of data x, y, w should be punched on each card according to the Format specification 3E10.4, where x and y are the

independent and dependent variables respectively and w is the weight factor for y . If w is not punched, the program assigns it the value 1.

Any set of data cards must be preceded by one header card on which is punched according to Format (14,E10.4,14); N , the number of data cards; TOL , the largest acceptable standard deviation from the regression line; and $LAST$, the highest order polynomial fitted.

Operating Instructions (for the I.B.M. 1620/22)

The following three procedures will be taken for granted in subsequent instructions.

- (a) Press **READER START** to load the last two cards of any pile of cards in the "read" hopper.
- (b) Three cards (2 blank and one punched) remain in the card punch at the end of any batch of punching. To remove them, lift the unpunched cards from the "punch" hopper and press **NON-PROCESS RUN OUT**. The two blank cards are discarded and the single punched card is added to the bottom of the deck which has been punched.
- (c) The overflow switch to **PROGRAM**; **I/O** and **PARITY** switches to **STOP**.

Part I (for fitting equations):

- 1. Clear Storage.
- 2. Place the Part I card deck in the read hopper and press **LOAD**.
- 3. Set the Sense Switches:

Switch	ON	OFF
1	Polynomial fitted	EXP (Polynomial fitted)
2	Punch output on cards	No punch output
3	List of observed Y and calculated Y is typed	List not typed
4	(Normally OFF)	

(If the 'fit' is not satisfactory after the specified highest order polynomial (LAST) has been reached, turn switch 4 ON. When the typewriter 'clicks' turn switch 4 OFF, type in new values for TOL (The allowed standard deviation) and LAST according to FORMAT (2E10.4), press RELEASE, SIE, SIE, START. The program then proceeds to calculate higher order polynomials.)

4. When the instruction "load data" is typed, put the data cards (preceded by the header card) in the read hopper.
5. Press READER START and START.

When the switches 2, 3 and 4 are OFF, the standard deviation and regression coefficients a_0, a_1, \dots, a_n are typed out each time a polynomial is fitted. When the 'fit' is sufficiently good, a table of Y_{obs} and Y_{calc} can be typed out by turning switch 3 ON, and/or punched out by turning switch 2 ON. Punch output is necessary if a graph is to be plotted with Part II.

Part II (for plotting graphs)

6. Clear storage.
7. Turn Sense Switch 1 ON if a grid is to be plotted.
Other Sense Switches are not used.
8. Place Part II card deck in the read hopper and press LOAD.
9. When "load data" is typed, put either the instruction cards for a 5" x 5" graph or those for a 10" x 10" graph followed by the card output from Part I, in the read hopper.
10. Put blank cards in the punch hopper.
11. Press PUNCH START, READER START and START.
12. When the typewriter carriage "clicks", ensure switch 4 is OFF and type in values for XMAX, YMAX, XMIN, YMIN according to FORMAT (4E10.4).
XMAX and XMIN are the values of x at the right hand and left hand

edges of the graph respectively. It is usually convenient for XMAX to be the first multiple of 10 (or 5) which exceeds the largest value to be plotted. It is usually convenient for XMIN to be zero or the first multiple of 10 (or 5) which is less than the smallest x value to be plotted. YMAX and YMIN are the corresponding Y axis quantities and are treated similarly. As an example, if x ranged from 0.5 to 9 and y ranged from 0.2 to 0.7, the figures bbbbbb1.E1bbbbbb1.E0bbbbbb0.E0bbbbbb0.E0 could be typed in.

13. Press RELEASE, SIE, SIE and START.
14. The cards now being punched are the input for the Library Autoplot program (CX-01X), which is executed as follows:-
15. Clear storage.
16. Put the Library Autoplot program (CX-01X) followed by the card output from step 9 in the read hopper.
17. Turn all Sense-Switches OFF.
18. Press PUNCH START and LOAD.
19. When "Load data" is typed, press START.
20. The puⁿch output is taken to the 870 graph-plotter.

To operate the 870 for graph plotting:

21. Insert the Autoplotter panel in the base of the machine, put a blank card on the program drum and lower the star-wheels on to the drum.
22. Turn the Power Switch ON, the Pulse Switch OFF and both Auto-feed switches ON and the Print Switch Off.

23. Turn ON the plotting typewriter (switch under R.H.S.), check that tabs are set at 1 inch intervals and square the paper in the typewriter.
 24. Put the card output from step 20 in the read hopper of the 870 and press FEED twice.
 25. Send the last card through by pressing REGISTER.
-

The curve fitting and graph plotting programs are listed on the following five pages.

Part I of the Curve Fitting and Graph Plotting Program

```
C POLYNOMIAL OR LOGARITHMIC REGRESSION ANALYSIS
C WITH OPTIONAL OUTPUT TO AUTOLOTTER.
C T. I. QUICKENDEN 1965
  DIMENSIONX(500),Y(500),A(16,16),SUMX(31),SUMY(15),W(500)
  1 READ 200,N,TOL,LAST
    DO 40 I=1,N
      READ 202,XX,YY,WW
      IF(SENSE SWITCH1) 21,22
21  X(I)=XX
    Y(I)=YY
      IF(WW) 3,2,3
  2  WW=1.
  3  W(I)=WW
    GO TO 40
22  X(I)=XX
    Y(I)=LOGF(YY)
      IF(WW) 5,4,5
  4  WW=1.
  5  W(I)=YY**2*WW
40  CONTINUE
70  SUMX(1)=0.
    SUMX(2)=0.
    SUMX(3)=0.
    SUMY(1)=0.
    SUMY(2)=0.
    DO 90 I=1,N
      SUMX(1)=SUMX(1)+W(I)
      SUMX(2)=SUMX(2)+W(I)*X(I)
      SUMX(3)=SUMX(3)+W(I)*X(I)*X(I)
      SUMY(1)=SUMY(1)+W(I)*Y(I)
90  SUMY(2)=SUMY(2)+W(I)*X(I)*Y(I)
    NORD=1
91  IF(SENSE SWITCH 4) 92,93
92  ACCEPT 214,TOL,LAST
93  L=NORD+1
    KK=L+1
    DO 101 I=1,L
      DO 100 J=1,L
        IK=J-1+I
100  A(I,J)=SUMX(IK)
101  A(I,KK)=SUMY(I)
    DO 140 I=1,L
      A(KK,I)=-1.
      KKK=I+1
      DO 110 J=KKK,KK
110  A(KK,J)=0.
      C=1./A(1,I)
      DO 120 II=2,KK
        DO 120 J=KKK,KK
120  A(II,J)=A(II,J)-A(1,J)*A(II,I)*C
      DO 140 II=1,L
        DO 140 J=KKK,KK
140  A(II,J)=A(II+1,J)
```

```
S 2=0.
DO 160 J=1,N
S 1=0.
S 1=S 1+A(1, KK)
DO 150 I=1,NORD
150 S 1=S 1+A(I+1, KK)*X(J)**I
160 S 2=S 2+(S 1-Y(J))*(S 1-Y(J))
B=N-L
S 2=(S 2/B)**.5
IF(SENSE SWITCH 1) 190, 191
190 PRINT 220
GO TO 192
191 PRINT 221
192 CONTINUE
PRINT 203
PRINT 204
PRINT 205, NORD, TOL, S 2, N
PRINT 210
PRINT 211
IF(SENSE SWITCH 2) 176, 177
176 PUNCH 216
PUNCH 217
IF(SENSE SWITCH 1) 185, 186
185 LLG=1
GO TO 187
186 LLG=2
187 PUNCH 213, LLG
PUNCH 213, NORD
PUNCH 213, N
177 DO 164 I=1, L
J=I-1
PRINT 206, J, A(I, KK)
IF(SENSE SWITCH 2) 178, 164
178 PUNCH 212, A(I, KK)
164 CONTINUE
IF(SENSE SWITCH 2) 179, 180
179 DO 175 I=1, N
IF(SENSE SWITCH 1) 181, 182
181 PUNCH 212, X(I), Y(I)
GO TO 175
182 XX=X(I)
YY=EXP(Y(I))
PUNCH 212, XX, YY
175 CONTINUE
180 IF(SENSE SWITCH 3) 167, 166
166 IF(S 2=TOL) 167, 167, 171
167 CONTINUE
PRINT 207
PRINT 208
DO 169 I=1, N
S 1=0.
S 1=A(1, KK)
DO 168 J=1, NORD
```

```

168 S1=S1+A(J+1, KK)*X(I)**J
    S3=Y(I)-S1
    IF(SENSE SWITCH1) 183, 184
183 PRINT 209, X(I), Y(I), S1, S3
    GO TO 169
184 YY=EXPF(Y(I))
    XX=X(I)
    SS1=EXPF(S1)
    S3=YY-SS1
    PRINT 209, XX, YY, SS1, S3
169 CONTINUE
    IF(NORD-LAST) 170, 173, 173
170 IF(S2-TOL) 173, 173, 171
171 NORD=NORD+1
    J=2*NORD
    SUMX(J)=0.
    SUMX(J+1)=0.
    SUMY(NORD+1)=0.
    DO 172 I=1, N
    SUMX(J)=SUMX(J)+X(I)**(J-1)*W(I)
    SUMX(J+1)=SUMX(J+1)+X(I)**J*W(I)
172 SUMY(NORD+1)=SUMY(NORD+1)+Y(I)*X(I)**NORD*W(I)
    GO TO 91
173 PAUSE
    IF(SENSE SWITCH 4) 171, 1
200 FORMAT(14, E10.4, 14)
202 FORMAT(3E10.4)
203 FORMAT(/46H ORDER OF          ALLOWED          OBSERVED
204 FORMAT(46HPOLYNOMIAL        STD DEV          STD DEV
205 FORMAT(4X, 14, 6X, E10.4, 4X, E10.4, 5X, 14, 2X)
206 FORMAT(3X, 14, 8X, E10.4)
207 FORMAT(49HINDEPENDENT        OBS DEP          FITTED DEP
208 FORMAT(34H VARIABLE          VARIABLE          VARIABLE)
209 FORMAT(E10.4, 2X, E10.4, 2X, E10.4, 2X, E10.4)
210 FORMAT(/24HINDEX OF          COEFFICIENT)
211 FORMAT(22H TERM              OF TERM)
212 FORMAT(2E10.4)
213 FORMAT(14)
214 FORMAT(E10.4, 14)
217 FORMAT(18HDEPENDENT VARIABLE)
216 FORMAT(20HINDEPENDENT VARIABLE)
220 FORMAT(/12HY=POLYN IN X)
221 FORMAT(/17HY=EXP(POLYN IN X))
    END

```

Part II of the Curve Fitting and Graph Plotting Program

```
C AUTO PLOT PROGRAM TO ACCEPT OUTPUT OF REGRESSION ANALYSIS PROGRAM
C T.I. QUICKENDEN, AUGUST 1964
  DIMENSION X(500), Y(500)
  READ 101, I1, I2, I3, I4, I5, I6
  READ 102, J1, J2
  READ 102, J3, J4
  READ 113, K1, K2, K3
  READ 113, K4, K5, K6
  READ 109, M1, M2, M3, M4, M5, M6
  READ 109, N1, N2, N3, N4, N5, N6
  READ 110, IX, IY
  READ 107
  READ 108
  READ 104, LLG
  READ 104, NORD
  READ 104, N
  DIMENSION A(16)
  LORD=NORD+1
  SIX=IX
  SIY=IY
  DO 11=1, LORD
1  READ 103, A(1)
  DO 61=1, N
6  READ 105, X(1), Y(1)
  ACCEPT 114, XMAX, YMAX, XMIN, YMIN
  PUNCH 100, 1, I1, I2, I3, I4, I5, I6
  PUNCH 100, 2, J1, J2
  SX=XMIN
4  SY=A(1)
  DO 21=2, LORD
  SYM=A(1)*SX**(1-1)
2  SY=SY+SYM
  GO TO (7, 8), LLG
7  SSY=SY
  GO TO 9
8  SSY=EXPF(SY)
9  JSX=100.*SIX*SX/(XMAX-XMIN)
  JSY=100.*SIY*SSY/(YMAX-YMIN)
  PUNCH 100, 3, JSX, JSY
  SX=SX+(XMAX-XMIN)/100.
  IF(SX-XMAX) 4, 4, 3
3  DO 51=1, N
  JAX=100.*SIX*X(1)/(XMAX-XMIN)
  JAY=100.*SIY*Y(1)/(YMAX-YMIN)
  PUNCH 100, 2, J3, J4
5  PUNCH 100, 3, JAX, JAY
  IF(SENSE SWITCH) 10, 22
10 I11=I1-100
  I22=I2-100
  DO 21 IAYY=100, I22, 100
  DO 20 IAXX=100, I11, 100
  IAX=IAXX-100
```

```
IAY=IAYY-100
PUNCH100,2,01,11
20 PUNCH100,3,IAX,IAY
21 CONTINUE
22 PUNCH100,4,K1,K2,K3
    PUNCH107
    PUNCH100,4,K4,K5,K6
    PUNCH108
    PUNCH100,5,M1,M2,M3,M4,M5,M6
    PUNCH100,5,N1,N2,N3,N4,N5,N6
100 FORMAT(7I5)
101 FORMAT(I4,1X,I4,1X,I3,1X,I3,1X,I1,1X,I1)
102 FORMAT(I2,1X,I2)
103 FORMAT(E10.4)
104 FORMAT(I4)
105 FORMAT(7E10.4)
107 FORMAT(49X)
108 FORMAT(49X)
109 FORMAT(I4,1X,I4,1X,I4,1X,I4,1X,I4,1X,I2)
110 FORMAT(2I3)
113 FORMAT(I4,1X,I4,1X,I2)
114 FORMAT(4E10.4)
    PUNCH100,6
    STOP
    END
```

INSTRUCTION CARDS FOR PART II, 5 INCH BY 5 INCH GRAPH

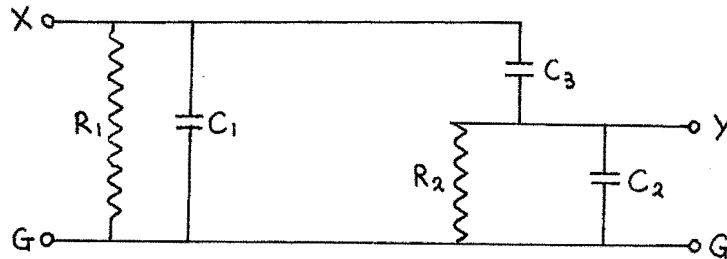
0700 0700 100 100 0 0
41 10
01 00
25 -60 00
-60 500 10
0 -10 6 0 2 00
-10 0 6 0 2 10

INSTRUCTION CARDS FOR PART II, 10 INCH BY 10 INCH GRAPH

1200 1200 100 100 0 0
41 10
01 00
100 -80 00
-80 650 10
-10 0 11 0 10 10
0 -10 11 0 10 00
10 10

APPENDIX 2. Analysis of the Photomultiplier Coupling Circuit

The photomultiplier coupling circuit (page 18) is equivalent to



The steady state impedance between X and G is

$$Z_{XG} = Z_1(Z_2+Z_3)/(Z_1+Z_2+Z_3)$$

where

$$Z_1 = R_1/(1+j\omega R_1 C_1) ; Z_2 = R_2/(1+j\omega R_2 C_2) ; Z_3 = 1/j\omega C_3$$

and ω is the angular frequency.

