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RADIOCHEMICAL
STUDIES OF HYDROGEN
EXCHANGE IN
ORGANIC COMPOUNDS

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DETERMINATION OF ACTIVE OR EXCHANGEABLE HYDROGEN

METHOD I

INTRODUCTION

A property of a new or unknown organic compound which must be determined once the empirical formula and molecular weight are known, is the number of active or replaceable hydrogen atoms which the compound contains. These include hydrogen atoms present in amine, hydroxyl, carboxyl and other groups, where the hydrogen is not bound to a carbon atom but to an oxygen, nitrogen or sulphur atom or is in a position where it can ionize.

The most general method by which this may be done quantitatively, is the one originally due to Zerewitinoff ⁽¹⁾ and since developed on a micro scale by Roth ⁽²⁾, Flaschentrager ⁽³⁾, whose method incorporates work by Tschugaeff ⁽⁴⁾ and the other two authors, and Soltys ⁽⁵⁾, and incorporates many of the latest improvements. This involves the quantitative evolution of methane from reaction of the Grignard reagent MeMgI on groups such as $-\text{SH}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{COOH}$ etc., i.e. those groups containing active or replaceable hydrogen atoms. Analysis by this method requires extreme care in technique and exact attention to experimental details. High results are obtained if the solvent or any part of the apparatus contains moisture and the whole determination must be carried out in an atmosphere of nitrogen to avoid reaction of the Grignard reagent with any oxygen present. Low results are obtained if the test solution

does not dissolve completely in the chosen solvent and it is essential to carry out a blank prior to each analysis. The procedure is labourious and painstaking and gives an accuracy of not greater than 5% using 3 - 5 mgm of organic compound. It also has the disadvantage that the Grignard *Reagent* will also react with other groups, such as carbonyl, aldehyde, nitrile etc., which may be present. This method cannot be applied to highly water soluble compounds which do not dissolve in ethers or other organic solvents and as the molecular size or complexity of the sample increases, the accuracy of the gasometric reactions becomes less, due to side reactions and incomplete reaction.

Other methods of assaying exchangeable hydrogen have also been reviewed by a number of authors.

J. Mitchell Jr. ⁽⁶⁾ has described a method by which active hydrogen may be determined using diazomethane, giving rise to nitrogen, the volume of which is measured quantitatively. More recently the same author has used LiAlH_4 to give hydrogen, the volume of which is again measured quantitatively.

The above methods suffer from the obvious disadvantage that they change, usually irreversibly, the starting material.

A possible alternative to these methods is an exchange of the active hydrogen with isotopic hydrogen and this type of procedure has received the attention of several authors.

Although the isotope methods were of obvious potential for some time, comprehensive evaluation for routine work was initially delayed,

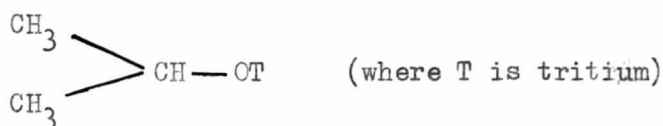
probably because of the early expense of isotopic hydrogen or the lack of any really convenient method of assaying a variety of organic compounds for the content of their hydrogen isotope. These drawbacks have since been overcome by the increased availability of high specific activity tritium and the introduction of good general techniques for the radio-assay of H^3 labelled organic compounds.

A large body of evidence is available to show that if a compound containing "active" hydrogen in functional groups such as amine, hydroxyl, carboxyl etc. is dissolved in a solvent containing isotopic hydrogen, e.g. D_2O T_2O CH_3OD (where D = deuterium 2H + T = tritium 3H), exchange occurs rapidly between the hydrogen atoms of the solute and the solvent. The replacement of hydrogen by isotopic hydrogen, is in many cases, not due to direct substitution, but to the fact that ionization of the hydrogen atoms of the compound takes place in solution and is followed by alternative recombination and dissociation. A detailed discussion of exchange reactions of deuterium with other molecules is given by Taylor et. al (7). Investigation of the behaviour of glycol (8) hydroquinone (9) succinic acid (10) and aniline (11) shows two hydrogen atoms exchange immediately while in the case of phenol (12) benzyl alcohol (10) benzoic acid (13) and dimethylamine (14), only one hydrogen atom was found to be exchangeable. In general, hydrogen ^{atoms} attached directly to a carbon atom do not undergo replacement. unless influenced by an adjacent activating group such as a carbonyl group, or under the (12) influence of some acids as shown by Ingold in work on replacement of

hydrogen in the aromatic nucleus.

Several estimations of active hydrogen in organic compounds have been described by various authors, based on the type of exchange outlined above.

R.J. Williams (15) described calculation of active hydrogen content by replacing it with deuterium and measuring the increase in weight after suitable drying, using a sample of ~ 10 mgm. W.H. Hamill and W. Freudenberg (16) report an estimation of exchangeable hydrogen in hexoses and their methyl glycosides using D_2O and determining the loss in weight of D_2O after exchange. Eastham and Raaen (17) dissolved the organic compound in -OH tritiated isopropyl alcohol, i.e.



evaporated the solvent and subjected the organic compound to radio-assay.

A recent paper by Giles (18) describes the micro determination of active hydrogen with tritiated ethyl alcohol, i.e.



by dissolving the organic compound (approximately 0.1 milliequivalents) in 2.00 ml tritiated alcohol, distilling off the ethyl alcohol and counting suitable aliquots in a scintillation counter. However, this would apparently give a count decrease of $\sim 0.1\%$ in the ethanol activity and in spite of the quoted results, this does not seem to be a very accurately measurable decrease.

COUNTING AND RADIO-ASSAY METHODS FOR TRITIUM

Accurate radio-assay and counting of tritium involves special techniques owing to the nature of the decay emanations of tritium. Tritium is the three isotope of hydrogen ^3H , and is radio-active with a half-life of approximately 12.3 ⁽²²⁾ years and for this reason it is eminently suited as a study tracer. It is a β emitter with an E_{max} for the β particles of 0.018 Mev ⁽²²⁾, a range in air of ~ 1 mm and it is this extremely weak radiation, weaker than either C^{14} or S^{35} , that makes special detection procedures necessary. The radiation is too weak to penetrate even the thinnest of end-window counters, but two counting techniques overcome the counting difficulty effectively, these being liquid scintillation and gas-phase counting.

Liquid scintillation counting has been widely developed over the past few years and has been discussed by a number of authors. ^{(19) (20) (21)} The sample is dissolved or suspended in the scintillating fluid or gel, thus producing a minimum of absorption, and essentially no barrier between sample and detector. Such a system can detect as little as 25 $\mu\text{c.}$ of tritium. An application of this type of counting procedure is described by Roucayrol ⁽²³⁾. However, liquid scintillation counters and their associated electronic equipment are expensive and their efficiency is too low to make them useful in kinetic studies.

The best method of assaying low energy β emitters is to make them the filling gas inside an ionization chamber, geiger counter or proportional counter.

In this method, tritium in the form of a gas is introduced into

a gas counting tube, thus overcoming the problem of absorption by having the radio active material within the counter itself. This gives rise to two kinds of gas-phase counting; conventional Geiger-Müller counting with the tritium within the counters initiating the discharges, and gaseous scintillation counting.

In the work done during these studies, the former method, that of Geiger-Müller, counting, was adopted.

Tritium has been counted by the Geiger-Müller method in the form of a number of gases. Eidinoff ⁽²⁴⁾ has described a method of counting tritium as hydrogen gas, using alcohol/argon gas mixture as a quenching gas in the counter tube. Tritium has also been assayed as active butane, ⁽²⁵⁾ HTO using propane in the counter ⁽²⁶⁾, and in several other gaseous forms including H_2/CH_4 mixture by a number of authors.

Counting Equipment and Methods

In the following work, the conventional method of Geiger-Müller counting was adopted, with the tritiated water vapour being introduced into the counting tube to be counted. The counters used were filled with a gas containing 40% ethylene and 60% argon to a pressure of 10 - 12 cm of mercury. This was found suitable for counting water vapour samples at the pressures used (~ 1 mm of mercury). This type of counter filling is described by Wolfgang and Libby ⁽²⁸⁾.

The counter was quenched by an external quench circuit, as the counters were not self quenching. A negative pulse of 210 v. was applied to the anode wire, the duration of this pulse ~ 1 millisecond, determining the paralysis time T of the counting assembly. This was

adjusted to the lowest possible T valve.

The counts lost during the interval T were computed from:

$$N = \frac{R}{1 - R T}$$

where N = true counting rate in counts/minute

R = observed rate

T = paralysis time in minutes

A nomogram based on the above equation as shown in a paper by Melhuish (29), was obtained, and paralysis corrections were calculated for all runs from this.

The paralysis time of the counting circuit used was determined by the paired source method described by Cooke and Duncan (30). The quench unit was set to the minimum value of T possible, and the counting apparatus and quench unit connected to a geiger tube. The background, activity of source A, activity of source B and combined activities of A and B determined by counting each over a period of 10 minutes. If the activities of sources A and B are comparable it can be shown mathematically that:

$$T \text{ (the circuit paralysis time)} = \frac{R_A + R_B - R_{AB} - b}{[R_{AB}]^2 - [R_A]^2 - [R_B]^2} \text{ minutes}$$

where R_A is the activity of source A in c/m

R_B is the activity of source B in c/m

R_{AB} is the activity of sources A and B in c/m

b is the background count

From these results the paralysis time of the circuit was calculated and found to be 0.73 m.secs. This was checked periodically and the differences each time found to be insignificant.

As the counters were all of similar dimensions for each class of counter used, no corrections were needed for counter geometry.

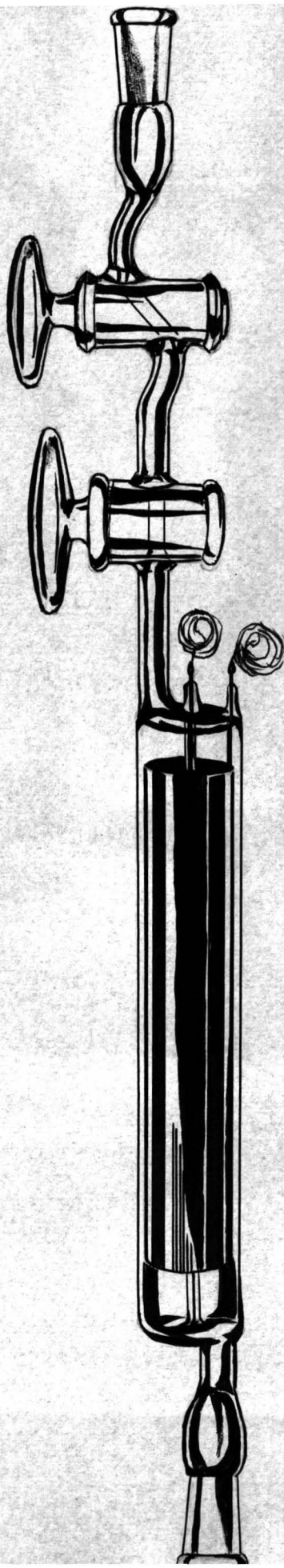
The counters used during the initial work were Type G.M. 10A4. Geiger Müller tubes as produced by Cinema Television Ltd., London, containing a copper sheet cathode with a tungsten anode centre wire and were supplied, sealed and filled with ethyl formate as a counting gas. These tubes were opened and a B14 Quickfit cone ground-glass joint sealed to one end and a B14 socket joint sealed to the other end.