To an input current i_m , the mutual impedance Z_m results in an output voltage appearing at Y of

$$E_{YG} = i_m \cdot Z_m \dots\dots\dots (1)$$

where $Z_m = Z_1 Z_2 / (Z_1 + Z_2 + Z_3)$

Substituting s for $j\omega$, and using the values of Z_1, Z_2, Z_3 above

$$Z_m = s C_3 / \{ s^2 (C_2 C_3 + C_1 C_3 + C_1 C_2) + s \cdot [\mu C_2 C_3 + \lambda C_1 C_3 + (\mu + \lambda) C_1 C_2] + \mu \lambda C_1 C_2 \} \dots\dots (2)$$

where $\lambda = 1/R_1 C_1$, $\mu = 1/R_2 C_2$.

Hence $Z_m = [C_3 / (C_2 C_3 + C_1 C_3 + C_1 C_2)] \cdot s / [(s + \alpha)(s + \beta)] \dots\dots (3)$

where α and β are the roots of the quadratic denominator of equation (2).

Solution of the integro-differential equations for the passage of a charge pulse from the photomultiplier through the coupling circuit may be simplified by observing that equation (3) is the Laplace transform¹⁰⁸ of the function

$$a_1(\beta e^{-\beta t} - \alpha e^{-\alpha t})/(\beta - \alpha)$$

and represents the output voltage at Y, t seconds after the arrival of a unit impulse (δ function) of charge at X. The constant a_1 is given by

$$a_1 = C_3/(C_1C_3 + C_1C_2 + C_2C_3).$$

As the photomultiplier pulses are narrow there is no delay between rise and fall, i.e. no sag due to C_3 , and further, as $C_1C_2 \ll C_3$, terms of the C_1C_2 product can be neglected so that

$$\alpha = 0, \beta = -(\lambda C_1 + \mu C_2)/(C_1 + C_2).$$

For a charge pulse of q coulombs from the photomultiplier the voltage appearing at Y is

$$E_{YG} = q/(C_1 + C_2) \cdot e^{-\beta t}$$

which implies that the voltage rises sharply to a maximum and falls with a decay constant

$$\beta = (R_1 + R_2)/(R_1R_2C_2 + R_1R_2C_1).$$

Usually, $\frac{1}{\beta}$ is the longest time constant encountered by pulses in the photon counter and hence pulse overlap is important in the coupling circuit. In the text, the decay constant β is denoted by A which equals $6.3 \times 10^4 \text{ sec.}^{-1}$ in the present equipment (page 18).

The dependence of the height of a multiple pulse on the photoelectron rate n and the decay constant A .

As shown in the text, the height of the last of a sequence of x pulses in the photomultiplier coupling circuit is

$$h = h_1 + h_2 e^{-At_2} + \dots + h_x e^{-At_x}.$$

The relationship of values of h to the individual pulse heights depends on A and on the distribution of times t_i ; the latter is dependent only on n . Since n and A are both of unit dimension in reciprocal time, it follows that this relation must depend only on the quotient $\frac{n}{A}$.

APPENDIX 3

THE DISTRIBUTION OF HEIGHTS OF THE LAST OF A SEQUENCE
OF OVERLAPPING PULSES. AN ALTERNATIVE METHOD

$P(1,h)dh$ is the fraction of isolated pulses of height h to $h+dh$, $Q(x,t)dt$ is the fraction of sequences of x pulses of total duration t to $t+dt$, and was calculated on page 20. From these will be calculated $S(x,h)dh$ which is the probability that the first pulse of such a sequence will contribute a height between h and $h+dh$ to the last pulse. Finally, from these distributions will be calculated the probability required, $P(x,h)dh$, which is the fraction of pulses which, together with the contributions of the preceding $x-1$ pulses reach a height between h and $h+dh$.

If the first pulse of a sequence of duration t has a residual height between h and $h+dh$ at the end of the sequence, its original height must have been between he^{At} and $(h+dh)e^{At}$, for which the probability is $e^{At} \cdot P(1,he^{At})dh$. Allowing for all possible durations, one writes

$$S(x,h)dh = \left[\int_0^{\infty} e^{At} \cdot P(1,he^{At}) \cdot Q(x,t)dt \right] dh$$

For sequence of two pulses, the height of the latter h is combined with the residual height of the former ($h-h'$) to get

$$P(2,h)dh = \left[\int_0^{\infty} S(2,h-h') \cdot P(1,h)dh' \right] \cdot dh$$

By considering in the same way the effect of the first pulse on the combined effect of the remainder of the sequence, one finds for

the height of the last pulse, that

$$P(x,h)dh = \left[\int_0^{\infty} S(x,h-h') \cdot P(x-1,h)dh' \right] \cdot dh$$

Numerical calculation proceeds stepwise as x increases, but soon makes excessive demands on computer storage and time.

APPENDIX 4

THE MONTE CARLO PROGRAM TO CALCULATE THE PROBABLE HEIGHTS OF

OVERLAPPING PULSES

This Fortran II program was outlined on page 20 and required a computer such as the I.B.M. 1620 with 40,000 units of storage. The output of this program is a number of multiple pulse heights punched on cards which are input to the Stochastic Integral Program which determines the fraction of these pulses exceeding various heights h .

Input Data Cards

The Monte Carlo program required three data cards which contain:

1st card A,B according to FORMAT (2E10.4);

2nd card RN, JIF, J2F, MF, K, UMH, UMT according to FORMAT (E10.4,
415, 2E10.4);

3rd card HFIN, TFIN(2), TFIN(3), TFIN(4), TFIN(5) where

A is the reciprocal time constant for the decay of the exponential pulses (page 53),

B is the constant in the height distribution of single pulses (page 18),

RN is the rate of single pulses,

JIF is the number of elements allocated to the array of heights,

J2F is the number of elements allocated to the array of times,

MF is the number of composite pulses to be generated,

K is the largest number the random number sub-program is allowed to generate,

UMH is the number of heights provided to occupy the height array,

UMT is the number of times provided to occupy the time array,

HFIN is the largest height to appear in the height array,

TFIN() is the largest time to appear in the appropriate time array.

These data cards are listed with the relevant arrays on page

Instructions for operating the Monte Carlo program on the I.B.M.1620 computer.

1. Clear storage.

2. Set the Switches:

Overflow to PROGRAM

I/O to STOP

PARITY to STOP

SENSE SWITCH	ON	OFF
1	Continue generating time arrays until 4 are generated.	Stop generating time arrays after the current one is completed.
2	Print output	Punch output
3	Return to the beginning	Continue
4	Print out the arrays as they are generated.	Don't.

3. Place the Monte Carlo program followed by the random number subprogram, followed by the Fortran II subroutines in the read hopper.
4. Press LOAD and whenever the computer enters manual mode press START.
5. After the data cards have loaded, the typewriter will 'click' and any number must be typed in according to the FORMAT (E10.4). This number is the starting number for the random number generator and is preferably a different number each time the program is run.
6. Press RELEASE, SIE, SIE, START.
7. If switch 4 is ON, the arrays will be typed as they are generated but in both settings of switch 4 the serial number of the array will be typed when it is completed.

8. When sufficient arrays have been generated, switch 4 can be turned OFF and the program will punch (switch 2 OFF) or print (switch 2 ON) the heights of MF multiple pulses which are input to the Stochastic Integral Program.

Operating Instructions for the Stochastic Integral Program

9. Clear Storage
10. Set the Switches

Overflow to PROGRAM

I/O to STOP

PARITY to STOP

<u>Sense Switch</u>	<u>ON</u>	<u>OFF</u>
1	Punch output	Print output
2	Go to beginning	Continue

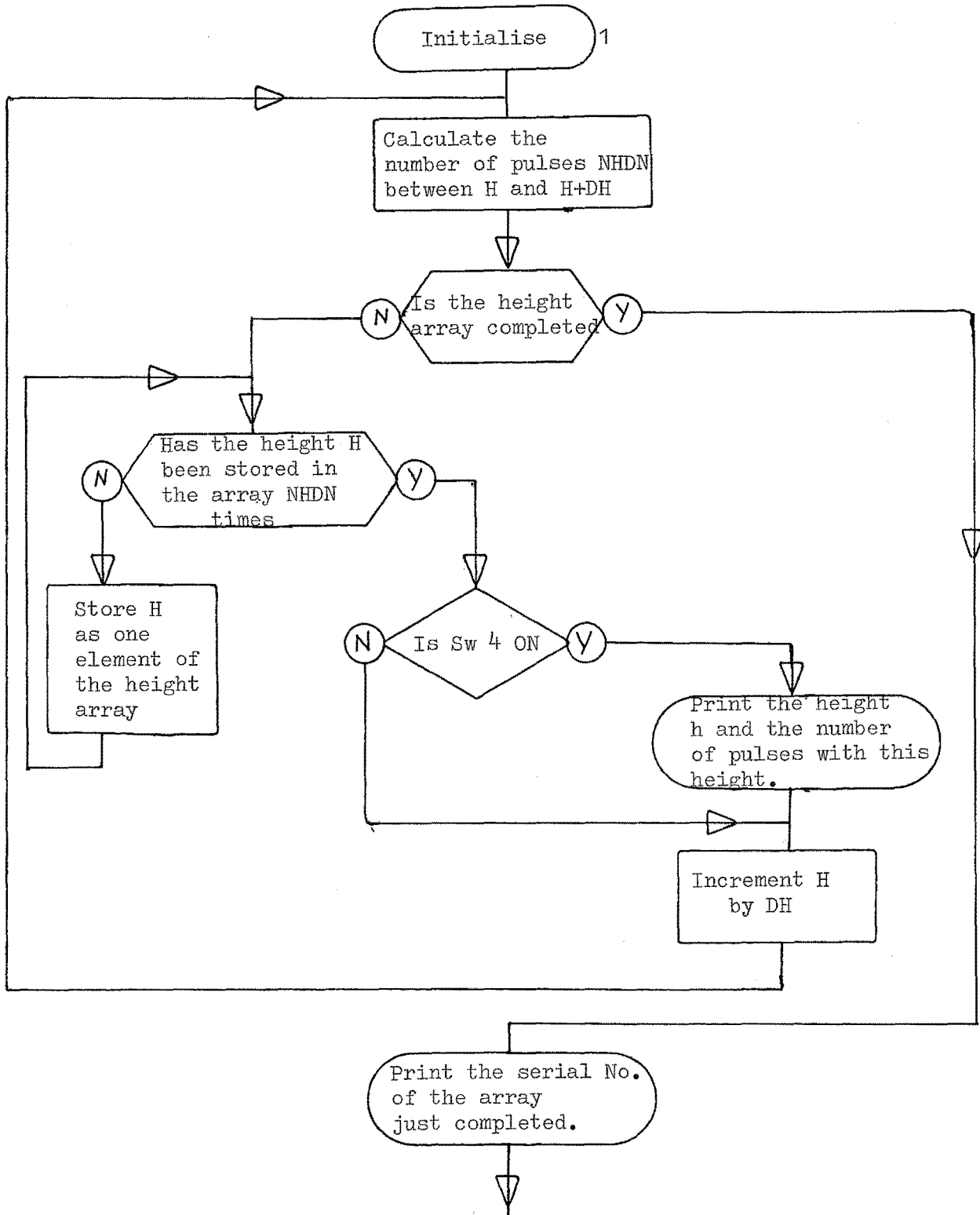
Switches 3 and 4 are not used.

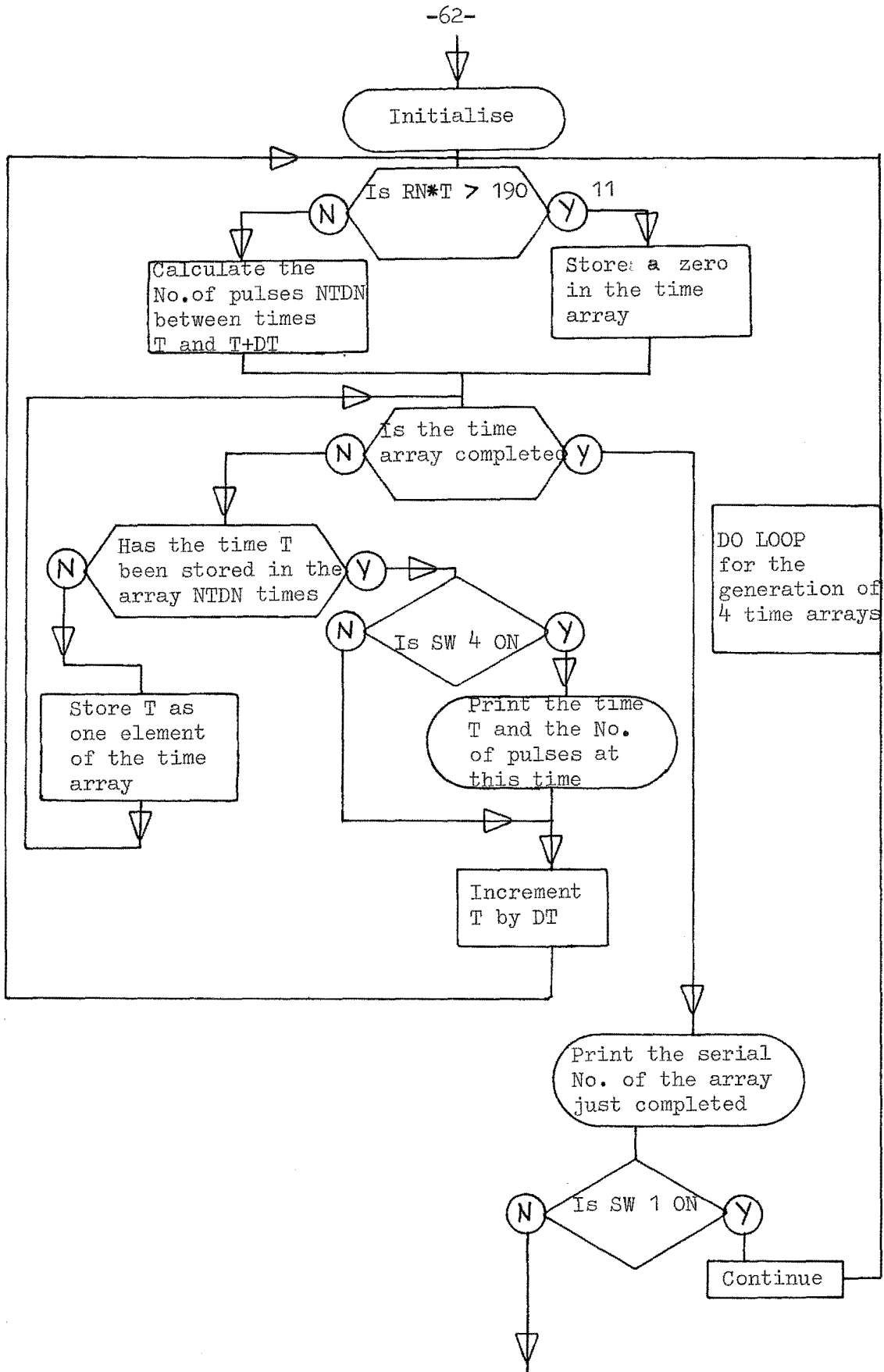
11. Place the Stochastic Integral Program in the read hopper, followed by the Fortran II subroutines followed by the card output from step 8.
12. Press LOAD and whenever the machine enters manual mode press START.
13. After the last cards have loaded, the tyewriter will 'click' and the number of cards NF and the height increment DH (0.0025 mV is satisfactory) must be typed in according to FORMAT (2E10.4).
14. Press RELEASE, SIE, SIE, START.
15. Put blank cards in the punch hopper and press PUNCH START.
16. The pulse height H and the number of multiple pulses exceeding this height are punched on each card, H increasing serially by DH from card to card.