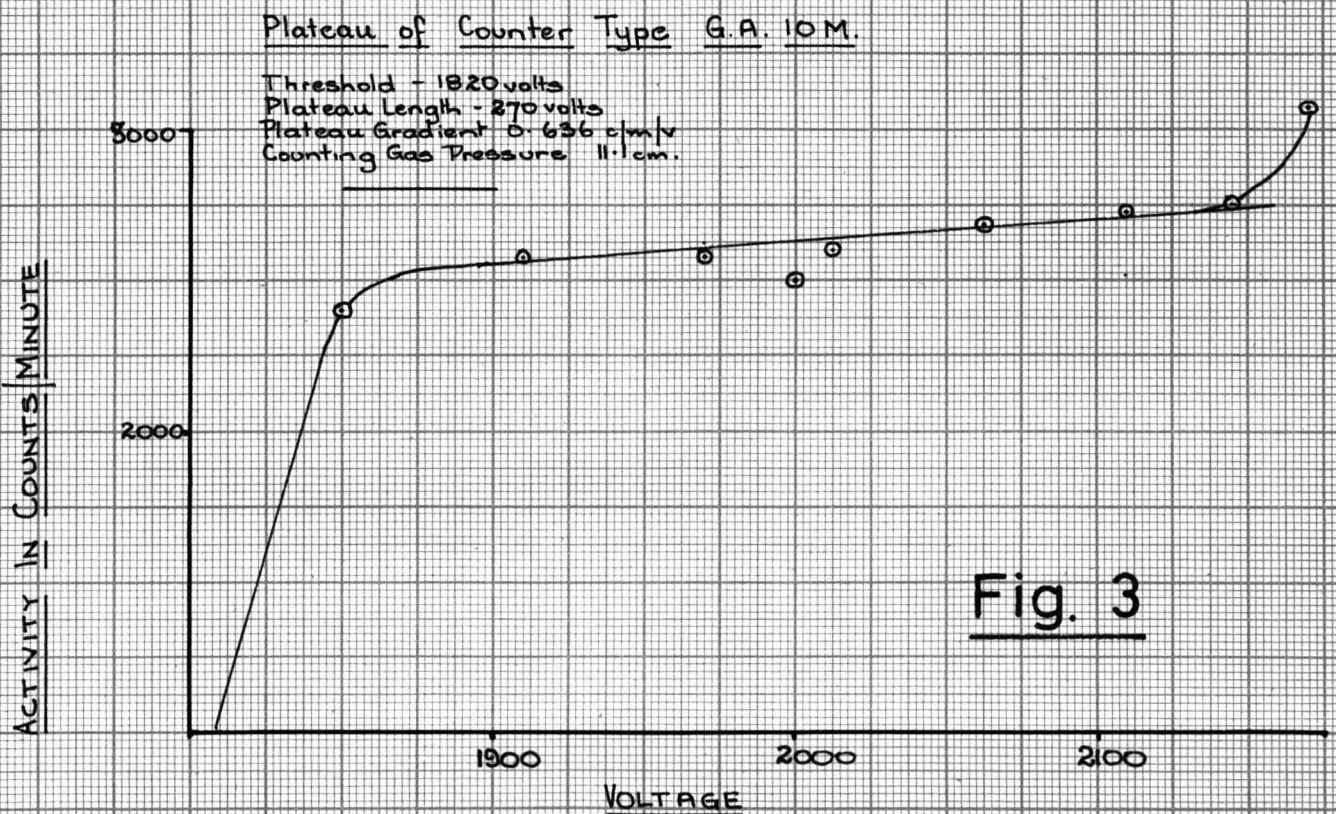
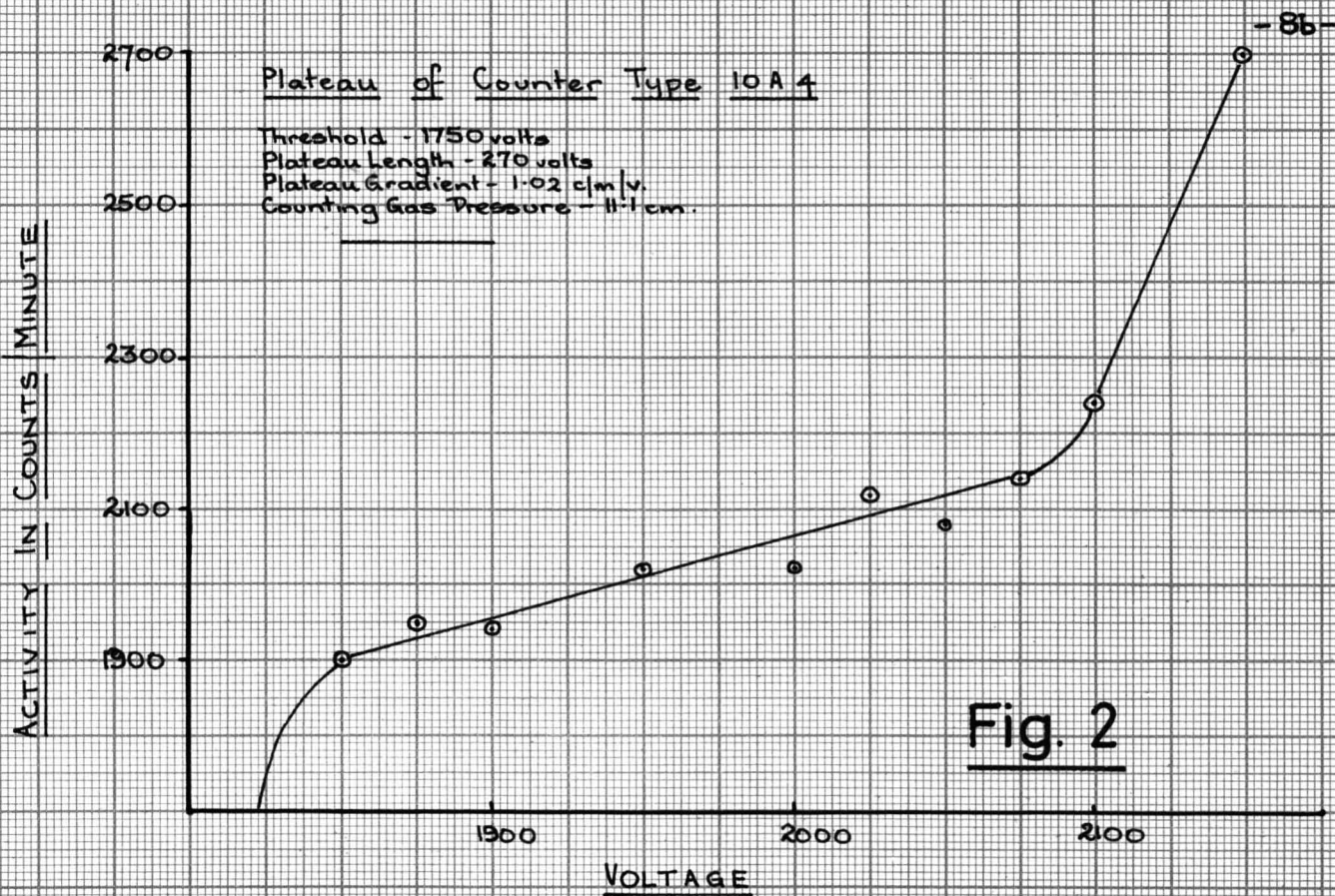
In later work (method II and Ketone work) a smaller counter of type G.A. 10M as supplied by Twentieth Century electronics was used. These counters had stainless steel cathodes and a tungsten wire anode, and were adapted in a similar fashion with a B14 quickfit socket at the base. At the top, however, it was found necessary to incorporate two taps as shown in the diagram (See Fig 1) thus enabling small quantities of tritiated water vapour to be introduced into the counter as will be explained in a following section.

Threshold voltage was about 1900V and the counters were operated at 100V above threshold. The counting and plateau characteristics of both counter types are shown in figs 2 and 3.

The counting equipment consisted of the bank shown in Plate I and comprising a Phillips FW4024/01 High Voltage Power supply giving up to 2500V, a Phillips FW4042 rate meter and a Phillips FW4032 Electronic counter. The output from the rate meter was fed into a Brown single

Fig. 1





channel recorder and plotted by means of a pen recorder. The rate meter was on a number of occasions, calibrated against the electronic counter and the difference between the two was found to be insignificant. In several of the runs performed the electronic counter was also coupled to a printing unit built by a fellow student, Mr F.P. Crotty. This apparatus described by F.P. Crotty (31) added all pulses received from the electronic counter over a period of 15 minutes, and then printed this total on paper. This was checked against the value for the same period obtained from the recorder pen trace.

The recorder pen trace was calibrated periodically throughout the work against the value given on the rate meter dial. This could be done by setting the rate meter selector switch to calibrate, thus feeding in a std. pulse which gave a reading of 0.3 full scale deflection on the rate meter dial. It was also calibrated at zero reading with the pen trace, and at high values on the scale and over the full range 0 - full scale deflection, little difference between the rate meter value and the recorder value on the pen trace was noted.

Shielding of Counters

In the work done with the keto-enol system, the counters were shielded by an improvised lead castle. This was formed of lead bricks, cast in a circular mould with a $1\frac{1}{2}$ inch hole left in the centre. These were machined so that they fitted together as shown in Plate 3. The counter was placed inside the lead castle with just the terminals protruding enabling leads to the quench unit to be attached to anode and

PLATE 1

- 9a -



KEY TO PLATE 1

- A Lead shielding for counter
 - B Thermos flask to maintain constant temperature
 - C Paralysis circuit unit
 - D Printing unit
 - E Scaler
 - F Ratemeter
 - G High voltage supply unit
 - H Strip chart recorder
-

cathode. The castle was mounted on a frame, enabling a thermos flask to be placed beneath the counter, thus providing a constant temperature in the small flask attached to the counter. (See Plate 3)

In the active hydrogen work Methods I and part of II shielding was not feasible, as in method I the counters were attached directly to the vacuum line, and could not be removed during experimental runs as in the later work. In part of method II it was necessary to incorporate a stirring mechanism beneath the counter (see Plate 4) and this made it difficult to shield the counter with the lead castle.

However, it was found that the background radiation did not change enough to be significant during the course of a determination, and thus provided the background was known accurately before the commencement of each run, the results were in no way affected by lack of shielding. The effect of the shielding was to reduce the background radiation (due to cosmic radiation etc.) from approximately 300 counts per minute to approximately 80 - 100 counts per minute.

Light Sensitivity of the Counters

From the observation that slightly higher background counts were recorded during the day or when the laboratory lights were on, it was suspected that the counters might be slightly light sensitive. This was overcome by painting the ends of the glass envelope of the counter with black Dulux enamel, thus making the interiors very nearly light-proof. After this had been done, no background differences were noted when the lights were switched on and off during an experimental run.

MANIPULATION OF RADIOACTIVE WATER VAPOUR

To facilitate the handling of the tritiated water samples used in this work, it was necessary to design and have made, a glass vacuum system in which the liquid water and water vapour samples used, could be handled. This system was designed by the author and a fellow research student and made in the glassblowing department of Victoria University. A photograph of the system is shown in Plate 2.

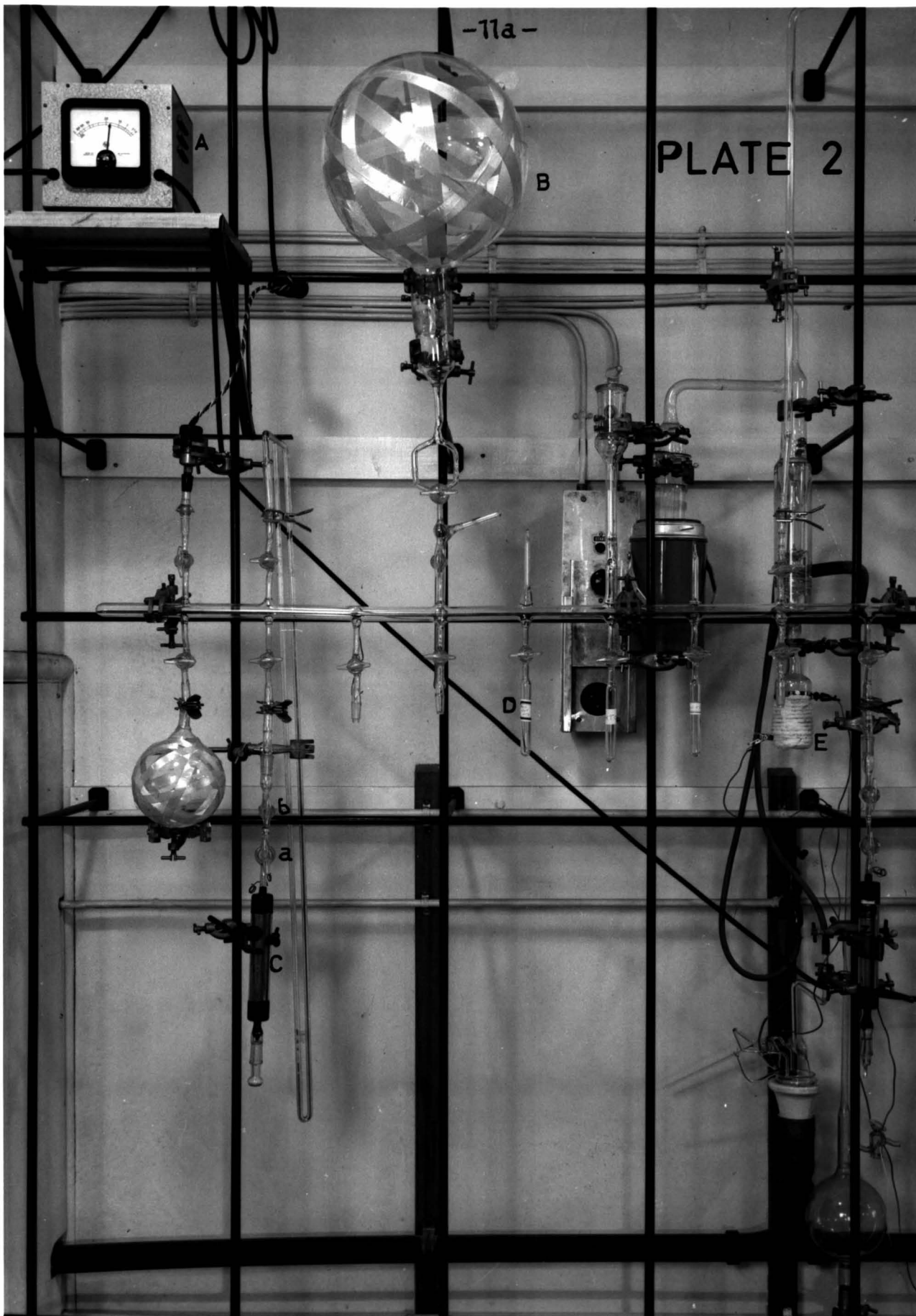
The main part of the vacuum system was composed of $\frac{3}{4}$ inch glass tubing and to this were attached various side arms for specific purposes. The majority of these incorporated a Quickfit glass tap to enable the arm to be isolated from the main system at will. To enable a counter to be attached to the system, it was found best to use an arm incorporating a tap and ending in a ball and socket joint. An adaptor was used incorporating the socket portion of the joint and having on the other end a B₁₄ Quickfit cone, over which the B₁₄ Quickfit socket on the top of the counter fitted. By this method, some movement of the glass connection was possible, thus decreasing the likelihood of glass fracture which would have resulted from any strain imposed on a rigid glass connecting system, such as a Quickfit cone and socket connection. Two counters connected to the system using the ball and socket connection can be seen in Plate 2. The whole vacuum line and mercury pump were clamped securely to an iron rod frame bolted to the Laboratory wall.

The tritiated water samples used, were stored in tubes attached permanently to the system and isolated from it by a tap.

The vacuum system was attached to a two stage mercury diffusion

-11a-

PLATE 2



KEY TO PLATE 2

- A Vacuum pressure gauge
 - B Counting gas reservoir
 - C Geiger counter attached to vacuum line
 - D Hot tritiated water reservoir
 - E Mercury-vapour pump
-

pump via a cooling trap, and then to a rotary oil backing pump. It was found necessary to incorporate a water switch in the water cooling circuit of the mercury vapour pump, to prevent continued boiling of the mercury, with consequent introduction of mercury vapour into the vacuum line, in the event of the water to the cooling jacket being cut off.

The pressure in the system was measured by means of an electrical thermocouple pressure gauge of the type produced by Hastings - Raydist Inc. of Virginia, U.S.A. The pressure in the system determines the current flowing in the head, and this in turn is fed directly to an ammeter, calibrated to read pressure in microns (10^{-4} cm). The pressure gauge was initially calibrated against a standard vacuum pressure gauge.

It was found, that after outgassing the system thoroughly over a period of approximately one week, a pressure of 15 microns could be obtained using the rotary oil backing pump only, and this could be reduced to approximately 1 micron if the mercury pump was used as well.

COUNTING GAS RESERVOIR

The counting gas used was stored, initially at a pressure of 1 atmosphere in the 5 litre flask attached to the top of the system. To introduce the counting gas into this reservoir, the cylinder containing the ethylene/argon mixture was attached to the vacuum line by means of rubber pressure tubing and the whole system, including the reservoir, evacuated to 20 microns. The system was flushed with the counting gas and re-evacuated several times to ensure removal of all air, and the tap on the top of the counting gas cylinder opened slowly. The reservoir was filled to a pressure of 1 atmosphere as determined from the large

manometer attached towards the left-hand end of the vacuum line. When this pressure had been obtained, the reservoir was isolated by turning off the taps, and the cylinder detached and removed. When the reservoir pressure became too low to give the required counting gas pressure when filling the counters, the reservoir was refilled in a like manner.

As a safety precaution, the reservoir was bound with fibre-glass adhesive tape as shown, and was normally enclosed in a sack, thus minimizing the risk to research staff, of flying glass, should the reservoir implode at low pressures or on evacuation.

FILLING OF COUNTERS

The method adopted in filling the counters was as follows:

The counters were first attached to the vacuum line by means of the socket/cone and clamped firmly in place by means of screw clamps as shown. The whole system and the counter were then evacuated to the lowest possible pressure, having first isolated the counting gas reservoir and its small manometer, by turning off the tap. After evacuation, the first tap, connecting the small manometer to the vacuum line was opened, and a check made to see that the manometer read zero at this stage. The reservoir tap was then opened slowly, and counting gas allowed to bleed into the manometer, vacuum line, and counter. When this counting gas pressure had reached 11 cm of Hg in the counter the reservoir was closed, the manometer reading taken and the manometer tap closed. The counter taps were then closed, and the vacuum line opened to the atmosphere. This allowed the counter to be removed easily from its side arm; the side arm tap was then closed and the vacuum line re-evacuated.

From the results obtained of trials of counting gas pressure in the counters vs. threshold voltage [Fig. 4], it was decided to run the counters at an operating pressure of $11 \text{ cm} \pm 0.2 \text{ cm}$ and this pressure was used in all work. Thus the counters had a threshold voltage of approximately 1850V and were operated 100 volts \pm 10 volts above the threshold voltage. Fig. 4 shows a typical graph of threshold voltage vs. counting gas pressure and although each counter varied slightly in its threshold voltage at various pressures, no major differences were noted in any of the counters used.