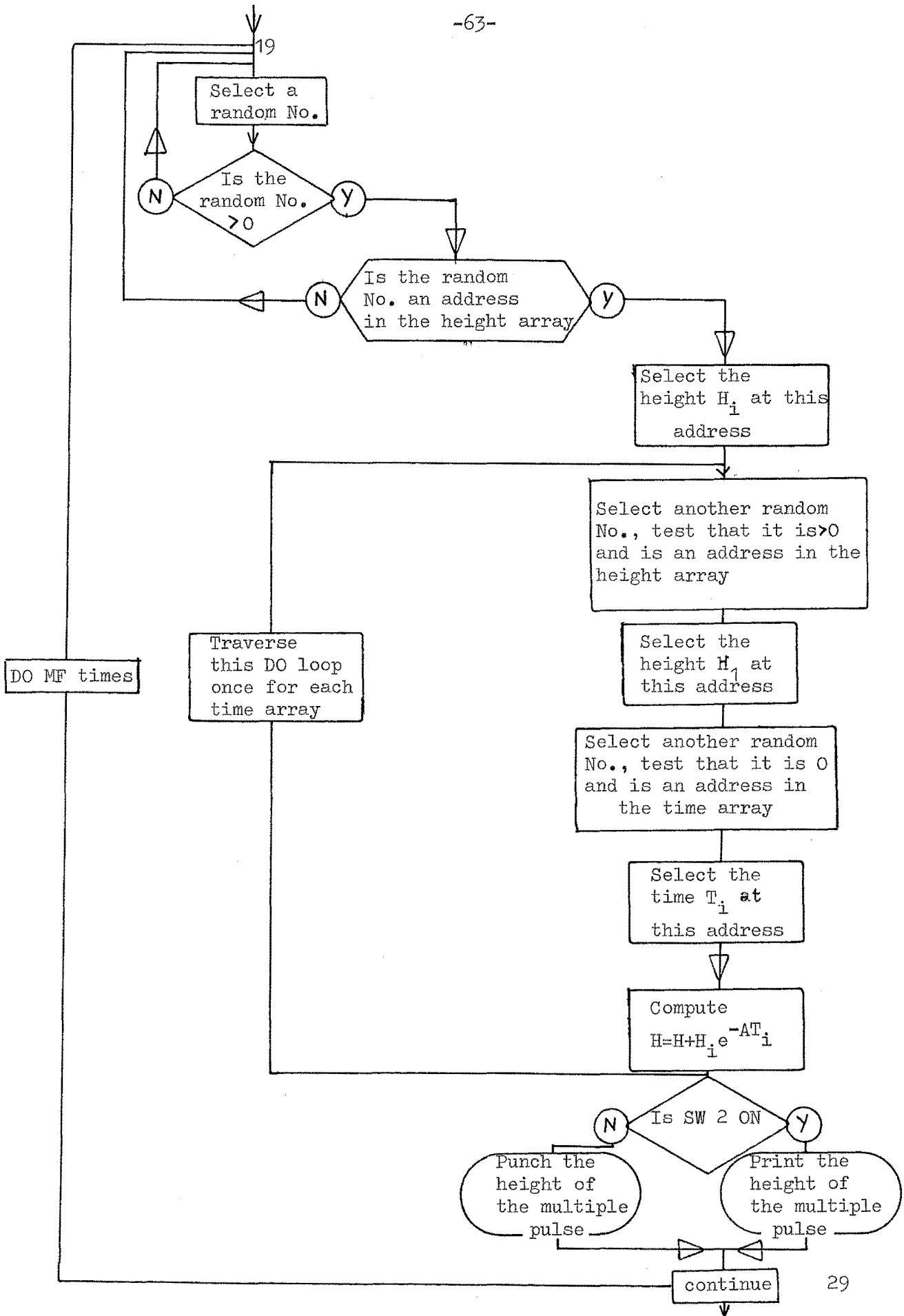
17. When the number of pulses reaches zero, the program can be stopped by turning switch 3 ON and the output cards can be used as input cards to the General Curve Fitting and Graph Plotting program in Appendix 1. The graphs produced are shown on figure 13 page 21.
-

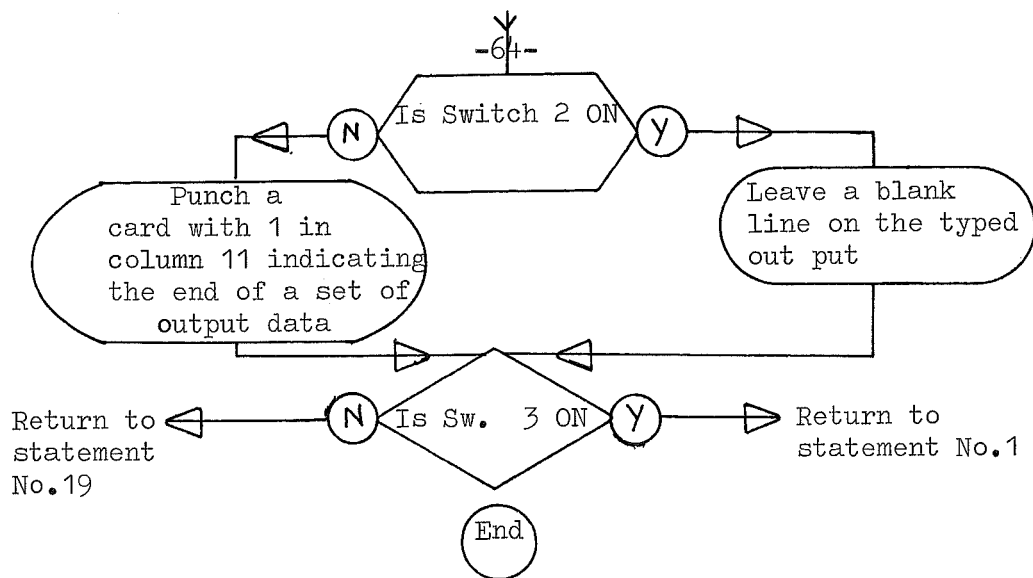
Flow charts for the Monte Carlo program and the Stochastic Integral program are contained on the next four pages and are followed by listings of the programs and the arrays generated by the Monte Carlo program.

FLOW CHART FOR THE MONTE CARLO PROGRAM

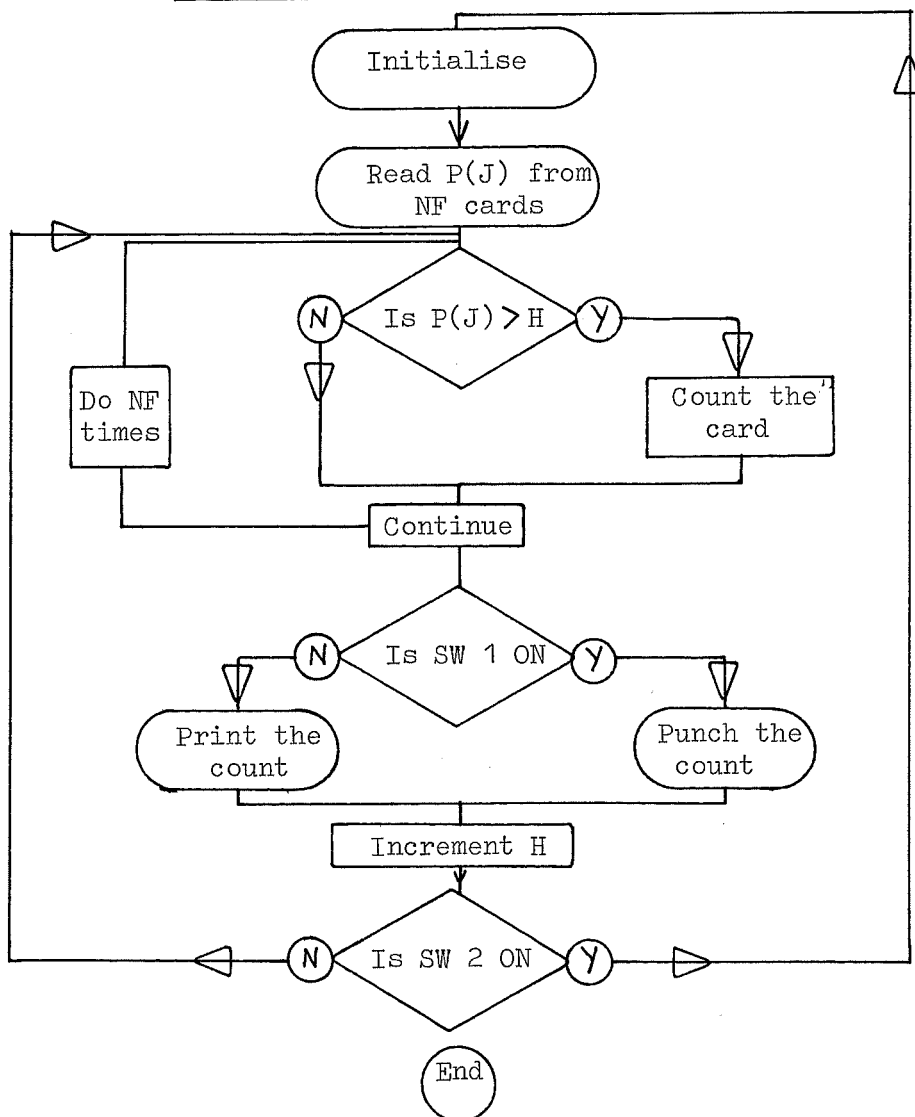








FLOW CHART FOR THE STOCHASTIC INTEGRAL PROGRAM



```
**1111 13.08 MONTE CARLO
C MONTE CARLO PROGRAM TO CALCULATE THE PROBABLE HEIGHTS
C OF OVERLAPPING EXPONENTIAL PULSES.
C T. I. QUICKENDEN 1966
DIMENSION Q1(400), Q(4,400), SUM(5), TFIN(5)
1 READ50, A, B
2 READ51, RN, J1F, J2F, MF, K, UMH, UMT
READ57, HF IN, TFIN(2), TFIN(3), TFIN(4), TFIN(5)
ACCEPT52, K1
K1=NRAND(-K1)
F1J=J1F
F2J=J2F
DH=HF IN/F1J
H=DH
J1=1
J11=J1-1
3 HDN=B*EXPF(-B*H)*DH
NHDN=UMH*HDN
311 IF(J1-J1F)4,4,10
4 IF(J1-J11-NHDN)6,6,5
5 IF(SENSE SWITCH4)7,8
7 PRINT56, NHDN, H
8 H=H+DH
J11=J1
GO TO 3
6 Q1(J1)=H
J1=J1+1
GO TO 311
10 I=1
PRINT53, I
FACX=1.
DO17I=2,5
DT=TFIN(I)/F2J
T=DT
X=1
FACX=FACX*X
J2=1
J22=J2-1
11 IF(190.-RN*T)112,112,110
112 NTDN=0
GO TO 111
110 TDN=(RN*T)**(X-1.)*EXPF(-RN*T)*DT*X*(X-1.)/(T*FACX)
NTDN=UMT*TDN
111 IF(J2-J2F)12,12,18
12 IF(J2-J22-NTDN)14,14,13
13 IF(SENSE SWITCH4)15,16
15 PRINT56, NTDN, T
16 T=T+DT
J22=J2
GO TO 11
14 Q(I, J2)=T
J2=J2+1
```

```
GO TO 111
18 PRINT53,I
   IF IN=1
   IF(SENSE SWITCH 1)17,19
17 CONTINUE
19 DO 29M=1,MF
20 IH=NRAND(K)
   IF (IH) 20, 20, 21
21 IF (J1F-IH) 20, 22, 22
22 SUM(1)=Q1(IH)
   DO 28L=2, IF IN
24 IH=NRAND(K)
   IF (IH) 24, 24, 25
25 IF (J1F-IH) 24, 26, 26
26 IT=NRAND(K)
   IF (IT) 26, 26, 27
27 IF (J2F-IT) 26, 28, 28
28 SUM(L)=SUM(L-1)+Q1(IH)*EXPF(-A*Q(L, IT))
   IF(SENSE SWITCH 2)34,35
34 PRINT52,SUM(IF IN)
   GO TO 29
35 PUNCH52,SUM(IF IN)
29 CONTINUE
   IF(SENSE SWITCH 2)32,31
31 PUNCH54
   GO TO 33
32 PRINT55
33 IF(SENSE SWITCH 3)1,19
50 FORMAT(2E10.4)
51 FORMAT(E10.4,4I5,2E10.4)
52 FORMAT(E10.4)
53 FORMAT(9HARRAY NO.,I4)
54 FORMAT(11X,1H1)
55 FORMAT(/)
56 FORMAT(I4,E10.4)
57 FORMAT(5E10.4)
END
```

```
**1111 13.08 STOCHASTIC INTEGRAL
C      PROGRAM TO CALCULATE  $FX = \int P(N,H)DH$ 
C      BY A STOCHASTIC METHOD
C      T.I.QUICKENDEN 1966
      DIMENSION P(1000)
1 ACCEPT 23,NF,DH
      FN=NF
      H=0.
      READ 21,(P(J),J=1,NF)
2 I=0
      DO 4 J=1,NF
        IF(P(J)-H)4,3,3
3 I=I+1
4 CONTINUE
      UI=I
      XI=UI/FN
      IF(SENSE SWITCH 1)5,6
5 PUNCH 23,H,XI
      GO TO 7
6 PRINT 23,H,XI
7 H=H+DH
      IF(SENSE SWITCH 2)1,2
21 FORMAT(E 10.4)
22 FORMAT(E 10.4,16)
23 FORMAT(2E 10.4)
      END
```

RANDOM NUMBER GENERATOR

```

01010****
01020****      RANDOM NOS.
01030****
01040      DC  1,@
01050      DC  1,@
01060      DORG 11124
01070      BNF B,A,0111
01080      TF  S,A,0111
01090      CF  S,,0
01100      TFM C,S+1,017
01110      S   C,404,0
01120      TF  FAC,S,1
01130      CF  C,,06
01140      B   RETRN,,06
01150      DORG*-4
01160B     TFM S-10,,010
01170      A   X,X,01
01180E     C   X,P,01
01190      BNI D,1100,0
01200      S   X,P,01
01210RETRN DS ,11051
02010      B   E,,0
02020      DORG*-4
02030D     A   Y,Y,01
02040G     C   Y,Q,01
02050      BNI F,1100,0
02060      S   Y,Q,01
02070      B   G,,0
02180      DORG*-4
02190X     DC  12,123456789
02200P     DC  12,999999883
02210Y     DC  12,987654321
02220Q     DC  12,999999893
02230S     DC  12,0
02240A     DS  ,11045
02250FAC   DS  ,485
02090F     A   S,X,01
02100      A   S,Y,01
02110      SF
02120C     DS  ,*-5
02130      TF  FAC,S,1
02140      CF  C,,06
02150      C   FAC,A,111
02160      BNL B,,0
02170      B   RETRN,,06
02260      DEND

```

The adjacent S.P.S. program is processed with the S.P.S. processor deck to give object deck No. 1. The Fortran 11 subprogram

```

FUNCTION NRAND(K)
RETURN
END

```

is then processed with the Fortran 11 processor to give object deck No.2.

Punch the number 13 in columns 79 and 80 of the last card of object deck No.2 and then place object deck No.1 between the third and fourth cards of object deck No.2.

The resulting deck is a complete random number subprogram which can be used by any Fortran 11 mainline program. Whenever a variable (e.g. X) in the mainline program is to be a random number, simply write the statement `X=NRAND(K)`

The maximum size of the random number must have been determined by

a previous statement `K1=NRAND(-K1)` where K1, which is the largest random number required must be defined in the mainline program as must K, any initial random No. which should be altered each time the program is used.