INTRODUCTION OF TRITIUM INTO COUNTERS

The method of introducing a small sample of tritiated water vapour into the counter already filled with 11 cm of counting gas was as follows:

The counters were fitted with a small adaptor (see Fig 5) and the whole apparatus attached to the vacuum line and clamped. The whole apparatus including the small space between the two taps on the Geiger counter, but excluding the counter itself, was then evacuated by means of the pumps. The tritiated water sample, enclosed in one of the vacuum system side arms, was then frozen lightly with liquid air and surrounded by a thermos of crushed ice and water, thus bringing the sample to a temperature of approximately 0°C . The tube was then opened and the tritiated water vapour allowed to fill the vacuum system and the small space between the two taps on the geiger counter. The upper tap on the counter was then closed, trapping a small sample between the two taps. A flask of liquid air was then placed around the tritiated water reservoir

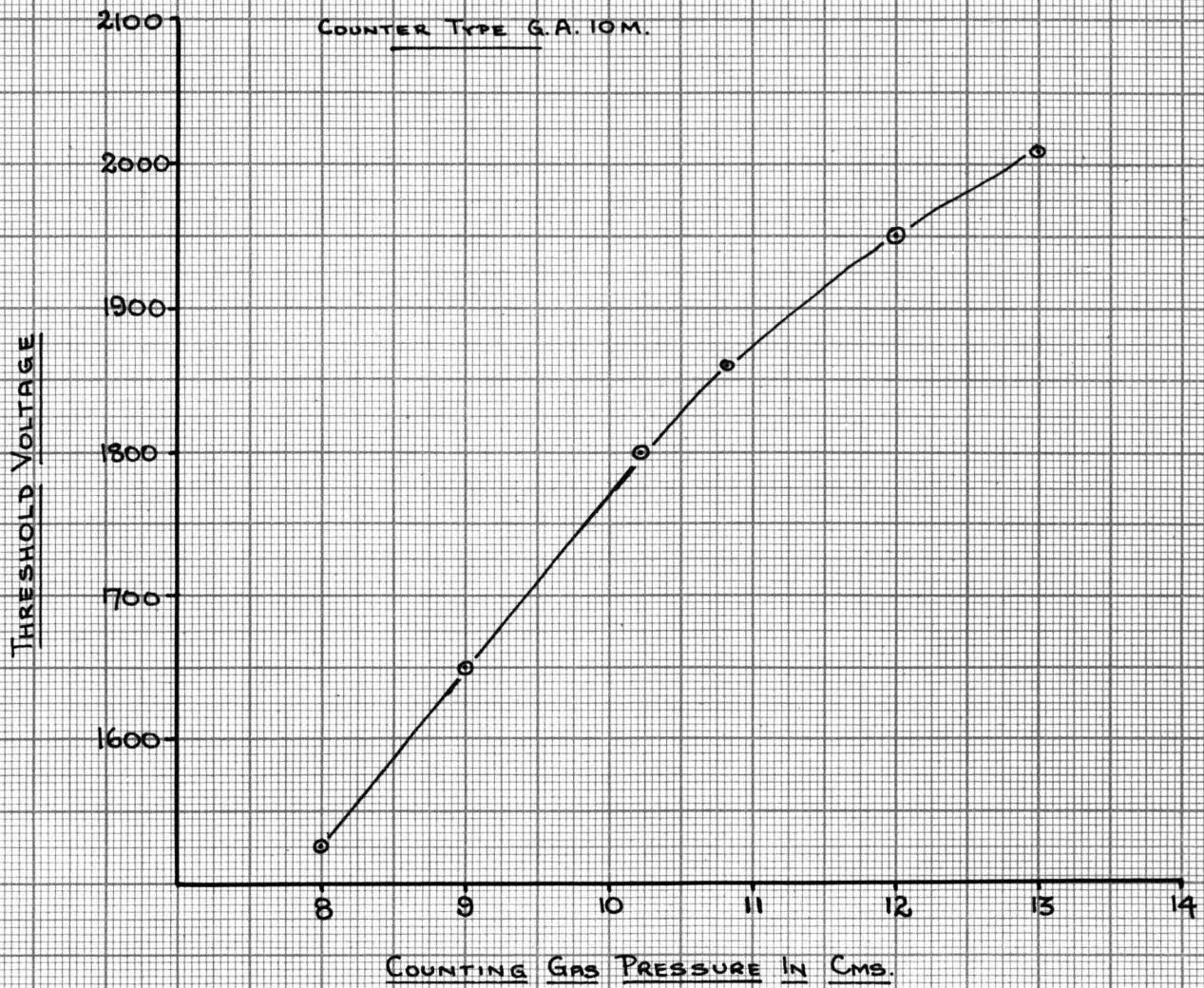


Fig. 4

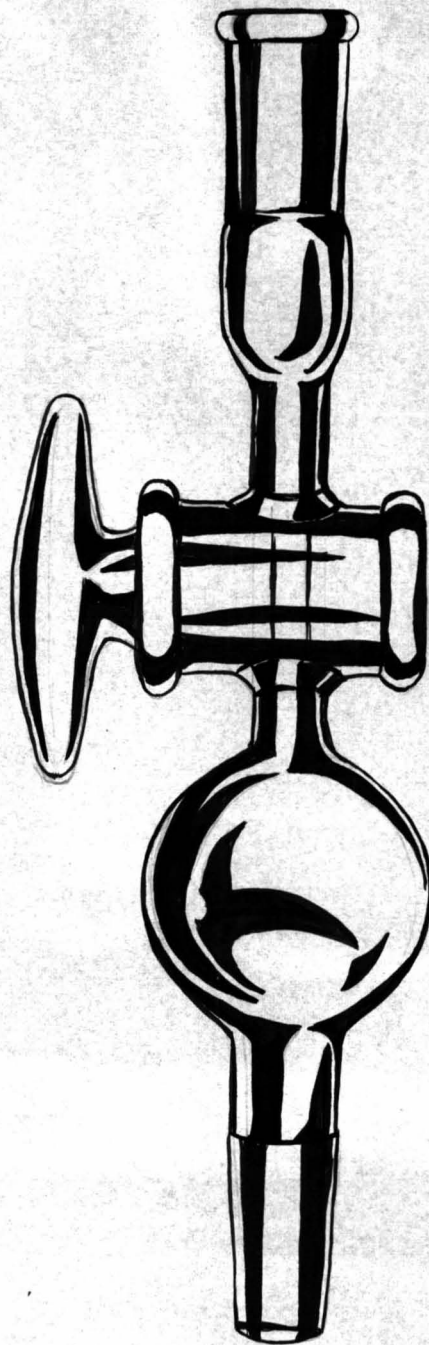


Fig. 5

in the side arm, thus distilling the remaining vapour back from the vacuum line into the side arm. This was continued until the pressure in the system was returned to its usual low value. The tap to the counting gas reservoir was then opened and the adaptor above the counter was filled with counting gas to a pressure one to two cm above the counting gas pressure already in the counter. The whole apparatus was then removed from the vacuum line and connected to the counting set up. To introduce the radioactive sample, the lower tap on the counter is opened and then the upper tap. As the counting gas pressure in the adaptor above the counter is greater than that in the counter, counting gas rushes into the counter, taking the radio active water sample with it. Distribution of the active sample within the counter was rapid and no diffusion effects were noticed after approximately one minute. These effects during this period were found to be insignificant in the results obtained.

In method II and ketone work, more active samples of water vapour were used and introduction of the whole sample contained between the two counter taps was too great for the scale of the rate meter used. High activities also result in high and inaccurate corrections for count loss during paralysis time. Thus endeavours were made to keep the initial activity in the counter to approximately 20000 counts/minute.

Two methods of introduction were used to achieve an activity of approximately this magnitude.

Method I

The sample enclosed between the two taps of the Geiger counter was divided between this space and the vacuum line i.e., after enclosing the sample and evacuating the vacuum line, the upper tap on the counter

was opened, allowing the sample to diffuse into the vacuum line. The tap was then closed and the remaining gas in the vacuum line returned to the HTO reservoir using liquid air. The portion remaining enclosed between the counter taps was then blown into the counter in the usual way.

Method II

The activity was blown into the counter by opening both counter taps very carefully and bleeding into the counter just sufficient sample of HTO to give the required initial activity. With care this method gave very satisfactory results and was used throughout the latter portion of the work.

TRANSFERRING OF HTO WITHIN THE SYSTEM

On numerous occasions it was necessary to transfer liquid or gaseous HTO samples within the system or to renew the HTO in the side arm reservoir. This was achieved rapidly and efficiently by surrounding the receiver with a thermos flask of liquid air and warming the container from which the HTO was being removed with a flask of warm water, thus causing distillation to the cooler receiver.

GREASING OF TAPS, JOINTS ETC.

All taps and joints permanently incorporated in the vacuum line were greased lightly with Apiezon "N" high vacuum grease, to ensure a high vacuum. Where greasing of other joints etc. was necessary (e.g. in attaching counters to the vacuum line for filling), Apiezon "L" vacuum grease was used.

BACKGROUND COUNTING

The natural "background" count i.e. the activity constantly present

from natural radiation, cosmic radiation etc, was determined prior to each of the different runs done. The background count rate was found and subtracted from each figure obtained during that particular run. The background figure was obtained by washing the counter to be used well with distilled water several times, rinsing twice with alcohol and removing the last traces of alcohol with a water pump. When the counter was dry, it was attached to the vacuum line and pumped to as low a pressure as possible. It was then filled with counting gas, to a pressure of ~ 11 cm and the background figure obtained by allowing the counter to count overnight prior to a run, with the counting rate being traced by the pen-recorder. The average count-rate over this period was taken as the background count for that particular run.

The washing of the counters with alcohol and water was found necessary, since evacuation of the counter tube is insufficient to obtain a return to normal background values, following the use of tritiated water samples. (32) This is due to activity adsorption on the counter walls etc. In spite of these cleaning procedures, as the counters received greater use, their backgrounds gradually became greater. Thus in some runs, higher backgrounds were recorded than in others. One counter, having a background of some 1000c/m was allowed to run overnight with a small flask of alcohol attached to the bottom. No decrease in background count rate was observed over a period of sixteen hours as would be expected were the activity causing the background count labile. Hence it was assumed that this higher activity was immobile in the counting tube and unlikely to affect the run following. It was shown in later work that

although it is desirable to keep the background activity as low as possible, counters with higher backgrounds could be used for a run without impairing the results, provided this background is known accurately by the method previously outlined.

DEGENERATION OF COUNTERS

After a period of constant use over several weeks, counters of both types used were found to gradually degenerate in their characteristics. Plateaus became very short and the counters very unstable, especially at high count rates. To avoid a heavy counter mortality rate by discarding the counter when this happened, attempts were made to clean the counters out and thus restore their original characteristics. The best method of doing this depended on the type of counter.

(a) In the case of the copper cathode counters, it was found best to fill these with 2N HNO_3 for 5 minutes, rinse well several times with distilled water, and wash with alcohol, removing the last traces of alcohol with a water pump.

(b) With the stainless steel counters, however, this treatment had little effect. It was found necessary to remove from the counter all traces of grease and paint and to heat them in an annealing oven to a temperature of 560°C , allowing them to cool over a period of about eight hours. Following this, the counters were pumped out for two hours and flushed well with counting gas. Following these respective treatments, the counters were found to have characteristics equal to and in some cases better than the original.

Initial counter instability was also experienced during early

work with counters sealed permanently to the vacuum line. This however, was found to be due to mercury vapour from the mercury diffusion pump leaking into the vacuum line and counters when a liquid air trap was not employed between the mercury pump and the vacuum line. Hence whenever it was necessary to fill a counter while the mercury diffusion pump was running, a liquid air trap was employed.

TECHNIQUE

The first method tried in the determination of active hydrogen in organic compounds consisted essentially of taking a very small, constant sample of tritiated water, counting a standard sample of it, allowing the remainder to react with a small sample of compound and then recounting a standard sample and measuring the decrease in activity due to exchange.

A very small sample of tritiated water was allowed to distill from the reservoir into a small side arm of the vacuum system, by the usual method of surrounding the receiver with liquid air and warming the reservoir. When about a drop had distilled over the reservoir and receiver were closed, after distilling back into the reservoir all excess water vapour from throughout the system. The small sample distilled over was lightly frozen with liquid air and surrounded by a flask of crushed ice/water for a period of two to three hours. It was then assumed that the sample was at 0°C . At this temperature the tap to the vacuum line was opened and tritiated water vapour at 0°C was allowed to escape into the vacuum line and counter. The counter was then isolated by closing the tap and the water vapour in the vacuum line returned to the sample tube using liquid air. When the pressure in the vacuum line had dropped to its usual value of about 5 microns, counting gas to a pressure of approximately 13 cm was allowed into the vacuum line. The tap to the counter was then opened, allowing counting gas into the counter. Filling the line with gas first had the effect of preventing any water vapour escaping from the counter when the tap was opened. The counter

was filled with counting gas to a pressure of 11 cm and the tap closed. The activity of the sample in the counter was then determined, the background of the counter having been determined previously. As the water vapour was always introduced from the sample holder into the vacuum line and counter at the same temperature (0°C) it was assumed that it was possible to meter into the counter a constant volume of water vapour whose activity could then be determined. The activity of the sample and the background count were obtained from the average of a series of ten one minute counts, suitably corrected for paralysis deviation where necessary. After obtaining these the counter was pumped out, flushed well with inactive water vapour and re-evacuated. The remaining tritiated water in the sample holder was then transferred to the small reaction flask shown in Fig. 6. This was attached to the vacuum line so that the teats hung vertically down and the flask could be revolved about this axis so that the teats could be successively brought into the vertical position. This flask had previously been carefully weighed and a small amount of a standard solution of the compound whose active hydrogen was to be determined, introduced into each teat. The flask was then placed in a dessicator and the water from the teats removed, leaving the crystalline test compound in the flask. The last traces of water were removed from the flask by pumping on the vacuum line until the pressure in the system no longer rose when the flask was opened to the line and the vacuum pump stopped. This was found to be the easiest method of introducing small amounts of material into the teats of the reaction flask.

The tritiated water was transferred successively from teat to



Fig. 6

teat by revolving the flask until one teat was hanging vertically and surrounding that teat with liquid air. This was done for each teat in turn, several times, each time allowing the water sample to equilibrate with the test compound in the teat over a period of fifteen minutes. The whole flask was then left overnight with the water sample distributed between the teats as evenly as possible. After a period of approximately sixteen hours, the whole flask was frozen lightly with liquid air and surrounded by crushed ice/water for an hour and the activity of a sample determined as before after obtaining the counter background again. After suitable correction, the activity in counts per minute was obtained, and the decrease in activity due to exchange with the test compound was obtained by subtraction of the second activity from the first. The number of active hydrogens in an unknown compound could then be determined by comparison of the activity decrease with the decrease due to a comparable number of gram-molecules of a test compound containing a known number of active hydrogen atoms.

Measuring the decrease in activity of the solvent rather than the increase in activity of the solute obviates the necessity of completely removing all the solvent from the compound, a factor which is difficult in many cases and which results in anomalously high activities in the compound if not done completely. This is avoided by this method; where the sample to be counted is obtained directly from the reaction flask.

EXPERIMENTAL

(a) Constant Sampling

Samples were metered out of the reservoir successively into the counter and counted for a period of ten minutes with the object of finding the accuracy to which repetitive samples could be measured out and counted. A table giving the results of the five runs performed is given below.

Background activity has been subtracted in each case.

<u>Run</u>	<u>Total Activity in ten minutes</u>	<u>Counts/min</u>	<u>Standard Deviation</u>
1.	60420	6042	28 c/m
2.	60930	6093	35 c/m
3.	61976	6198	51 c/m
4.	62674	6267	30 c/m
5.	61911	6191	38 c/m

Average Value 6158 c/m

Standard deviation 36 c/m

From the results obtained above it was shown that a sampling accuracy of $\pm 2\%$ could be obtained and this figure was assumed in all future runs.

EXPERIMENTAL DATA

Repetitive runs were done with three flasks containing oxalic acid, samples of which were introduced in aqueous solution from a pipette and the solvent removed at low pressure. These flasks were each of the teat-type as in Fig. 6.

The weight of oxalic acid sample (anhydrous) in each flask was as follows:

Flask	I	5.38 mgms
Flask	II	5.37 mgms
Flask	III	5.39 mgms.

and for the purposes of this run, it was assumed there was the same weight of sample in each flask. In each run the HTO sample was transferred successively from teat to teat and allowed to react with the oxalic acid in each. The flask was then left overnight and the activity in the HTO determined the following morning. The results of the three runs are summarized as follows:

	<u>Run I</u>	<u>Run II</u>	<u>Run III</u>
	Flask I	Flask II	Flask III
Counter Background	134 c/m	150 c/m	160 c/m
<u>Before Reaction</u>			
HTO Sample Count	16530 c/m	16759 c/m	16553 c/m
Plus Coincidence Correction	19290 c/m	19469 c/m	19303 c/m
Less Background	19156 c/m	19319 c/m	19143 c/m

After equilibration with oxalic acid sample for approximately twenty hours

	<u>Flask I</u>	<u>Flask II</u>	<u>Flask III</u>
Counter Background	140 c/m	143 c/m	190 c/m
HTO Sample Count	14367 c/m	14628 c/m	14486 c/m
Plus Paralysis	16497 c/m	16708 c/m	16576 c/m
Less Background	16357 c/m	16565 c/m	16386 c/m
<u>Decrease in Activity</u>	<u>2799 c/m</u>	<u>2754 c/m</u>	<u>2757 c/m</u>

Hence from the results of this run 5.38 mgm oxalic acid lowered the activity of the HTO sample by an average of 2770 c/m.

i.e. 7.24×10^{-2} millimoles of oxalic acid decrease the activity by 2770 counts/minute

where $\frac{\text{weight of oxalic used}}{\text{molecular weight}} = \text{millimoles}$

These runs were then repeated, this time using benzoic acid. The weights of benzoic acid used are given in the table below.

	<u>Flask I</u>	<u>Flask II</u>	<u>Flask III</u>
Weight of Benzoic Acid	10.01 mgm	10.05 mgm	10.04 mgm

The results of these runs were as follows:

	<u>Flask I</u>	<u>Flask II</u>	<u>Flask III</u>
Counter Background :-	200 c/m	185 c/m	193 c/m
HTO Sample Count :-	16653 c/m	16492 c/m	16329 c/m
Plus Paralysis :-	19383 c/m	19252 c/m	19139 c/m
Less Background :-	19138 c/m	19067 c/m	18946 c/m

After equilibration with benzoic acid:

Counter Background :-	203 c/m	206 c/m	197 c/m
HTO Sample Count :-	14985 c/m	14893 c/m	14784 c/m
Plus Paralysis :-	17785 c/m	17693 c/m	17574 c/m
Less Background :-	17582 c/m	17487 c/m	17377 c/m
Decrease :-	<u>1601 c/m</u>	<u>1580 c/m</u>	<u>1569 c/m</u>

Average decrease = 1583 c/m

10.03 milligrams benzoic give 1583 c/m decrease

$$= 8.20 \times 10^{-2} \text{ millimoles}$$

Comparing this with the 7.24×10^{-2} millimoles used in the experiments of oxalic acid

7.24×10^{-2} millimoles of benzoic would cause a decrease of

$$\frac{7.24}{8.20} \times 1583 \text{ c/m} = \underline{1398 \text{ c/m}}$$

Hence the ratio of active hydrogen atoms in benzoic acid to active hydrogen atoms in oxalic acid is $1398 : 2770 = 1 : 1.985$

or 1 : 2

These runs were then repeated, this time using phthalic acid and these results are tabulated as follows:

The weights of phthalic acid in each flask were:-

	<u>Flask I</u>	<u>Flask II</u>	<u>Flask III</u>
	5.02 mgm	5.01 mgm	5.05 mgm
Counter Background	247 c/m	210 c/m	225 c/m
HTO Sample Count	16300 c/m	16335 c/m	16576 c/m
Plus Paralysis	19130 c/m	19135 c/m	19316 c/m
Less Background	18883 c/m	18925 c/m	19091 c/m

After equilibration with phthalic acid for approximately twenty hours.

Counter Background	260 c/m	208 c/m	220 c/m
HTO Sample Count	14856 c/m	14798 c/m	14939 c/m
Plus Paralysis	17646 c/m	17588 c/m	17729 c/m
Less Background	17386 c/m	17380 c/m	17509 c/m
Decrease	<u>1497 c/m</u>	<u>1545 c/m</u>	<u>1582 c/m</u>
Average decrease	<u>1541 c/m</u>		

5.02 mgm causes a decrease of 1541 c/m

$$= 4.18 \times 10^{-2} \text{ millimoles}$$

7.24×10^{-2} millimoles would give a decrease of 2670 c/m

Hence the ratio of active hydrogen atoms in phthalic acid to active hydrogen atoms in oxalic acid is 2670 : 2770

or 1 : 1

DISCUSSION OF RESULTS

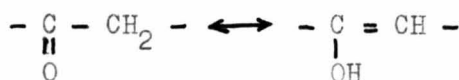
From the above results it seems that the number of active hydrogen atoms in a compound can be determined fairly readily by reference to the decrease in activity in comparison with a known compound, under conditions similar to those outlined in the above experiments. This type of experiment could be extended to include not only compounds which could equilibrate with tritiated water, but compounds which will equilibrate with tritiated ethanol and other activated solvents. With compounds containing relatively few active hydrogen atoms, great accuracy in counting and equilibration is unnecessary as the number of active hydrogen atoms must be in simple ratio to each other. It is only with more complex molecules that greater accuracy is required. This method also obviates the necessity of completely removing all traces of solvent from the test compound, an operation which is often difficult, if not impossible. In this method a standard sample may be metered directly from the reaction flask. Once the equipment is initially set up, determinations may be made rapidly and without destruction of the crystalline sample. During the course of the preceding experiments it was not possible to perform a determination on an unknown compound, and determinations were therefore carried out on well known organic compounds of known composition and known active-hydrogen content.