Arrays of Times and Heights

(Generated by the Monte Carlo Program)

The distribution of pulse heights $dn_h = n_h P(1,h)dh$ was digitised according to $\Delta n_h = n_h P(1,h) \cdot \Delta h$. The following table gives the number of pulses Δn_h lying between heights h and $h + \Delta h$ for $n_h = 400$ and $h = 0.0025$ m.v. This identical array was generated every time the Monte Carlo program was executed. The time arrays depend on the pulse rate and are listed overleaf and on subsequent pages.

No. of Pulse
pulses height
 Δn_h h

21	.7500E-01	7	.1500E+01	2	.2850E+01
20	.1500E-00	6	.1575E+01	2	.2925E+01
19	.2250E-00	6	.1650E+01	2	.3000E+01
17	.3000E-00	6	.1725E+01	2	.3075E+01
16	.3750E-00	5	.1800E+01	2	.3150E+01
16	.4500E-00	5	.1875E+01	1	.3225E+01
15	.5250E-00	5	.1950E+01	1	.3300E+01
14	.6000E-00	4	.2025E+01	1	.3375E+01
13	.6750E-00	4	.2100E+01	1	.3450E+01
12	.7500E-00	4	.2175E+01	1	.3525E+01
12	.8250E-00	4	.2250E+01	1	.3600E+01
11	.9000E-00	3	.2325E+01	1	.3675E+01
10	.9750E-00	3	.2400E+01	1	.3750E+01
10	.1050E+01	3	.2475E+01	1	.3825E+01
9	.1125E+01	3	.2550E+01	1	.3900E+01
9	.1200E+01	3	.2625E+01	1	.3975E+01
8	.1275E+01	2	.2700E+01	1	.4050E+01
8	.1350E+01	2	.2775E+01	1	.4125E+01
7	.1425E+01				

6 .3120E-03
6 .3240E-03
5 .3360E-03
5 .3480E-03
4 .3600E-03
4 .3720E-03
3 .3840E-03
3 .3960E-03
3 .4080E-03
3 .4200E-03
2 .4320E-03
2 .4440E-03
2 .4560E-03

4th pulses

0 .1600E-04
2 .3200E-04
4 .4800E-04
6 .6400E-04
9 .8000E-04
11 .9600E-04
13 .1120E-03
14 .1280E-03
15 .1440E-03
16 .1600E-03
17 .1760E-03
17 .1920E-03
17 .2080E-03
17 .2240E-03
16 .2400E-03
16 .2560E-03
15 .2720E-03
14 .2880E-03
14 .3040E-03
13 .3200E-03
12 .3360E-03
11 .3520E-03
10 .3680E-03
10 .3840E-03
9 .4000E-03
8 .4160E-03
7 .4320E-03
7 .4480E-03
6 .4640E-03
6 .4800E-03
5 .4960E-03
5 .5120E-03

4 .5280E-03
4 .5440E-03
3 .5600E-03
3 .5760E-03
3 .5920E-03
2 .6080E-03

5th pulses

0 .2000E-04
0 .4000E-04
1 .6000E-04
3 .8000E-04
4 .1000E-03
6 .1200E-03
9 .1400E-03
11 .1600E-03
12 .1800E-03
14 .2000E-03
15 .2200E-03
16 .2400E-03
17 .2600E-03
17 .2800E-03
17 .3000E-03
17 .3200E-03
17 .3400E-03
16 .3600E-03
16 .3800E-03
15 .4000E-03
14 .4200E-03
13 .4400E-03
13 .4600E-03
12 .4800E-03
11 .5000E-03
10 .5200E-03
9 .5400E-03
8 .5600E-03
7 .5800E-03
7 .6000E-03
6 .6200E-03
5 .6400E-03
5 .6600E-03
4 .6800E-03
4 .7000E-03
3 .7200E-03
3 .7400E-03
2 .7600E-03

Time Arrays at 2×10^4 p.p.s. (see page 70)

Input Data Cards

6.2E4 0.7509E0

2.E4 400 400 400 400 4.E2 4.E2

3.E1 9.E-4 24.E-4 32.E-4 40.E-4

No.of Time pulses (sec.)

2nd pulses

17 .2250E-05	3 .8550E-04	17 .6000E-04
16 .4500E-05	3 .8775E-04	16 .6600E-04
15 .6750E-05	2 .9000E-04	16 .7200E-04
15 .9000E-05	2 .9225E-04	15 .7800E-04
14 .1125E-04	2 .9450E-04	15 .8400E-04
13 .1350E-04	2 .9675E-04	14 .9000E-04
13 .1575E-04	2 .9900E-04	13 .9600E-04
12 .1800E-04	2 .1012E-03	12 .1020E-03
12 .2025E-04	2 .1035E-03	11 .1080E-03
11 .2250E-04	2 .1057E-03	11 .1140E-03
10 .2475E-04	2 .1080E-03	10 .1200E-03
10 .2700E-04	1 .1102E-03	9 .1260E-03
10 .2925E-04	1 .1125E-03	9 .1320E-03
9 .3150E-04	1 .1147E-03	8 .1380E-03
9 .3375E-04	1 .1170E-03	7 .1440E-03
8 .3600E-04	1 .1192E-03	7 .1500E-03
8 .3825E-04	1 .1215E-03	6 .1560E-03
8 .4050E-04	1 .1237E-03	6 .1620E-03
7 .4275E-04	1 .1260E-03	5 .1680E-03
7 .4500E-04	1 .1282E-03	5 .1740E-03
6 .4725E-04	1 .1305E-03	4 .1800E-03
6 .4950E-04	1 .1327E-03	4 .1860E-03
6 .5175E-04	1 .1350E-03	3 .1920E-03
6 .5400E-04	1 .1372E-03	3 .1980E-03
5 .5625E-04	1 .1395E-03	3 .2040E-03
5 .5850E-04		3 .2100E-03
5 .6075E-04	3rd pulses	2 .2160E-03
5 .6300E-04		2 .2220E-03
4 .6525E-04	5 .6000E-05	2 .2280E-03
4 .6750E-04	9 .1200E-04	
4 .6975E-04	12 .1800E-04	4th pulses
4 .7200E-04	14 .2400E-04	0 .8000E-05
4 .7425E-04	15 .3000E-04	2 .1600E-04
3 .7650E-04	16 .3600E-04	4 .2400E-04
3 .7875E-04	17 .4200E-04	6 .3200E-04
3 .8100E-04	17 .4800E-04	9 .4000E-04
3 .8325E-04	17 .5400E-04	

11	.4800E-04	17	.1500E-03
13	.5600E-04	17	.1600E-03
14	.6400E-04	17	.1700E-03
15	.7200E-04	16	.1800E-03
16	.8000E-04	16	.1900E-03
17	.8800E-04	15	.2000E-03
17	.9600E-04	14	.2100E-03
17	.1040E-03	13	.2200E-03
17	.1120E-03	13	.2300E-03
16	.1200E-03	12	.2400E-03
16	.1280E-03	11	.2500E-03
15	.1360E-03	10	.2600E-03
14	.1440E-03	9	.2700E-03
14	.1520E-03	8	.2800E-03
13	.1600E-03	7	.2900E-03
12	.1680E-03	7	.3000E-03
11	.1760E-03	6	.3100E-03
10	.1840E-03	5	.3200E-03
10	.1920E-03	5	.3300E-03
9	.2000E-03	4	.3400E-03
8	.2080E-03	4	.3500E-03
7	.2160E-03	3	.3600E-03
7	.2240E-03	3	.3700E-03
6	.2320E-03	2	.3800E-03
6	.3400E-03	2	.3900E-03
5	.2480E-03		
5	.2560E-03		
4	.2640E-03		
4	.2720E-03		
3	.2800E-03		
3	.2880E-03		
3	.2960E-03		
2	.3040E-03		

5th pulses

0	.1000E-04
0	.2000E-04
1	.3000E-04
3	.4000E-04
4	.5000E-04
6	.6000E-04
9	.7000E-04
11	.8000E-04
12	.9000E-04
14	.1000E-03
15	.1100E-03
16	.1200E-03
17	.1300E-03
17	.1400E-03

Time Arrays at 5×10^4 p.p.s. (see page 70)

Input Data Cards

6. 2E4 0.7509E0
5. E4 400 400 400 400 4.E2 4.E2
3. E1 14.E-4 12.E-4 16.E-4 20.E-4

No.of Time
pulses (sec.)

2nd pulses

19 .1000E-05	3 .3300E-04	19 .1200E-04
18 .2000E-05	3 .3400E-04	21 .1500E-04
17 .3000E-05	3 .3500E-04	21 .1800E-04
16 .4000E-05	3 .3600E-04	22 .2100E-04
15 .5000E-05	3 .3700E-04	21 .2400E-04
14 .6000E-05	2 .3800E-04	20 .2700E-04
14 .7000E-05	2 .3900E-04	20 .3000E-04
13 .8000E-05	2 .4000E-04	19 .3300E-04
12 .9000E-05	2 .4100E-04	17 .3600E-04
12 .1000E-04	2 .4200E-04	16 .3900E-04
11 .1100E-04	2 .4300E-04	15 .4200E-04
10 .1200E-04	2 .4400E-04	14 .4500E-04
10 .1300E-04	2 .4500E-04	13 .4800E-04
9 .1400E-04	2 .4600E-04	11 .5100E-04
9 .1500E-04	1 .4700E-04	10 .5400E-04
8 .1600E-04	1 .4800E-04	9 .5700E-04
8 .1700E-04	1 .4900E-04	8 .6000E-04
8 .1800E-04	1 .5000E-04	8 .6300E-04
7 .1900E-04	1 .5100E-04	7 .6600E-04
7 .2000E-04	1 .5200E-04	6 .6900E-04
6 .2100E-04	1 .5300E-04	5 .7200E-04
6 .2200E-04	1 .5400E-04	5 .7500E-04
6 .2300E-04	1 .5500E-04	4 .7800E-04
6 .2400E-04	1 .5600E-04	4 .8100E-04
5 .2500E-04	1 .5700E-04	3 .8400E-04
5 .2600E-04	1 .5800E-04	3 .8700E-04
5 .2700E-04		2 .9000E-04
4 .2800E-04	3rd pulses	2 .9300E-04
4 .2900E-04		2 .9600E-04
4 .3000E-04	7 .3000E-05	2 .9900E-04
4 .3100E-04	13 .6000E-05	
4 .3200E-04	17 .9000E-05	

4th pulses

1 .4000E-05
4 .8000E-05
7 .1200E-04
11 .1600E-04
14 .2000E-04
17 .2400E-04
19 .2800E-04
20 .3200E-04
21 .3600E-04
21 .4000E-04
21 .4400E-04
20 .4800E-04
20 .5200E-04
19 .5600E-04
17 .6000E-04
16 .6400E-04
15 .6800E-04
14 .7200E-04
12 .7600E-04
11 .8000E-04
10 .8400E-04
9 .8800E-04
8 .9200E-04
7 .9600E-04
6 .1000E-03
5 .1040E-03
5 .1080E-03
4 .1120E-03
4 .1160E-03
3 .1200E-03
3 .1240E-03
2 .1280E-03

5th pulses

0 .5000E-05
1 .1000E-04
3 .1500E-04
6 .2000E-04
9 .2500E-04
12 .3000E-04
15 .3500E-04
18 .4000E-04
20 .4500E-04
21 .5000E-04

22 .5500E-04
22 .6000E-04
22 .6500E-04
21 .7000E-04
20 .7500E-04
19 .8000E-04
18 .8500E-04
16 .9000E-04
15 .9500E-04
14 .1000E-03
12 .1050E-03
11 .1100E-03
10 .1150E-03
8 .1200E-03
7 .1250E-03
6 .1300E-03
6 .1350E-03
5 .1400E-03
4 .1450E-03
3 .1500E-03
3 .1550E-03

Time Arrays at 1×10^5 p.p.s. (see page 70)

Input Data Cards

6.2E4 0.7509E0
1.E5 400 400 400 400 4.E2 4.E2
3.E1 2.E-4 6.E-4 8.E-4 10.E-4

No. of Time
pulses (sec.)

2nd pulses

19 .5000E-06	3 .1750E-04	21 .1200E-04
18 .1000E-05	3 .1800E-04	20 .1350E-04
17 .1500E-05	3 .1850E-04	20 .1500E-04
16 .2000E-05	2 .1900E-04	19 .1650E-04
15 .2500E-05	2 .1950E-04	17 .1800E-04
14 .3000E-05	2 .2000E-04	16 .1950E-04
14 .3500E-05	2 .2050E-04	15 .2100E-04
13 .4000E-05	2 .2100E-04	14 .2250E-04
12 .4500E-05	2 .2150E-04	13 .2400E-04
12 .5000E-05	2 .2200E-04	11 .2550E-04
11 .5500E-05	2 .2250E-04	10 .2700E-04
10 .6000E-05	2 .2300E-04	9 .2850E-04
10 .6500E-05	1 .2350E-04	8 .3000E-04
9 .7000E-05	1 .2400E-04	8 .3150E-04
9 .7500E-05	1 .2450E-04	7 .3300E-04
8 .8000E-05	1 .2500E-04	6 .3450E-04
8 .8500E-05	1 .2550E-04	5 .3600E-04
8 .9000E-05	1 .2600E-04	5 .3750E-04
7 .9500E-05	1 .2650E-04	4 .3900E-04
7 .1000E-04	1 .2700E-04	4 .4050E-04
6 .1050E-04	1 .2750E-04	3 .4200E-04
6 .1100E-04	1 .2800E-04	3 .4350E-04
6 .1150E-04	1 .2850E-04	2 .4500E-04
6 .1200E-04	1 .2900E-04	2 .4650E-04
5 .1250E-04		2 .4800E-04
5 .1300E-04		2 .4950E-04
5 .1350E-04		
4 .1400E-04		
4 .1450E-04		
4 .1500E-04		
4 .1550E-04		
4 .1600E-04		
3 .1650E-04		
3 .1700E-04		
	3rd pulses	
	7 .1500E-05	4th pulses
	13 .3000E-05	1 .2000E-05
	17 .4500E-05	4 .4000E-05
	19 .6000E-05	7 .6000E-05
	21 .7500E-05	11 .8000E-05
	21 .9000E-05	14 .1000E-04
	22 .1050E-04	

17	.1200E-04	20	.3750E-04
19	.1400E-04	19	.4000E-04
20	.1600E-04	18	.4250E-04
21	.1800E-04	16	.4500E-04
21	.2000E-04	15	.4750E-04
21	.2200E-04	14	.5000E-04
20	.2400E-04	12	.5250E-04
20	.2600E-04	11	.5500E-04
19	.2800E-04	10	.5750E-04
17	.3000E-04	8	.6000E-04
16	.3200E-04	7	.6250E-04
15	.3400E-04	6	.6500E-04
14	.3600E-04	6	.6750E-04
12	.3800E-04	5	.7000E-04
11	.4000E-04	4	.7250E-04
10	.4200E-04	3	.7500E-04
9	.4400E-04	3	.7750E-04
8	.4600E-04		
7	.4800E-04		
6	.5000E-04		
5	.5200E-04		
5	.5400E-04		
4	.5600E-04		
4	.5800E-04		
3	.6000E-04		
3	.6200E-04		
2	.6400E-04		

5th pulses

0	.2500E-05
1	.5000E-05
3	.7500E-05
6	.1000E-04
9	.1250E-04
12	.1500E-04
15	.1750E-04
18	.2000E-04
20	.2250E-04
21	.2500E-04
22	.2750E-04
22	.3000E-04
22	.3250E-04
21	.3500E-04

Time Arrays at 5×10^5 p.p.s. (see page 70)

Input Data Cards

6.2E4 0.7509E0

5.E5 400 400 400 400 4.E2 4.E2

3.E1 0.4E-4 1.3E-4 1.7E-4 2.1E-4

No. of pulses (sec.)