ACTIVE HYDROGEN DETERMINATION IN COMPOUNDS CONTAINING

A CARBONYL GROUP

INTRODUCTION

It was suggested to the author that perhaps much information about the environment of a carbonyl group in an organic compound could be gained by allowing it to exchange with HTO under suitable conditions. An organic compound containing a carbonyl group adjacent to a methylene group would presumably exchange through a keto-enol mechanism:



Keto-form

enol form

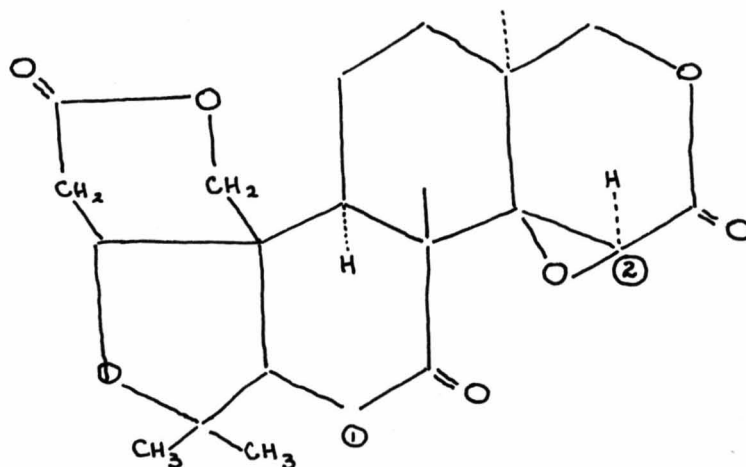
In the enol form the hydroxyl hydrogen atom is labile and undergoes immediate exchange with the hydrogen atoms of HTO. Hence if the enol form is readily accessible to the organic compound, the tritium could be rapidly incorporated in the compound, in a manner characteristic of the environment of the activating carbonyl group. Hence it may be possible, by allowing an organic compound containing a carbonyl or similar activating group to exchange with HTO, to determine the nature of the two groups flanking the activating group.

e.g. in a compound such as $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOEt}$ the $[-\text{CH}_2-]$ and $[\text{CH}_3-]$ groups would presumably have different rates of exchange due to their different molecular environments.

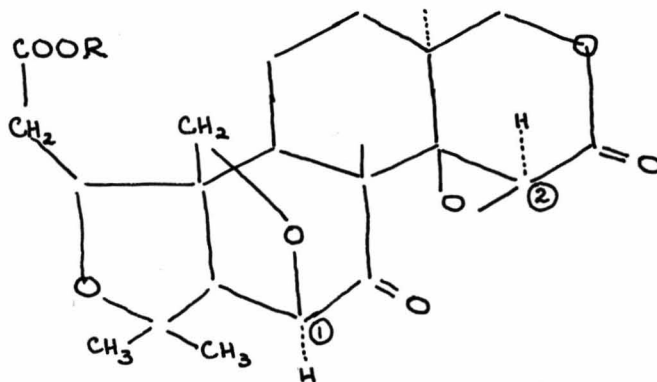
The $-\text{CH}_2-$ group could be expected to exchange more rapidly than the CH_3- group as the former is situated between the two activating carbonyl groups, while the CH_3- group is adjacent only to the single carbonyl activating group, the carboxyl group exerting no activating

influence.

In addition, the amount of exchange in each group should be proportional to the number of hydrogen atoms in that group, i.e. in the compound illustrated on Page 30, ethyl acetoacetate, there should be two rates of exchange, in the ratio of 3 : 2, corresponding to exchange in the methyl and methylene groups respectively. Barton, Cory, Jeger et. alia (33) have noted that



rapidly absorbs two atoms of deuterium, a third more slowly under alkaline conditions at room temperature but that



no longer exchanges rapidly.

This apparently shows that the methylene group in the four membered ring adjacent to the carbonyl exchanges rapidly with deuterium, presumably through a keto-enol mechanism. The slower exchange apparently takes place at either position I or II, neither of which is affected by ring opening.

METHOD

The apparatus used in work on the carbonyl exchange was as shown in Plates 1, 2 and 3. A small 25 ml conical flask, with the neck made from a B₁₉ Quickfit socket, and containing the sample, which in the first few trials consisted of a liquid ketone, was attached to the base of a

geiger counter of the type shown in Fig 4. To the top of the counter was attached the filling apparatus shown in Fig 5. The liquid sample was then frozen using liquid air, and the whole apparatus attached to the vacuum system. Following outgassing of the sample the counter was filled with counting gas to a pressure of 11 cm. and the counter background was determined. In order to ensure that the sample was kept a constant temperature throughout the experiment, the flask was immersed in crushed ice/water contained in a Dewar flask. This was replenished as necessary throughout the experiment, thus ensuring that each experiment was carried out at the constant temperature of 0°C.

To keep the background of the counting setup to a minimum the counter during the experiment was surrounded with lead bricks.

Having run the counter at background activity for approximately one hour, and determined the background, the counter was again attached to the vacuum system, and after tap A in Plate 2 had been closed, the system was pumped out and a small sample of HTO vapour was introduced and contained between taps A and B of the counter. The filling apparatus was then filled with counting gas to a pressure approximately 1.2 cm above that in the counter. The apparatus was then removed from the vacuum line and the leads from the counter terminals were attached to the counting assembly. Taps A and B were then opened simultaneously and the rush of gas from the filling apparatus into the counter carried with it the HTO vapour. In this way the HTO vapour was introduced rapidly and smoothly into the counting space of the counter. The counter voltage was adjusted immediately to 150 volts about threshold and the activity, instead of being counted on a scaler unit as previously, was fed into a ratemeter set at a time/base

-33a-

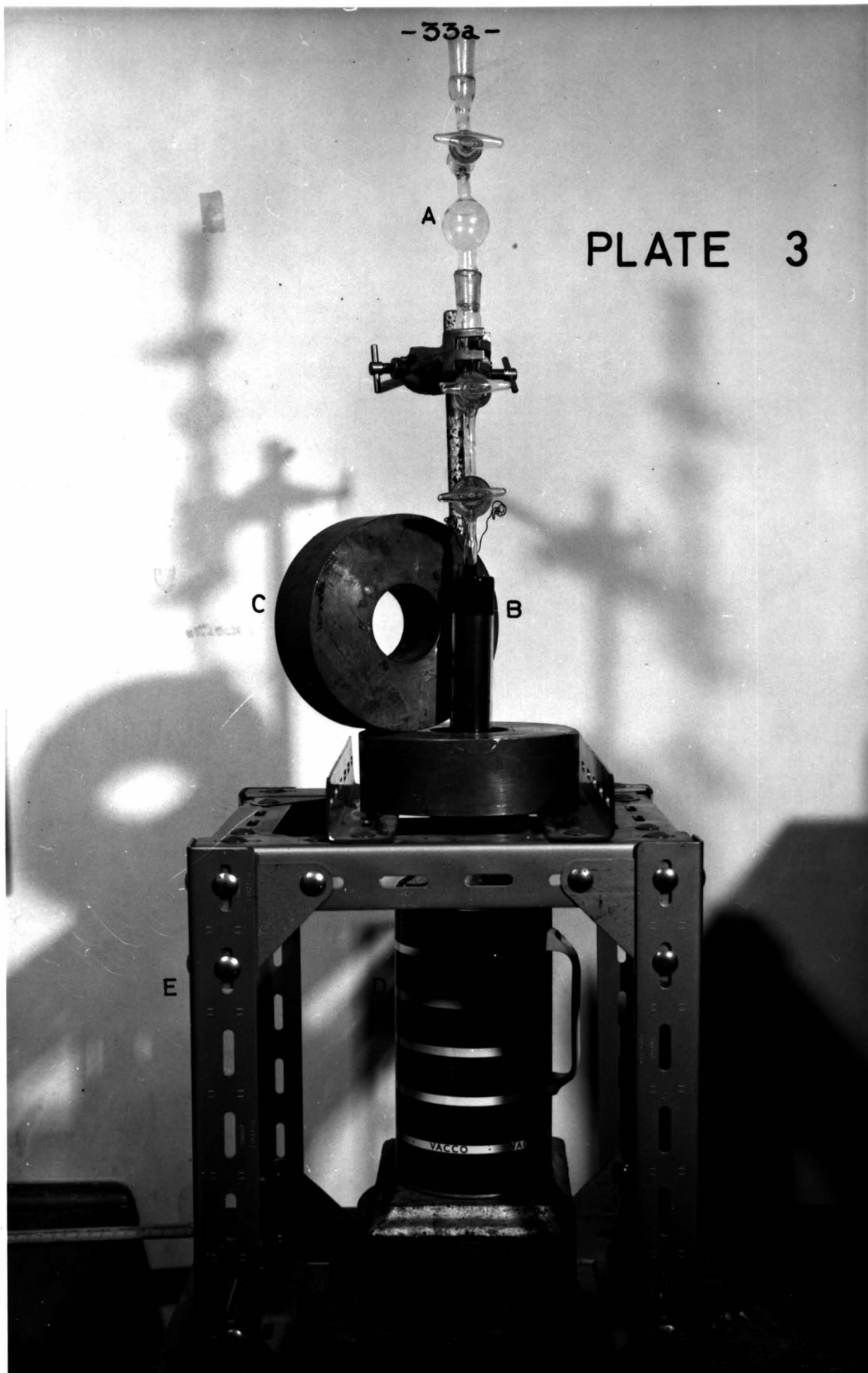
A

PLATE 3

C

B

E



KEY TO PLATE 3

- A Counting gas impellor
 - B Geiger counter tube
 - C Lead-shielding brick
 - D Dewar flask for maintaining constant temperature
 - E Equipment chassis
-

constant of 100 seconds. The output of the ratemeter was in turn fed onto a recorder, thus producing a continuous pen-trace of activity versus time.

As the activity was incorporated into the sample in the flask on the base of the counter, it is of course, removed from the counting space of the counter and hence the count rate on the meter falls with time. Hence a plot of activity versus time consists of a long curve, falling gradually until it reaches a steady value. The chart, when activity had reached a steady value, was removed from the recorder. The activity at various time intervals was noted, corrected for paralysis and background, and replotted on 3-cycle logarithmic graph paper, as shown in Figs 7 to 8. On subtracting from each value on the graph, the final steady value to which the graph fell, a second plot was obtained, which can be shown to be a curve falling to a continuous straight line after an interval of time. The values of this straight line are then subtracted from the initial curve to give another plot and this process is repeated until the whole plot is broken down into a series of straight lines of different slopes and different intercepts on the axes. It was possible to show that for each different type of active hydrogen exchange, there is a corresponding straight line appears on analysis of the plot.

It was hoped that it would be possible to show that the various intercepts and line slopes were related to the mode, rate and extent of exchange of each kind.

With this object in mind, the following mathematical treatment of the plot was performed, and showed that the number of lines produced from the plot indeed corresponded to the number of different types of exchange, that the plot should indeed fall to a constant value, and that

the intercepts were related to the amount of the corresponding exchange. It also showed that each exchange type could be represented by a straight line, and if these were successively extracted from the total plot, the plot itself could be broken up into a number of straight lines, the number being equal to the number of different types of exchanging hydrogen.

MATHEMATICS OF EXCHANGE

- (i) Total water vapour in counter and flask = A gm
- (ii) Total volume of counter = V ccs
- (iii) Sensitive volume of counter = v ccs

$$\text{Water in sensitive volume of the counter} = \frac{v}{V} \times A.$$

If it is considered that there are two types of labile hydrogen in the compound, with different exchange rates

- (iv) then: the amount of the more labile hydrogen = B
- (v) and the amount of the less labile hydrogen = C
- (vi) The initial activity in the sensitive volume of the counter (i.e. the activity at the moment of entry of the activated water sample) = T_0
- (vii) The activity at any time t in the sensitive volume = T

Now the slope of the curve obtained from the plot is equal to the rate at which the activity is leaving the sensitive counting space of the counter, i.e. the rate at which the activity is being incorporated in the sample.

$$\frac{dT}{dt} = \frac{RT}{A} - \frac{R(T_0 - T)}{B} \quad \text{where R is the specific rate}$$

constant for the decrease in activity

$$\frac{dT}{dt} = T \left(\frac{R}{A} + \frac{R}{B} \right) - \frac{RT_0}{B} \quad \text{_____} 1.$$

and this expression is of the form $\alpha T + \beta$ where α and β are arbitrary constants, and may thus be integrated as such

$$\alpha = \left(\frac{R}{A} + \frac{R}{B} \right)$$

$$\text{and } \beta = - \frac{RT_0}{B}$$

Integration of this expression gives:

$$\frac{T}{T_0} = \frac{A}{A+B} + \frac{B}{A+B} \frac{-R(A+B)t}{AB} \dots\dots\dots 2.$$

Hence it can be seen that this expression consists of an exponential term plus a constant, and thus showing that the plot indeed consist of an exponential curve falling to a constant value.

Let this constant = $Z = \frac{A}{A+B}$ and this will be the value at $t = \infty$ Subtracting Z, i.e. the final falloff value or value at $t = \infty$ of the plot, from the integrated expression, leaves the exponential term,

$$\frac{T}{T_0} = \frac{B}{A+B} \frac{-R(A+B)t}{AB}$$

which gives an exponential curve.

The graph of $\log_e T$ versus t is now prepared and shows a curve falling to a steady value. This may be subtracted from the rest of the curve, the resultant graph being a straight line. As the falloff value $Z = \frac{T_0 A}{A+B}$ the straight line corresponds to the exponential part of equation 2.

This line cuts the $t = 0$ axis at y and $Z = \frac{AT_0}{A+B}$ has been subtracted from the curve.

If an exponential curve is obtained, this is exhaustively treated as above until it is reduced to a series of straight lines, with different intercepts on the $t = 0$ axis. In the course of this work, only two modes of hydrogen exchange were ever obtained, and the corresponding three straight lines have the intercept values z , y and x on the ordinate axis.

Hence z is the final falloff value on the ordinate, y is the

intercept on the ordinate of the less labile hydrogen and x is the intercept on the ordinate of the more labile hydrogen. Had there been more modes of hydrogen exchange, there would have been correspondingly more lines in the plot and more intercepts.

Now for the more labile hydrogen:-

$$\frac{x}{T_0} = \frac{B}{A+B} \quad \text{_____} \quad (i)$$

and for the less labile hydrogen:-

$$\frac{y}{T_0} = \frac{C}{A+B+C} \quad \text{_____} \quad (ii)$$

But in evaluating (ii) one counts only A counts out of $A+B$ as an amount of activity = B has already been incorporated in the compound. Hence the counts in (ii) must be increased by a factor $\frac{A+B}{A}$

$$\frac{y}{T_0} \cdot \frac{(A+B)}{A} = \frac{C}{A+B+C} \quad \text{_____} \quad (iii)$$

Hence from (i) and (iii) one can, by suitable rearrangement of the terms, obtain an expression for $\frac{B}{C}$ in terms of x , y , z and T_0 , all of which are known from the graph obtained experimentally from the run.

Thus one is able to find the ratio of:-

the labile hydrogen in the less labile group to the labile hydrogen in the more labile group.

This is found to be:

$$\frac{C}{B} = \frac{\left(\frac{y}{T_0 - x} \right) \cdot \frac{T_0}{x}}{1 - \left(\frac{y}{T_0 - x} \right)}$$

but as $T_0 = x + y + z$

(i.e. total activity = activity in more labile group plus activity in less labile group + final activity at the end of the run), the expression may be rewritten in its most simple form as

$$\frac{C}{B} = \frac{yT_0}{xz}$$

and it was in this form that ratio values for exchanging groups were calculated in the following runs.

As the numbers of exchanging hydrogen atoms in the groups must be in simple numerical ratio to one another, extreme accuracy in calculation of experimental values is not required. This was especially so in this work, where only two labile groups were ever present in one compound. However in compounds of greater complexity containing greater numbers of exchanging hydrogen groups, more careful evaluation would be necessary, especially where the structure or number of labile groups was undetermined.

EXPERIMENTAL

In order to test some of the foregoing suppositions and theories, a trial run was performed as outlined previously, using as a check compound, ethyl acetoacetate.

19.7 mgms of ethylacetoacetate (2 drops) were placed in the bottom of a small soda-glass flask and the flask attached to the base of the counter. The compound was frozen and the counter and flask evacuated. After several outgassings of the trial compound, counting gas was fed into the counter and its background determined. Activated water vapour was then fed into the counter and the output of the counting device fed on to the recorder. The experiment was allowed to proceed for forty-two hours, after which time no further exchange could be observed.

After suitable correction for background and counter dead-time, a plot on logarithmic graph paper was made of activity against time.

As predicted from previous theory, by suitable analysis of the resulting plot, two distinct straight lines were obtained, presumably due to the exchange of the two groups $[-CH_2-]$ and $[CH_3-]$. The exchange half-times of these two modes of exchange were 32 minutes and 185 minutes respectively.

As this was in the nature of a trial run, no plot was kept of the run and the figures obtained were used only as an indication that the general techniques and theory behind the experiment were correct.

The experiment was repeated several times under more closely controlled conditions and a closer study was made of the results.

Several attempts to repeat this experiment gave anomalous results, with sometimes only one mode of exchange being observed and that was both faster and more extensive than expected. No constructive results were

obtained from these runs and it was suggested that the ethylacetoacetate sample might be contaminated with some proton labile impurity. It was noted that the ethylacetoacetate sample used during these experiments was obtained from a different source, to that used in the initial check experiment.

PURIFICATION OF SAMPLE

In order to check the purity of the ethylacetoacetate used, a gas chromatogram was run on a small sample of the compound. The flow gas used was argon at a rate of 30cc/minute and the immobile phase 10% Apiezon "M" grease on celite. The results of this showed one large positive peak, due to the ethylacetoacetate, and a smaller negative peak, presumed to be an impurity, possibly water. In order to remove this impurity, the ethylacetoacetate was distilled at a vacuum of 9 mm, the fraction distilling between 66°C and 70°C collected and dried over calcium chloride as outlined in Vogel (34).

Two further runs were performed on the purified ethylacetoacetate, with the following results. In order to allow the counter to settle down and reach equilibrium at the commencement of each run, no readings were taken over the first half hour. T_0 was calculated from $x + y + z = T_0$.

EXPERIMENTAL DATA

Ethyl acetoacetate Run II

Weight of Ethyl acetoacetate = 5.33 mgms

Counting Gas Pressure in Counter 1.23 cms

Voltage 2100 volts

Background 517 counts/minute

Time (Hours)

* Count Rate (counts/minute)

0.50 26150

0.75 19640

1.00 15700

1.25 12830

1.50 10850

1.75 9550

1.00 8250

2.50 6660

3.00 5570

3.50 4870

4.00 4450

4.50 4125

5.00 3920

5.50 3810

6.00 3720

6.50 3665

7.00 3630

8.00 3600

9.00 3580

Equilibrium 3420

after 10 hours

* Corrected for background and coincidence

Half times of
exchange 25 minutes
and 128 minutes

From the corrected results were subtracted (i) the equilibrium activity count z , resulting in the isolation of the straight line presumably due to the CH_3 - group exchange, intercept y (ii) by subtraction of the values of the first line y , a second straight line with intercept x was isolated.

<u>Time Corrected</u>	<u>Count Rate</u>	<u>-Value at Equilibrium</u>	<u>-Values of line y</u>
0.5	26150	22730	19330
0.75	19640	16220	13150
1.0	15700	12280	9550
1.25	12830	9410	6980
1.50	10850	7430	5230
1.75	9550	6130	4170
2.0	8250	4830	3070
2.50	6660	3240	1840
3.0	5570	2150	1040
3.5	4870	1450	550
4.0	4450	1030	310
4.5	4125	705	125
5.0	3920	500	
5.5	3810	390	
6.0	3720	300	
6.5	3665	245	
7.0	3630	210	
8.0	3600	180	
9.0	3580	160	
Equilibrium	3420	-	

From the ensuing graph the following values were obtained:

$$T_o = x + y + z = 34120 \text{ counts/minute}$$

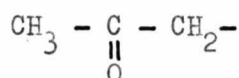
$$x = 26500 \text{ c/m}$$

$$y = 4200 \text{ c/m}$$

$$z = 3420 \text{ c/m}$$

$$\text{Hence } \frac{C}{B} = \frac{yT_o}{xz} = 1.58$$

Hence the ratio of exchange in the slower group **C** $[\text{CH}_3-]$ to the exchange in the faster group B $[-\text{CH}_2-]$, is 1.58 : 1 or 3.16 : 2. Hence the ketone group responsible for the activation causing the exchange is flanked by two proton-labile groups in the ratio 3 : 2. Hence the partial structure of the compound must be :-



In order to confirm the results obtained during this run, a further run using ethyl acetoacetate was performed under similar conditions. In graphing the results (see Fig 7) the initial values on the run, i.e. those over the first hour, are omitted for convenience. In the original plot, they were plotted on a separate sheet.