2nd pulses

19.	.1000E-06	3	.3700E-05	18	.3900E-05
18	.2000E-06	2	.3800E-05	16	.4225E-05
17	.3000E-06	2	.3900E-05	15	.4550E-05
16	.4000E-06	2	.4000E-05	13	.4875E-05
15	.5000E-06	2	.4100E-05	12	.5200E-05
14	.6000E-06	2	.4200E-05	11	.5525E-05
14	.7000E-06	2	.4300E-05	10	.5850E-05
13	.8000E-06	2	.4400E-05	9	.6175E-05
12	.9000E-06	2	.4500E-05	8	.6500E-05
12	.1000E-05	2	.4600E-05	6	.7150E-05
11	.1100E-05	1	.4700E-05	5.	.7575E-05
10	.1200E-05	1	.4800E-05	5	.7800E-05
10	.1300E-05	1	.4900E-05	4	.8125E-05
9	.1400E-05	1	.5000E-05	4	.8450E-05
9	.1500E-05	1	.5100E-05	3	.8775E-05
8	.1600E-05	1	.5200E-05	3	.9100E-05
8	.1700E-05	1	.5300E-05	2	.9425E-05
8	.1800E-05	1	.5400E-05	2	.9750E-05
7	.1900E-05	1	.5500E-05		
7.	.2000E-05	1	.5600E-05		
6	.2100E-05	1	.5700E-05		
6	.2200E-05	1	.5800E-05		
6	.2300E-05				
6	.2400E-05				
5	.2500E-05				
5	.2600E-05				
5	.2700E-05				
4	.2800E-05				
4	.2900E-05				
4	.3000E-05				
4	.3100E-05				
4	.3200E-05				
3	.3300E-05				
3	.3400E-05				
3	.3500E-05				
3	.3600E-05				

3rd pulses

4th pulses

1	.4250E-06
5	.8500E-06
9	.1275E-05
13	.1700E-05
16	.2125E-05
19	.2550E-05
21	.2975E-05
22	.3400E-05
22	.3825E-05
22	.4250E-05
22	.4675E-05
21	.5100E-05
20	.5525E-05
19	.5950E-05
17	.6375E-05

16 .6800E-05
14 .7225E-05
13 .7650E-05
12 .8075E-05
10 .8500E-05
9 .8925E-05
8 .9350E-05
7 .9775E-05
6 .1020E-04
5 .1062E-04
5 .1105E-04
4 .1147E-04
3 .1190E-04
2 .1232E-04
2 .1275E-04
2 .1317E-04

5th pulses

0..5250E-06
1 .1050E-05
3 .1375E-05
7 .2100E-05
10 .2625E-05
14 .3150E-05
17 .3675E-05
19 .4200E-05
21 .4725E-05
22 .5250E-05
23 .5775E-05
23 .6300E-05
22 .6825E-05
22 .7350E-05
20 .7875E-05
19 .8400E-05
17 .8925E-05
16 .9450E-05
14 .9975E-05
13 .1050E-04
11 .1102E-04
10 .1155E-04
9 .1207E-04
8 .1260E-04
6 .1312E-04
6 .1365E-04
5 .1417E-04
4 .1470E-04
3 .1522E-04
3..1575E-04

Time arrays at 1×10^6 p.p.s. (see page 70)

Input Data Cards

6.2E4 0.7509E0

1.E6 400 400 400 400 4.E2 4.E2

3.E1 2.4E-5 5.2E-5 6.8E-5 8.4E-5

No.of Time
pulses (sec.)

2nd pulses

22 .6000E-07	2 .2280E-05	13 .2080E-05
21 .1200E-06	2 .2340E-05	12 .2210E-05
20 .1800E-06	2 .2400E-05	11 .2340E-05
18 .2400E-06	2 .2460E-05	10 .2470E-05
17 .3000E-06	1 .2520E-05	10 .2600E-05
16 .3600E-06	1 .2580E-05	9 .2730E-05
15 .4200E-06	1 .2640E-05	8 .2860E-05
14 .4800E-06	1 .2700E-05	7 .2990E-05
13 .5400E-06	1 .2760E-05	7 .3120E-05
13 .6000E-06	1 .2820E-05	6 .3250E-05
12 .6600E-06	1 .2880E-05	5 .3380E-05
11 .7200E-06	1 .2940E-05	5 .3520E-05
11 .7800E-06	1 .3000E-05	4 .3640E-05
10 .8400E-06	1 .3060E-05	4 .3770E-05
9 .9000E-06	1 .3120E-05	4 .3900E-05
9 .9600E-05	0 .3180E-05	3 .4030E-05
8 .1020E-05	0 .3240E-05	3 .4160E-05
8 .1080E-05		3 .4290E-05
7 .1140E-05	3rd pulses	2 .4420E-05
7 .1200E-05		2 .4500E-05
6 .1260E-05	5 .1300E-06	2 .4680E-05
6 .1320E-05	10 .2600E-06	
6 .1380E-05	13 .3900E-06	4th pulses
5 .1440E-05	16 .5200E-06	0 .1700E-06
5 .1560E-05	17 .6500E-06	2 .3400E-06
4 .1620E-05	18 .7800E-06	5 .5100E-06
4 .1680E-05	19 .9100E-06	7 .6800E-06
4 .1740E-05	19 .1040E-05	10 .8500E-06
3 .1800E-05	18 .1170E-05	12 .1020E-05
3 .1920E-05	18 .1300E-05	14 .1190E-05
3 .1980E-05	17 .1430E-05	16 .1360E-05
3 .2040E-05	17 .1560E-05	17 .1530E-05
2 .2100E-05	16 .1690E-05	17 .1700E-05
2 .2160E-05	15 .1820E-05	18 .1870E-05
2 .2220E-05	14 .1950E-05	

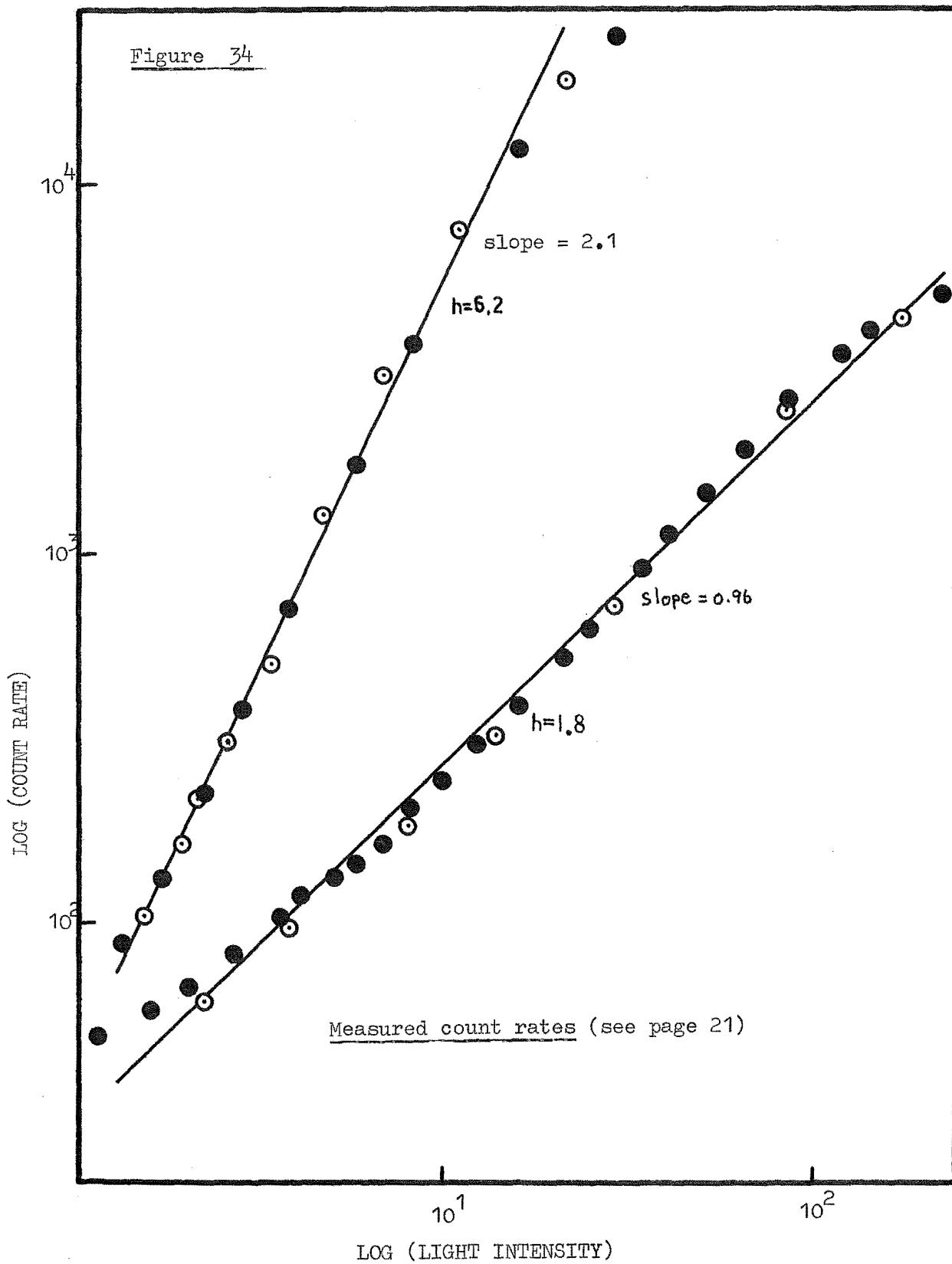
18	.2040E-05	13	.4620E-05
18	.2210E-05	12	.4830E-05
17	.2380E-05	11	.5040E-05
17	.2550E-05	10	.5250E-05
16	.2720E-05	9	.5460E-05
15	.2890E-05	8	.5670E-05
14	.3060E-05	7	.5880E-05
14	.3230E-05	7	.6090E-05
13	.3400E-05	6	.6300E-05
12	.3570E-05	5	.6510E-05
11	.3740E-05	5	.6720E-05
10	.3910E-05	4	.6930E-05
9	.4080E-05	4	.7140E-05
8	.4250E-05	3	.7350E-05
7	.4420E-05	3	.7560E-05
7	.4590E-05	2	.7770E-05
6	.4760E-05		
5	.4930E-05		
5	.5100E-05		
4	.5270E-05		
4	.5440E-05		
3	.5610E-05		
3	.5780E-05		
3	.5950E-05		
2	.6120E-05		

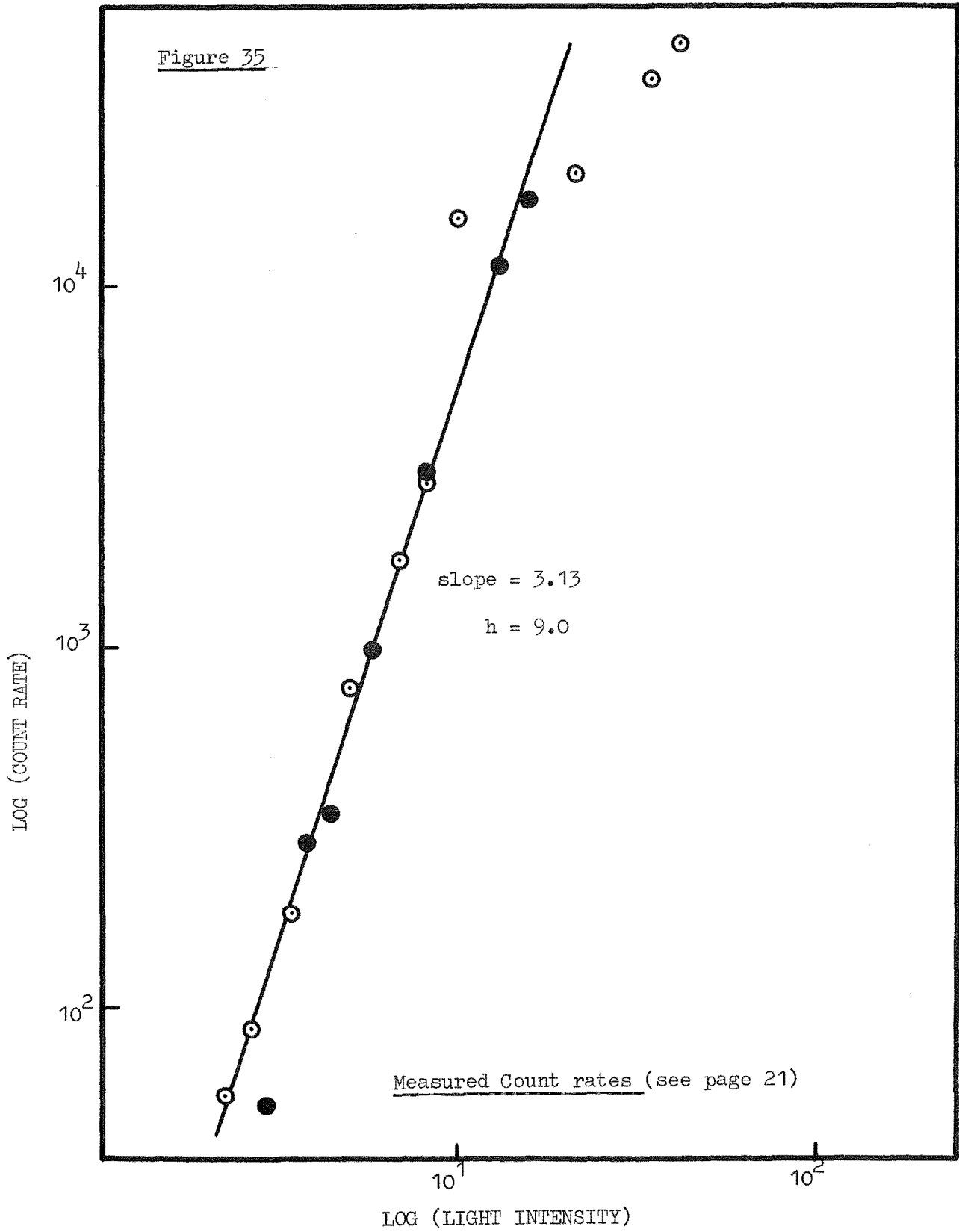
5th pulses

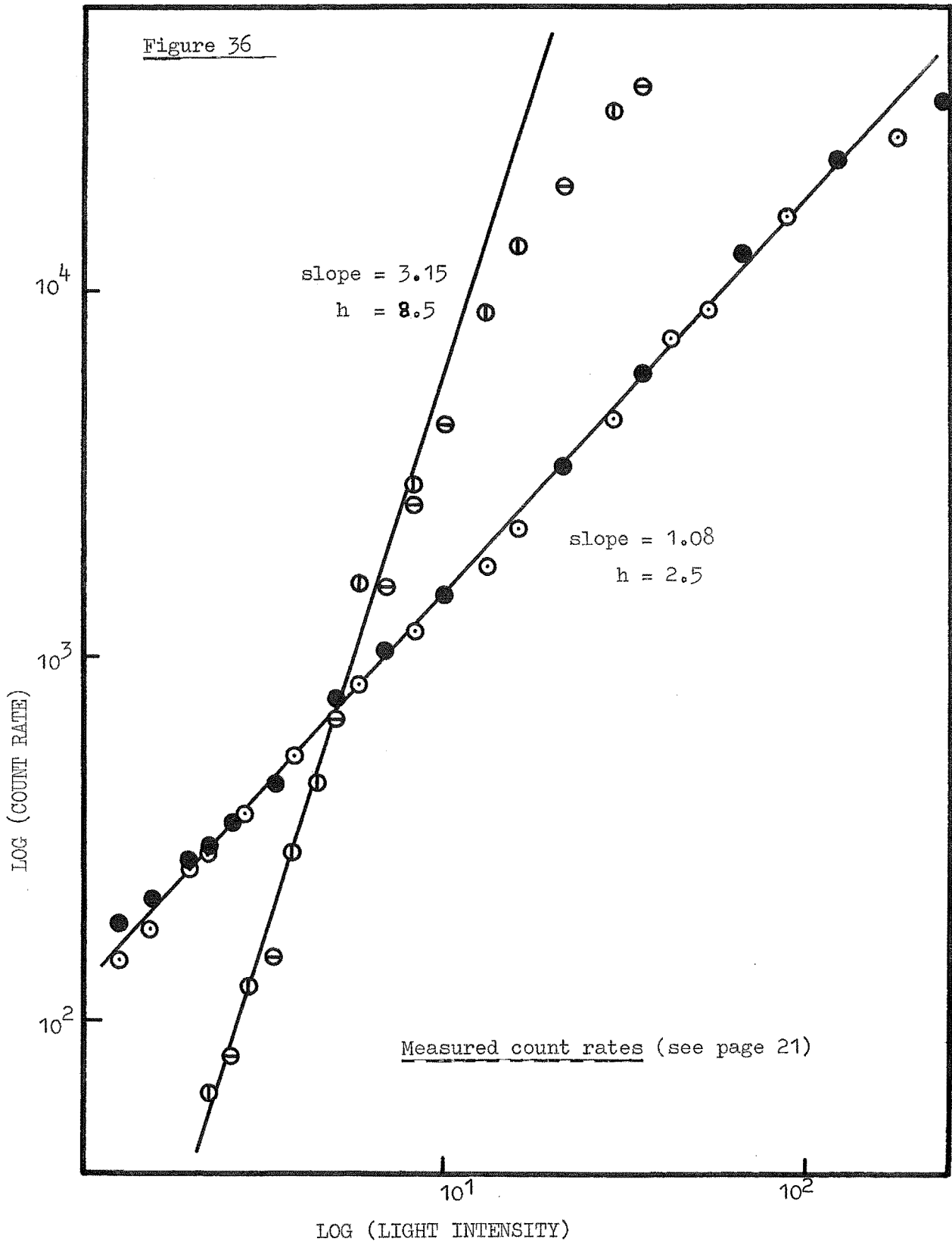
0	.2100E-06
0	.4200E-06
1	.6300E-06
3	.8400E-06
5	.1050E-05
7	.1260E-05
10	.1470E-05
12	.1680E-05
14	.1890E-05
15	.2100E-05
17	.2310E-05
18	.2520E-05
18	.2730E-05
18	.2940E-05
18	.3150E-05
18	.3360E-05
17	.3570E-05
17	.3780E-05
16	.3990E-05
15	.4200E-05
14	.4410E-05

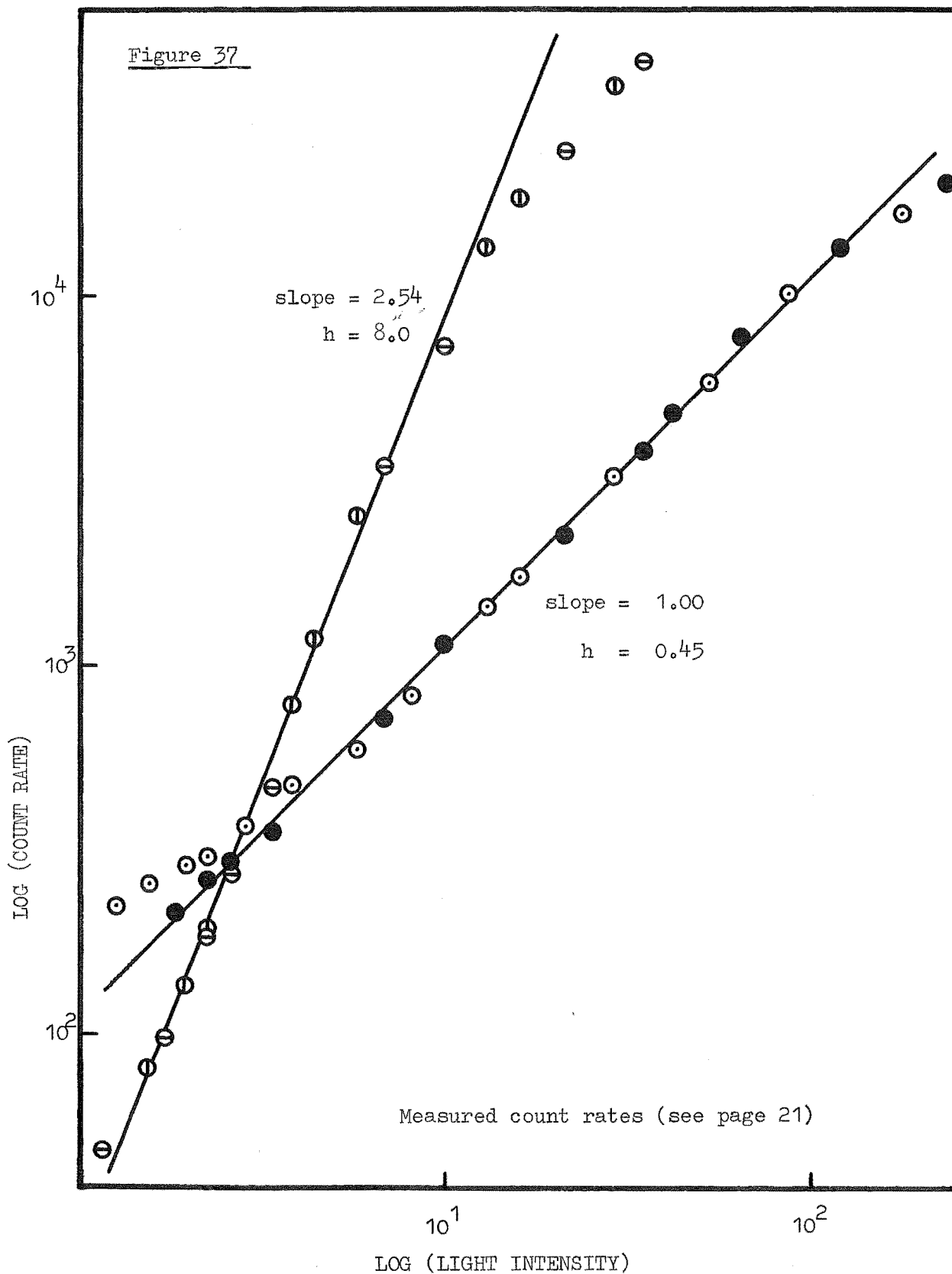
APPENDIX 5

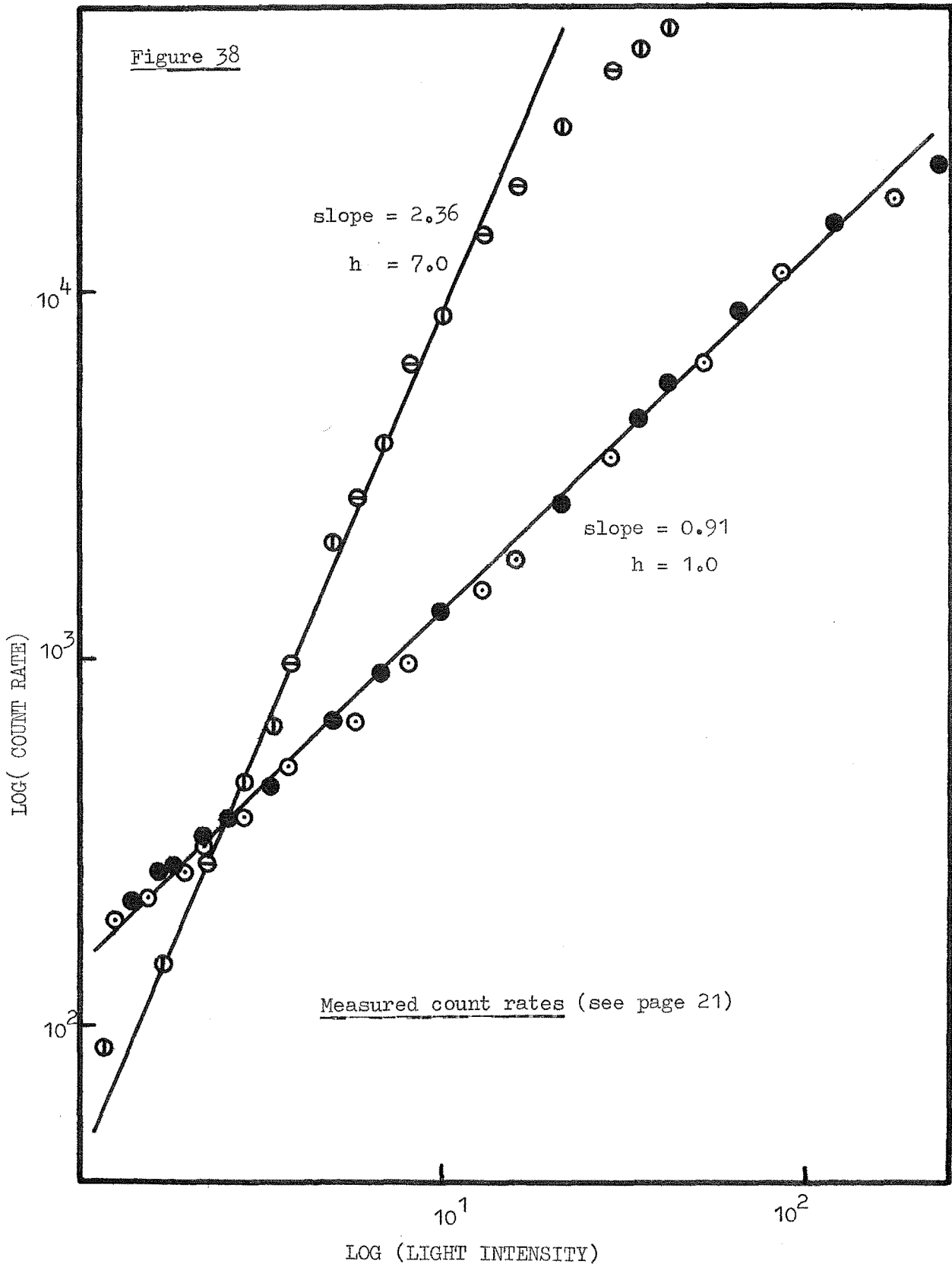
Pages 83 to 90 contain graphs of the measured pulse rate versus light intensity at various discriminator heights of the photon counter. The slopes of these graphs are compared with calculated slopes in figure 15 page 21.

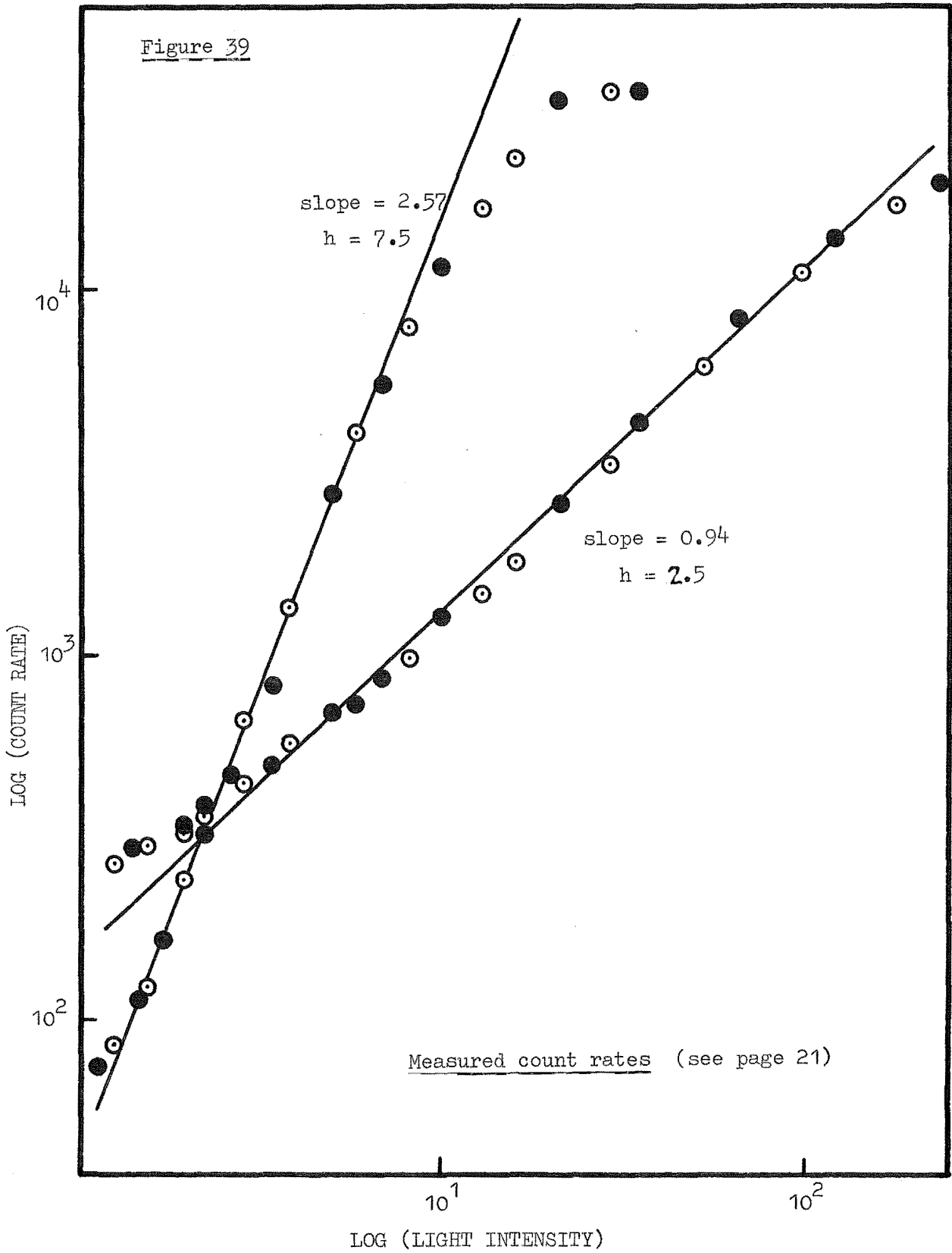












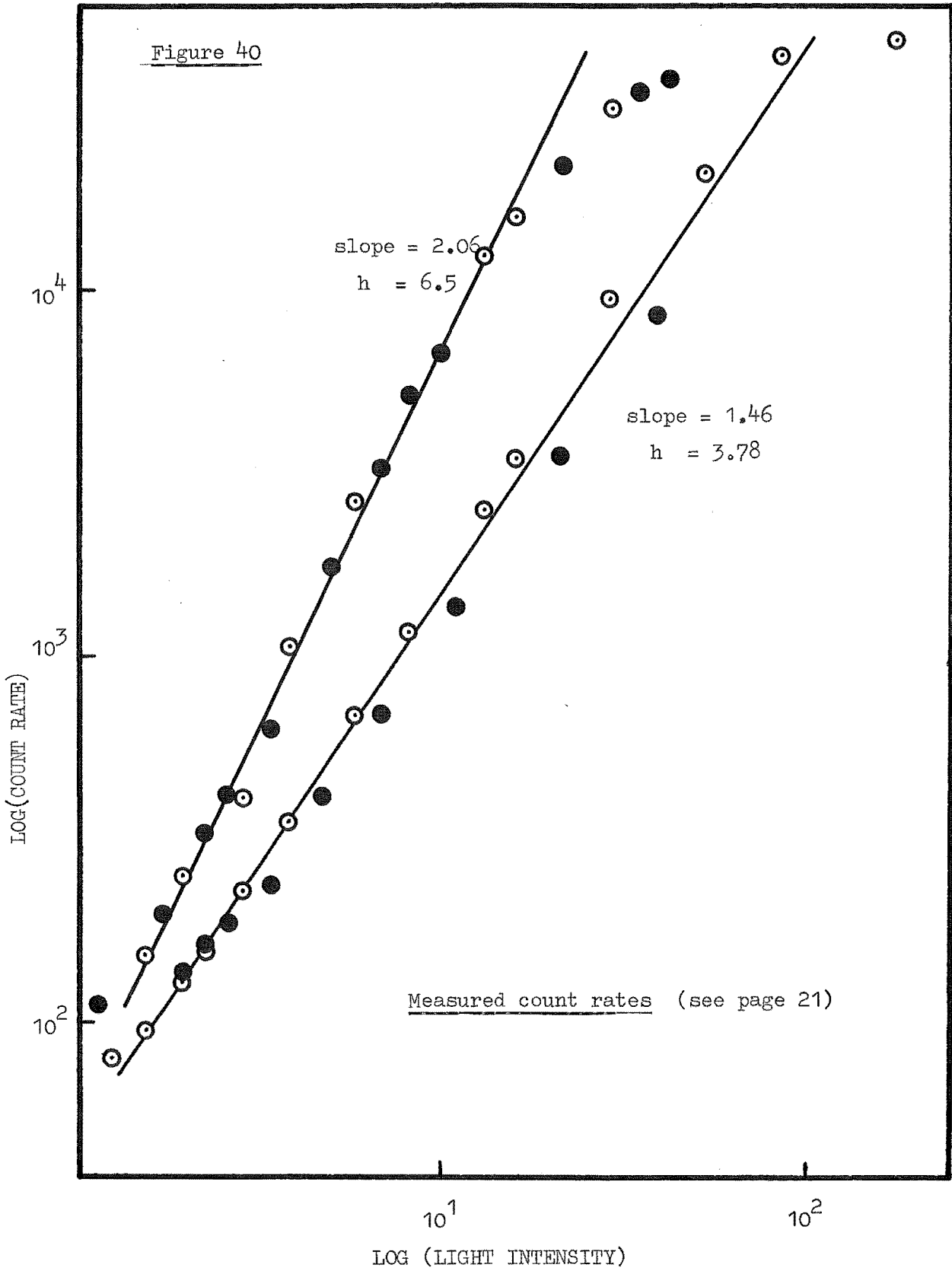
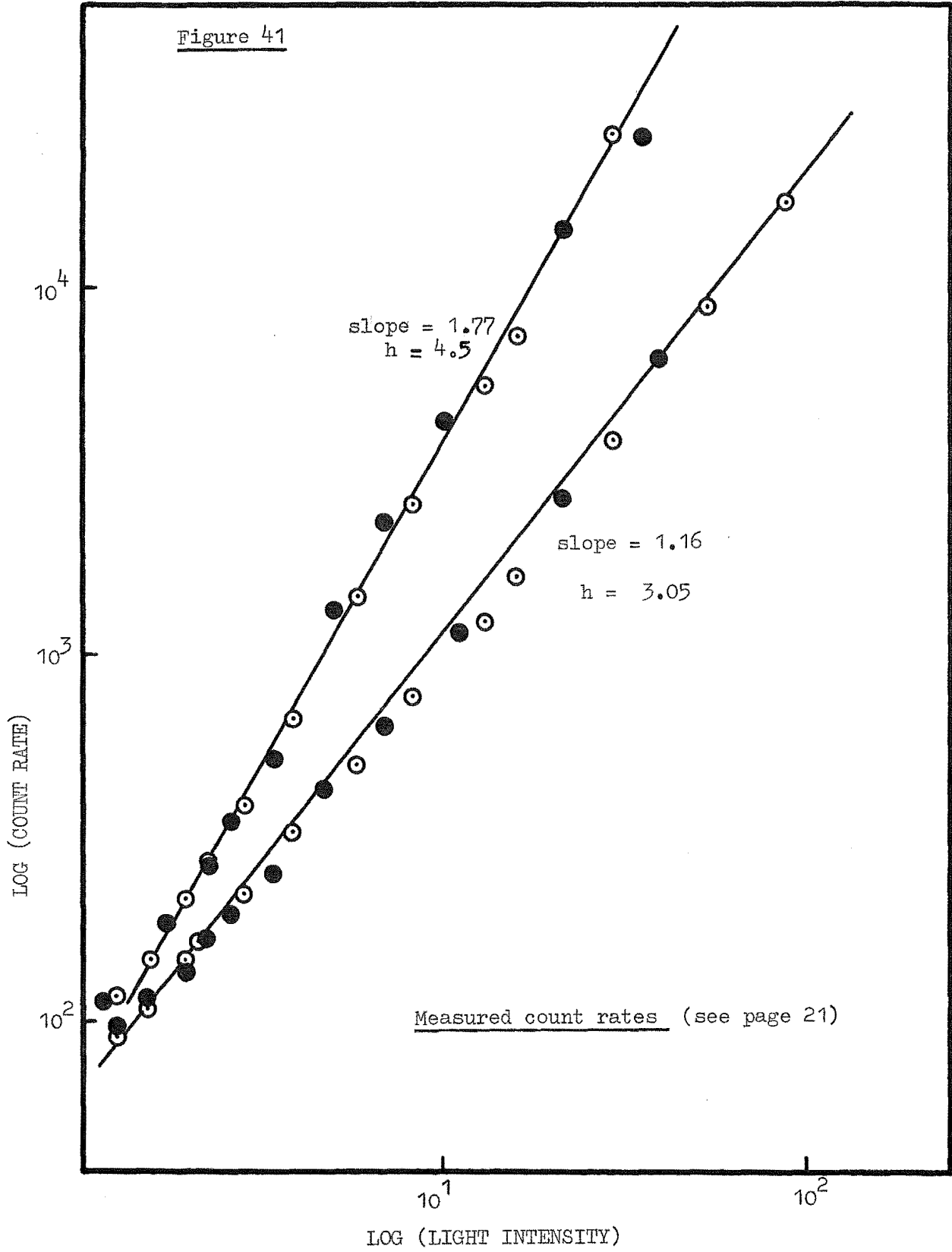
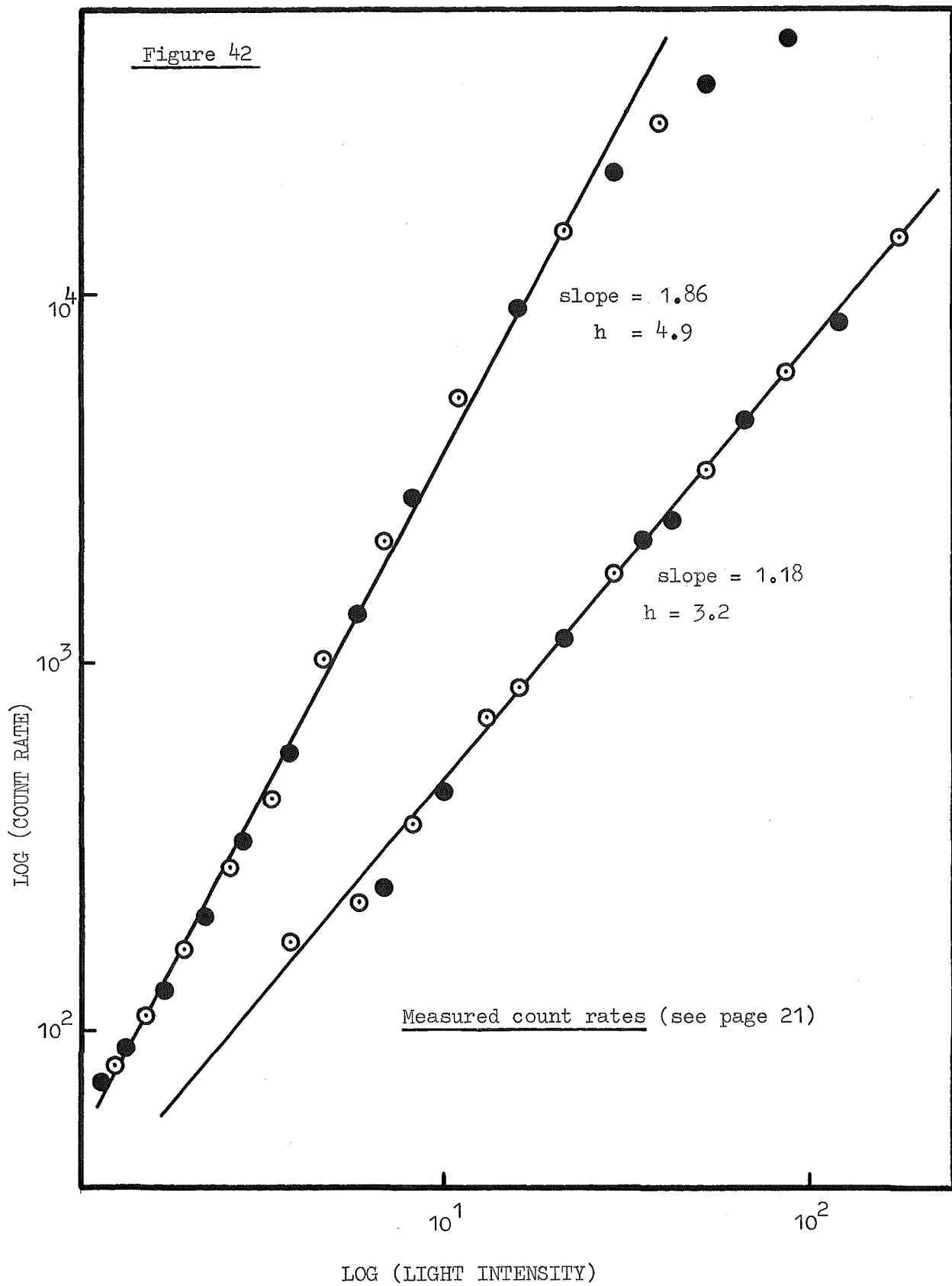


Figure 41





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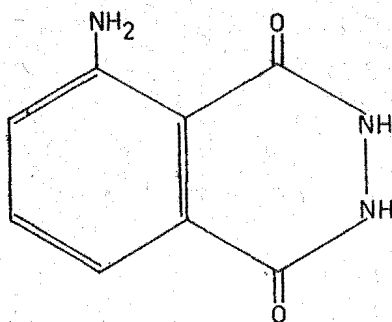
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Pamphlet to accompany
thesis by T. Quirk
Light from reactions in
solution.

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Di-imide and the Chemiluminescence of Luminol

THE light emitted when luminol reacts with hydrogen peroxide is usually¹ supposed to come from excited luminol molecules formed by the decomposition of a luminol peroxide². However, Albrecht³ has proposed that luminol molecules are excited by di-imide, N_2H_2 , formed during the oxidation of luminol by hydrogen peroxide.



Luminol

Di-imide was formerly postulated only as a reactive intermediate, but it has recently been isolated as a solid at low temperatures⁴ and detected in gases by mass spectrometry⁵. A growing body of evidence indicates that it is the reducing agent in solutions containing either hydroxylamine - *O* - sulphonic acid⁶, or hydrazine⁷ or *p*-toluene-sulphonyl hydrazine⁸, which are used to hydrogenate unsaturated compounds.

We find that luminol in aqueous solutions of these reagents emits light even in the absence of hydrogen peroxide, providing the solutions are alkaline and all the reactants are in the concentration range 10^{-2} - 10^{-4} M. The rate of formation of di-imide in these solutions is reported to increase on warming⁶⁻⁸. So also does the brightness of the light from luminol dissolved in them. The luminescence is comparable with that obtained from luminol in solutions of either hydrogen peroxide or of potassium ferricyanide, but it is less than that emitted when both these substances are present. The light-emitting solutions contained dissolved air. When it was removed by a stream of hydrogen, the chemiluminescence was reduced in each case. It has been reported⁷ that the yield of di-imide from hydrazine is less if oxygen is excluded.

These observations are as expected if Albrecht's mechanism³ is correct, and they show that the formation of a peroxide is not essential.

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¹ Reid, C., *Excited States in Chemistry and Biology* (Butterworth, London, 1957).

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CHEMILUMINESCENCE IN LIQUIDS

T. I. QUICKENDEN

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CHEMILUMINESCENCE IN LIQUIDS

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Some chemical reactions in liquids emit a faint glow called chemiluminescence. These reactions give products having a surplus of electronic energy which is emitted as light. Chemiluminescence occurs at ordinary temperatures, which contrast sharply with the high temperatures needed for incandescence. The glow of fluorescence also occurs at ordinary temperatures, but is caused by absorbed light and not by a chemical reaction.

Chemiluminescence can be conveniently demonstrated by adding 3-30% hydrogen peroxide to a mixture of equal quantities of 0.1 M pyrogallol, 1 M sodium carbonate and 10 M formaldehyde solution. A faint rosy glow is visible in complete darkness. A much brighter display can be made by adding a solution containing 0.01 M potassium ferricyanide and 0.01 M hydrogen peroxide to a solution of 0.0005 M luminol (5-amino phthalhydrazide) in 0.02 M sodium hydroxide. Huntress *et al.* (1) describe a convenient synthesis of luminol, and give a number of suggestions for displaying its luminescence.

Reactions in living matter sometimes emit light, a phenomenon known as bioluminescence. This attractive glow is found in luminous bacteria, glow-worms, fire-flies, some fungi and certain crustacea. Robert Boyle (2) examined a number of living systems which give out light and found that when oxygen was removed, the light gradually faded. We now know that oxygen is necessary in some form or other for nearly all chemiluminescent reactions, whether in living or non-living systems.

A number of reviews (3, 4, 5) summarize our present knowledge of bioluminescence, and a more popular account (6) contains many coloured plates of luminous plants and animals. A recent development is the discovery by Strehler (7) of a feeble light which is given out during photosynthesis.

The mechanisms of bioluminescence are often inferred from the simpler reactions which emit light in non-living systems. This review is largely concerned with the latter, and the following table contains the more common chemiluminescent reactions that occur in liquids.

Reaction	Typical Reactants	Conditions for Maximum Light
Oxidation of the phthalic hydrazides (8)	Hydrogen peroxide with ferricyanide in alkali	An NH ₂ group in the ortho position to the phthalhydrazide ring
Oxidation of the biacridinium salts (9)	Hydrogen peroxide with stannites	N,N'-dimethyl biacridinium nitrate (Lucigenin)
Oxidation of pyrogallol (10)	Formaldehyde with H ₂ O ₂ in alkali	
Oxidation of Grignard reagents (11)	Air	When magnesium is joined to an unsaturated carbon atom
Oxidation of xanthene dyes (12)	Hydrogen peroxide in alkali; also (13) pyrogallol with O ₂	When the dye is fluorescein, rhodamine, eosin or uranin
Decomposition of anthracene peroxides (14)		9,10 diphenyl anthracene
Oxidation of siloxene (Si _n H _{2n} O _n) (15)		Most Redox systems

THE PROCESS OF CHEMICAL EXCITATION

When a molecule has a surplus of electronic energy it is said to be excited. This excess energy may be radiated as light, lost to other molecules during collisions or degraded internally to heat.

A scheme for a simple chemiluminescent reaction is shown in Fig. 1.

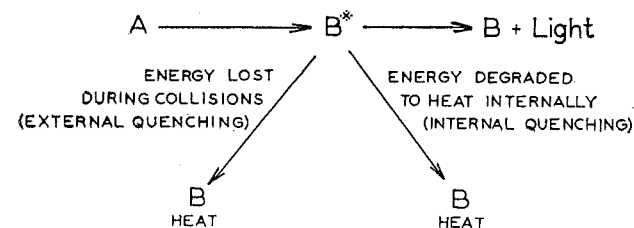


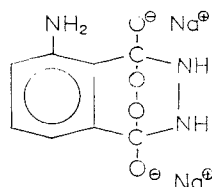
FIG. 1: Scheme for a simple chemiluminescent reaction.

In this scheme, A represents the reactants, B* the products which are in an excited state, and B the products in their usual ground state, which can be reached by light emission or by the two types of quenching.

The structure most commonly associated with chemiluminescence is the peroxide bridge. It is well known (14, 16), for instance, that the photo-oxides of anthracene can decompose to give anthracene, oxygen and a faint light.



Similarly, Drew and Garwood (17) have isolated a bridge peroxide of luminol, which also emits light on decomposition. In both cases, which are typical of organic peroxides, the highly electronegative peroxide bridge presumably polarizes the electrons of the molecule into the configuration of the excited state. When the peroxide bridge breaks, the electrons evidently return to their ground state with the emission of light.



Luminol peroxide

The electronic structure of the excited molecules which emit chemiluminescence is not clear. Two types of structure are possible. The molecule may have an unpaired electron and hence be in a triplet state, or alternatively, may have only paired electrons and be in a singlet state. Eyring (18) proposes that chemiluminescence comes from a molecule in a triplet state, while Reid (19) favours emission from a singlet level. The question is difficult to settle experimentally, because the techniques for detecting triplet states—*e.g.*, optical absorption (20), electron spin resonance (21)—require a higher concentration of triplet species than can be conveniently secured.

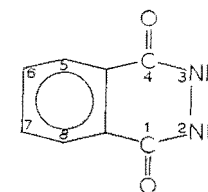
LIGHT FROM THE PHTHALIC HYDRAZIDES

Of the reactions previously listed, those of the phthalic hydrazides have received most study. In 1928 Albrecht (8), following Lommel (8), observed that the ortho-amino phthalic hydrazide (luminol) would emit a bright light in the presence of hydrogen peroxide and a catalyst such as potassium ferricyanide or haemin.

By comparing the light from various phthalhydrazide derivatives, a number of workers (22–26) have tried to find why luminol, with NH_2 in the 5-position, should glow so brightly.

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Phthalic hydrazide

A few derivatives, the 5-OH, the 5-NHCH₃ and the 6-N(C₂H₅)₂ show a light comparable with the glow of luminol. The unsubstituted phthalic hydrazide is only about 1/5,000 as bright. It is often concluded that electron donating substituents enhance chemiluminescence, most especially when in the 5-position of luminol. These rules have many exceptions, however.

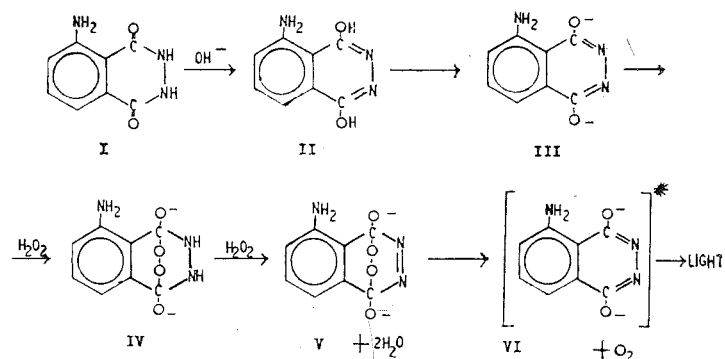
Because a substituent alters the intensity of chemiluminescence it is sometimes incorrectly supposed that excited molecules are necessarily being formed at a different rate. This need not be so, as the rate of internal quenching of excited molecules (Fig. 1) may also change with the substituent. As a result, the proportion of excited molecules which emit light may differ. Changes in the rate of internal quenching could be detected with measurements of fluorescence efficiency, and it is unfortunate that studies of substituent effects have not included these.

A wide variety of added substances, K₃Fe(CN)₆ (8), NaOCl (27), MnO₂, and colloidal metals such as platinum (28), many copper and cobalt complexes (29, 30), RuCl₃ and VOSO₄ (31) and haemin (8) increase the intensity of light when luminol is oxidized with hydrogen peroxide, but decrease its duration. Other substances such as KCN and Na₂S (32) and hydroquinone and phenol (23) reduce the light intensity. As in the case of substituents, the effect of added substances can be twofold. As well as affecting the reactions which lead to excitation, an added substance may quench the excited molecules once they are formed (Fig. 1). Measurements of the reduction of fluorescence by an added substance could be used to discover any such quenching process.

A technique recently described by Bersis (33) could be used to measure fluorescence and chemiluminescence at the same time, in the presence of added substances. Bersis excited the fluorescence with intermittent light and separated the steady, background chemiluminescence from the fluctuating fluorescence.

THE MECHANISM OF THE CHEMILUMINESCENCE OF LUMINOL

Some workers (8, 34) consider that the energy of the light from luminol comes from the decomposition of luminol molecules. Others (18, 35) claim that the decomposition of hydrogen peroxide provides the energy, and that luminol is lost only in side reactions. The mechanism of Drew (35), modified slightly by Reid (19), follows the second suggestion and is based on the isolation of the luminol peroxide IV by Drew and Garwood (17).



The substances II and III are accepted as intermediates because when their formation is prevented by methylating nitrogen and oxygen atoms respectively, chemiluminescence is almost absent (25). An alkaline solution is necessary for light emission, probably because it favours the formation of III. The next step is the formation of the luminol peroxide (IV) which is oxidized by a further molecule of hydrogen peroxide to V. When the peroxide bridge breaks, excited luminol molecules (VI) are formed and these subsequently emit light.

At some stage which is not clear, the luminol ion must again become protonated, despite the adverse pH of the alkaline solution. This is indicated by the spectrum of the chemiluminescence, which is similar to (8, 36) or identical with (37) the spectrum of the fluorescence from an acid solution of luminol. The fluorescence of an acid solution is generally attributed (18) to the protonated species II.

Ojima (29, 30) proposes that some ions, which catalyse the chemiluminescence, aid the formation of the peroxide bridge by chelating with a luminol ion as shown in the following diagram.

if it is real, indicates radiation in the far ultra-violet, which passes through air or quartz, but not glass.

Much of this type of work is unsatisfactory, and criticisms have been levelled at inadequate control of experiments (60, 61) and at the statistics used (62, 63). Some work has not been reproducible (60, 61). Nevertheless, a few papers (64, 70) withstand these criticisms.

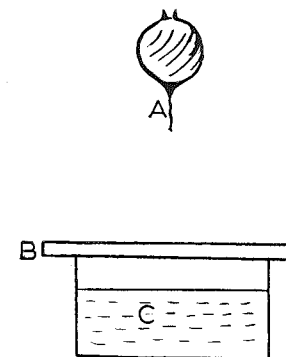


FIG. 2: Detection of mitogenetic radiation. A, emitter which contains rapidly dividing cells (e.g., onion root); B, interchangeable glass or quartz plate; C, detector (e.g., growing yeast).

A number of workers, some successful (52, 65) and others unsuccessful (66), have attempted to detect the emitted light with U.V. sensitive Geiger tubes. There have been some recent and successful attempts (67-69) to detect the radiation with sensitive photon counters, similar to that previously described. The signal-to-background ratios have not, however, been high.

There are a number of impartial reviews (70, 71) of this subject, particularly the account by Hollaender and Claus (71). The case against mitogenetic radiation has been forcibly presented by Lorenz (66). If, as seems indicated, most chemical reactions emit a feeble chemiluminescence, it should not surprise us if living systems behave similarly.

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In brief, each photon incident on the cathode of a photomultiplier tube may liberate an electron which triggers a pulse of some millions of electrons from the tube. These pulses are amplified further, separated from small noise pulses and counted. With the best photomultipliers, about 1 in 5 of the incident photons is counted. With shielding from cosmic rays and γ radiation, with cooling and with anticoincidence circuits, the inevitable background counts can be reduced to five or six per minute. Under these conditions light fluxes of fifty to one hundred photons per second per square centimetre can be measured reliably.

Recent measurements with such equipment indicate that some at least of the reactions that Audubert described, do emit light. This work is proceeding and will be published.

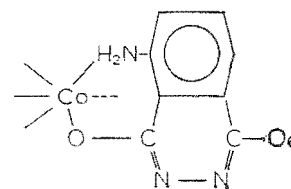
The theoretical challenge is to account for the production of high energy photons from a diversity of reactions. Eyring (58) suggests that black-body radiation excites the reactants which then yield products also excited, but with a higher excitation energy than before the reaction. Hence ultraviolet or visible photons could be emitted when only infra-red were absorbed, the energy difference coming from the free energy of reaction.

It is also conceivable that in the microscopic region surrounding two reacting molecules, very high, transient temperatures may be generated. These "hot regions" might be the source of visible and ultraviolet radiation, which is a quite insignificant part of black body radiation at room temperature.

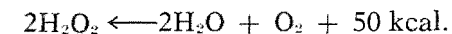
MITOGENETIC RADIATION

Some biophysicists, notably Gurwitsch (59), claim that cell division is accompanied by emission and stimulated by absorption of ultraviolet photons. The literature supporting this view extends to over five hundred papers and reviews, mostly in the Russian and German languages. Biological detectors are often used to detect the ultraviolet emission, and the following outline of a typical experiment illustrates their use (Fig. 2).

A material containing rapidly dividing cells, such as an onion root, is directed at a detector such as a yeast culture. The number of cell divisions in the yeast are counted, and compared with those in a control experiment where the onion root is not present. Under such conditions, Gurwitsch (59) has claimed a significant increase of cell growth above that of the control, when air or quartz separated the specimens, but not when glass was interposed. This observation



The major defect of the previous mechanism is its inability to account for all the energy of the emitted light. As luminol is regenerated in the last step, the overall reaction is simply



Unfortunately, the blue light emitted by luminol would require at least 65 kilocalories per mole of oxygen formed. To overcome this difficulty, Eyring (18) has proposed a mechanism involving four molecules of hydrogen peroxide, two of which form a hypothetical intermediate with luminol, having the triplet structure championed by Eyring. The intermediate has not been isolated.

Several free radical mechanisms (38, 39, 40) have been proposed to explain the inhibiting effect which free radical scavengers have on the light intensity. Weber *et al.* (40) combine a free-radical scheme with the peroxide mechanism of Drew (35).

Albrecht (8) originally suggested a mechanism in which a molecule of luminol was destroyed to provide the energy of each photon given out. According to Albrecht, hydrogen peroxide oxidizes a luminol molecule to give the reactive intermediate, di-imine, $\text{NH}=\text{NH}$ which reacts with and excites another luminol molecule.

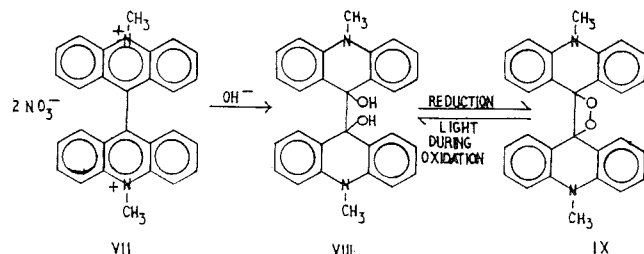
This mechanism is usually rejected for two reasons. One is that the rates of light emission and luminol decomposition are not obviously related (23). The other reason, which would be convincing if true, is that the number of photons emitted per molecule of luminol destroyed is too large for the above mechanism, which could not permit more than one photon per molecule. A survey of the literature shows that the highest photon yield reported, is about 0.33 photons per molecule (39), and that the usual yields (27, 41-43) are about 0.005 or less, depending on the conditions. On these grounds and because of recent work on di-imine (44) this mechanism, which could account for all the energy of the emitted light, merits further study.

THE CHEMILUMINESCENT BIACRIDINES

The salts of the biacridines (9) are the only known chemiluminescent materials which equal or surpass luminol in brightness. Lucigenin (VII) which has the structure shown, is the best known example. It glows feebly in a

solution containing hydrogen peroxide and the light is greatly intensified if osmium tetroxide or reducing agents such as stannites are added (9).

The following mechanism was advanced by Gleu and Petsch (9) to explain the observation that both an oxidizing and a reducing agent were essential for chemiluminescence. Hydrogen peroxide, which behaves both as an oxidizing agent and a reducing agent, evidently meets the requirements of the reaction less effectively.



The peroxide intermediate IX is analogous to the luminol peroxide of Drew, but has not been isolated. According to this mechanism, no lucigenin (VII) is destroyed, because in the light-emitting step the peroxide is reduced back to the carbinol (VIII). As in one of the luminol mechanisms (35), the overall reaction is the decomposition of two molecules of hydrogen peroxide to water and oxygen, with the liberation of 50 kcal per mole of oxygen. Once again, this is insufficient energy to account for light in the blue region of the spectrum.

Kautsky and Kaiser (45) propose a different mechanism in which VIII decomposes into N-methyl acridone during the light-emitting step.

SOME TECHNICAL USES OF CHEMILUMINESCENCE

Because the emission of light often depends on pH and sometimes depends on Redox potential, chemiluminescent materials have been used as indicators in volumetric analysis. The light emitted at the end point is easily visible, even in highly coloured or turbid solutions, where colorimetric or fluorescent indicators fail. Two reviews (46, 47) give access to details of the methods, and a recent modification (48) seems particularly effective.

As the haemoglobin in blood causes luminol to glow brightly, it has been used (49) in forensic chemistry to detect traces of blood. Chemiluminescent materials have

also been used to study mixing and flow in liquids (50). Mayneord *et al.* (51) have used the light emitted by the phthalic hydrazides to detect and estimate traces of hydrogen peroxide formed by X-rays in water.

WIDESPREAD CHEMILUMINESCENCE OF VERY LOW INTENSITY

It is sometimes stated that nearly all reactions in liquids emit minute amounts of light — about one photon from 10^{14} – 10^{15} molecules. These comments date from the interesting but neglected work of Audubert (52), who detected ultraviolet photons in very low yield, from a wide range of chemical reactions summarized below.

1. The reaction of NaOH with H₂SO₄, and other acid-base reactions in water.
2. Atmospheric oxidation of sulphites and of pyrogallol.
3. Reactions of the alkali metal amalgams with water.
4. Oxidation of glucose by potassium permanganate.
5. Oxidation of ethanol by chromic acid.
6. Electrochemical oxidation of aluminium.
7. The reactions in various living systems.

To count the emitted photons, Audubert used ultraviolet-sensitive Geiger tubes (53), in which a pulse is caused by the emission of electrons from a photo-sensitive surface. Such tubes are sensitive to wavelengths around 2,000Å. As this work was done over 25 years ago, the methods of light detection were not always reliable. Nevertheless, several observations similar to Audubert's have recently been made. Strehler (7) has observed a feeble chemiluminescence accompanying photosynthesis, Ahnstrom (54) (1961) has detected photons from the anode during electrolysis of organic compounds and Shliapintokh *et al.* (55) (1960) have detected feeble light from a number of organic reactions. Dimbat and Harlow (56) (1962) have recently observed feeble light from acid-base titrations in non-aqueous solvents.

The reactions of Audubert (52) are at present being investigated by the writer in conjunction with Dr W. S. Metcalf. The major experimental problem in this work is to measure reliably very small light intensities which will produce only minute photocurrents in a detector. Under these conditions, the pulses due to individual light photons are large compared with the mean photocurrent, and it is better to count the pulses than to measure the current.