ETHYL ACETOACETATE RUN III

Weight of ethyl acetoacetate = 7.26 mgms

Counting gas pressure in the counter = 11.8 cm

Voltage 2150 volts Background 232 counts/minute

<u>Time (Hours)</u>	<u>Corrected Count</u>	<u>- Equilibrium Value</u>	<u>- Values of y Line</u>
0.33	14700	13605	12475
0.66	9580	8485	7485
1.00	6410	5315	4375
1.33	4890	3795	2933
1.66	3765	2670	1887
2.00	3005	1910	1170
2.33	2665	1570	885
2.66	2265	1170	535
3.00	2016	921	341
4.00	1727	632	172
5.00	1553	458	98
6.00	1422	327	
7.0	1328	233	
8.0	1276	181	
9.0	1266	171	
10.0	1205	110	
11.0	1174	79	
12.0	1161	66	
13.0	1151	56	
14.0	1137	42	
Equilibrium value	1095		

$T_0 = 21560$
 $\chi = 19500$

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ETMYL ACETO ACETATE / HTO
RUN III

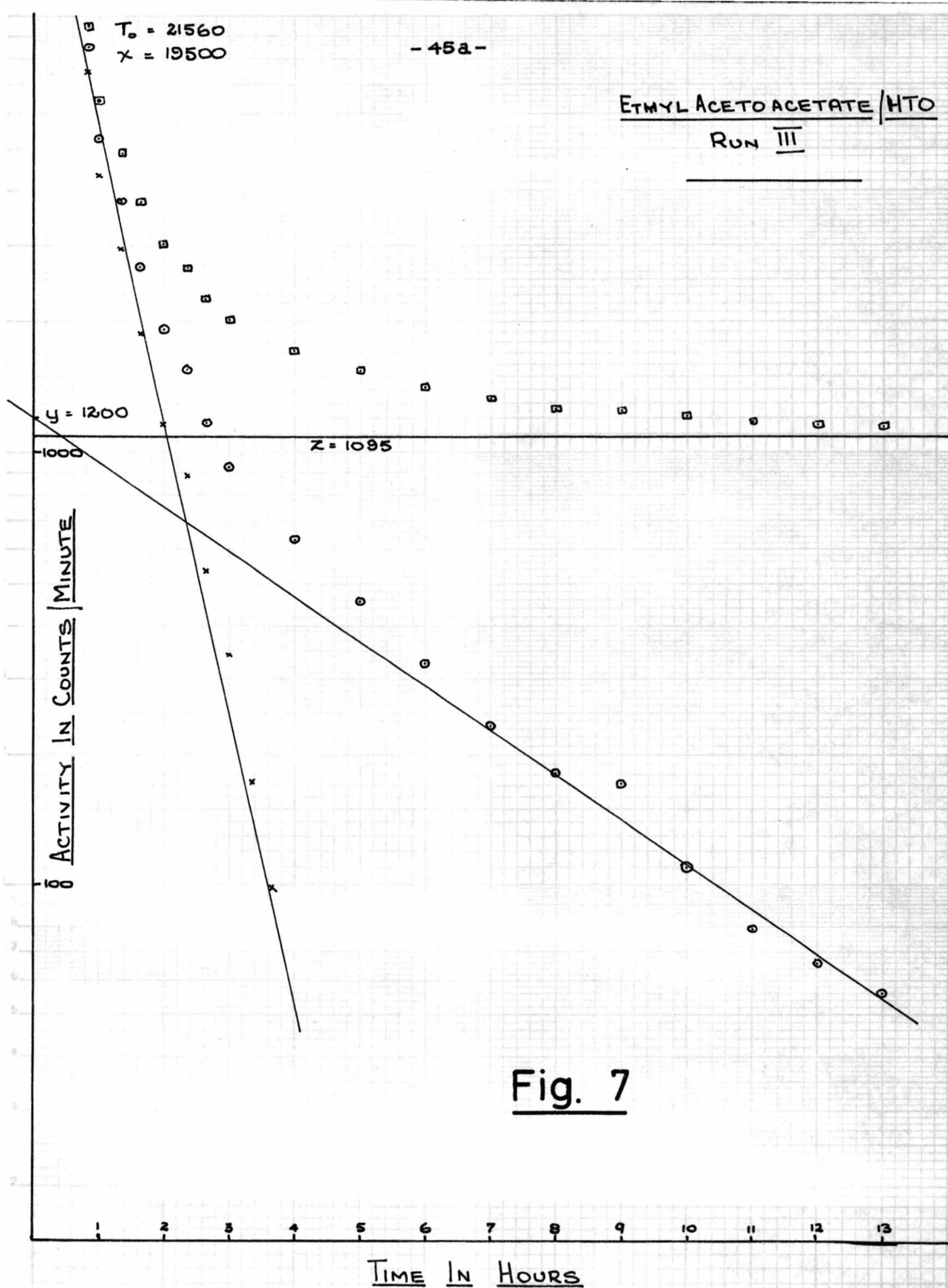


Fig. 7

From this run the following results were obtained:

$$T_o = x + y + z = 21563$$

$$z = 863$$

$$y = 1200$$

$$x = 19500$$

$$\frac{C}{B} = \frac{yT_o}{xz} = 1.53$$

The ratio of exchange in the slower (CH_3 -) group to the exchange in the faster ($-\text{CH}_2$ -) group is 1.53 : 1 or 3.06 : 2. confirming the results obtained by the previous run.

As a point of interest, this run was recalculated using a value of 963 counts/minute for z instead of the correct value of 863. This gave a ratio of 2.77 : 2 instead of 3.06 : 2, thus exphasizing the importance of knowing accurately the equilibrium value of the run. To obtain this during these experiments, the run was allowed to proceed until a steady count-rate was obtained over a period of 5 - 6 hours. This was then taken as the equilibrium value z.

Two further runs were performed, this time using acetyl-acetone

$\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{CH}_3$ as the test compound.

In each case, three distinct straight lines were obtained from the run plot, and it was not possible to obtain the theoretical ratio of exchanging groups from these lines. The evidence points to contamination of the acetyl acetone with some other hydrogen labile compound and this theory was supported by gas-chromatographic analysis, which showed two distinct peaks, some distance apart. The details of the gas chromatography column

were the same as for the analysis of ethyl acetoacetate.

Further runs were performed using methyl ethyl ketone

$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{CH}_3$ and mesityl oxide,

$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C} = \text{CH} - \text{CO} - \text{CH}_3 \\ \diagup \\ \text{CH}_3 \end{array}$ the latter compound being used to ascertain

whether any exchange occurs in the $\text{C} = \text{CH} - \text{CO} -$ grouping.

It was presumed unlikely that this would exchange as it would assume

a temporary allene-type configuration $\begin{array}{c} \diagdown \text{C} = \text{C} = \text{C} - \\ \diagup \quad \quad \quad | \\ \quad \quad \quad \text{OH} \end{array}$

during exchange which appeared unlikely.

In spite of several attempts to resolve these compounds, each run had to be abandoned due to the instability of the counting tubes being used in the runs. It was assumed that the reason for the instability, was that the vapour of the compound under examination, was rising from the sample holder on the counting tube base and entering the sensitive region of the counting tube. Here it interfered with the quenching of the counters or the filling gas of the counters and caused them to become unstable and on several occasions, to go into continuous discharge. Thus by the method outlined, it is apparent that the only ketonic compounds able to be determined by this method, would be liquids, traces of whose vapours have no effect on the characteristics or behaviour of the counting tubes and quench gas being used.

ANALYSIS OF SOLID KETONES

Early in work on this subject, it was realized that the methods outlined previously were restrictive in that they were applicable only to liquid ketones and from the above results it is apparent that the method is restricted even further to liquids whose vapours do not interfere with the stable operation of the counter. It was quickly apparent, that for this method to be applicable to ketones in general, it must be capable of adaption for analysis of solid ketones. To do this, it was apparent that a solvent of some kind would be necessary. This solvent would need to possess the following properties:

- (i) It would need to be a good solvent to ketone compounds as it would be desirable to keep the amount of solvent to a minimum.
- (ii) It should either have a very low vapour pressure or its vapours must have no effect on the operation of the counting tube.
- (iii) The solvent must contain no labile hydrogen in its molecular structure.

Several solvents were tried by placing a small sample in the counter flask, determining the background and then introducing a small sample of HTO vapour and following the course of the uptake, if any, of the HTO by the solvent. Among the solvents tried under these conditions were benzene, carbon tetrachloride, chloroform and pyridine. These solvents were also checked to see if the counter would operate satisfactorily using the solvent vapour only as a quench gas in the counter.

Of the solvents tried, it appeared that carbon tetrachloride and chloroform were unsuitable in that their vapours apparently had serious

effects on the counting stability of the geiger tubes. The counters were unstable, both when the usual quench gas was present, and when the counters were run with just the solvent vapour as the quench gas.

However, with benzene, the counter operated satisfactorily at 11 cm counting gas pressure in the tube and pyridine gave similar results at similar pressures. The operating voltage with benzene rose considerably above the usual 1950 volts and for the runs performed with benzene it was found that the operating voltage was just above the maximum obtainable from the power source used.

This was overcome by running the earth terminal of the counters at a negative potential of 180 volts through the use of two ninety volt dry-cell accumulators connected in series. In this way, the maximum potential difference between the counter terminals was raised to approximately 2700 volts, enabling runs using benzene, with a threshold voltage of approximately 2500 volts, to be carried out satisfactorily. Using Pyridine as a solvent, however it was found that the threshold voltage was raised only about 100 volts, to approximately 2050 volts, and so use of the above procedure was unnecessary. Initial attention was placed on the use of benzene as a solvent.

BENZENE AS A SOLVENT

A run was tried using benzene in the sample flask and blowing in the HTO sample with 9.5 cm of counting gas. The flask was surrounded with crushed ice/water at 0°C during the run but at this temperature it was found that the benzene, with a melting point of 4°C, was partially solid. However, it was found that the laboratory in which the work was carried out, did not suffer greatly from temperature variation, due to

good insulation. The temperature was found to remain fairly constant over a period and further runs were performed without insulation or constant temperature devices surrounding the sample flask.

A run was performed at 22°C with 250 mgm benzene in the flask, and resulted in very little falloff in activity due to incorporation in the benzene. The counter had a plateau of 260 volts during this run and the threshold became stable at 2480 volts within five minutes of the beginning of the run. Hence it was apparent that the benzene vapour in the sensitive region of the counter, had no adverse effects. Consequently it appeared that benzene might be a good solvent for use with compounds containing polar groups, -OH etc. e.g. steroids which have little solubility in some other organic solvents.

TESTOSTERONE IN BENZENE RUN I

32.5 mgms of testosterone was dissolved in 1.2153 gms of benzene (A.R. grade) previously dried over anhydrous sodium sulphate. A previous run with a similar amount of benzene only in the flask had shown that the loss of activity from reaction with the benzene was almost negligible. The HTO sample was blown into the counter with 10 cm of counting gas and the subsequent decrease in activity plotted and analysed in the usual way. The resultant curve was resolved into two straight lines (see Fig. 8) with exchange half times of 24 minutes and 344 minutes respectively.

From this run the following values were obtained.

x 5260 c/m

y 1750 c/m

z 1280 c/m

To 8290 c/m

$$\frac{C}{B} = \frac{yTo}{xz}$$

$$\frac{C}{B} = \frac{2.15}{1}$$

This showed the ratios of exchanging hydrogens was 2 : 1 and this can be related to exchange involving the hydrogen atom of the -OH group at C₁₇ and the two hydrogen atoms at C₂, activated by the ketone group at C₃.

The middle portion of this run, i.e. 8 hours onwards, was lost due to the counter falling off its plateau during a weekend. The final value of 1280 counts/minute was obtained after 3 days.

<u>Time (Hours)</u>	<u>Corrected Count</u>	<u>Equilibrium Value</u>	<u>Values of γ Line</u>
0.33	5880	4600	2900
0.66	4535	3255	1605
1.00	3895	2615	1015
1.33	3460	2180	640
1.66	3160	1780	290
2.00	2930	1650	190
2.33	2754	1474	84
2.66	2641	1360	
3.00	2603	1320	
3.33	2551	1270	
3.66	2508	1220	
4.00	2470	1190	
5.00	2340	1060	
6.0	2240	960	
7.0	2145	865	
8.0	2065	785	
Equilibrium Value	1280		

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TESTOSTERONE / BENZENE / HTO
RUN I

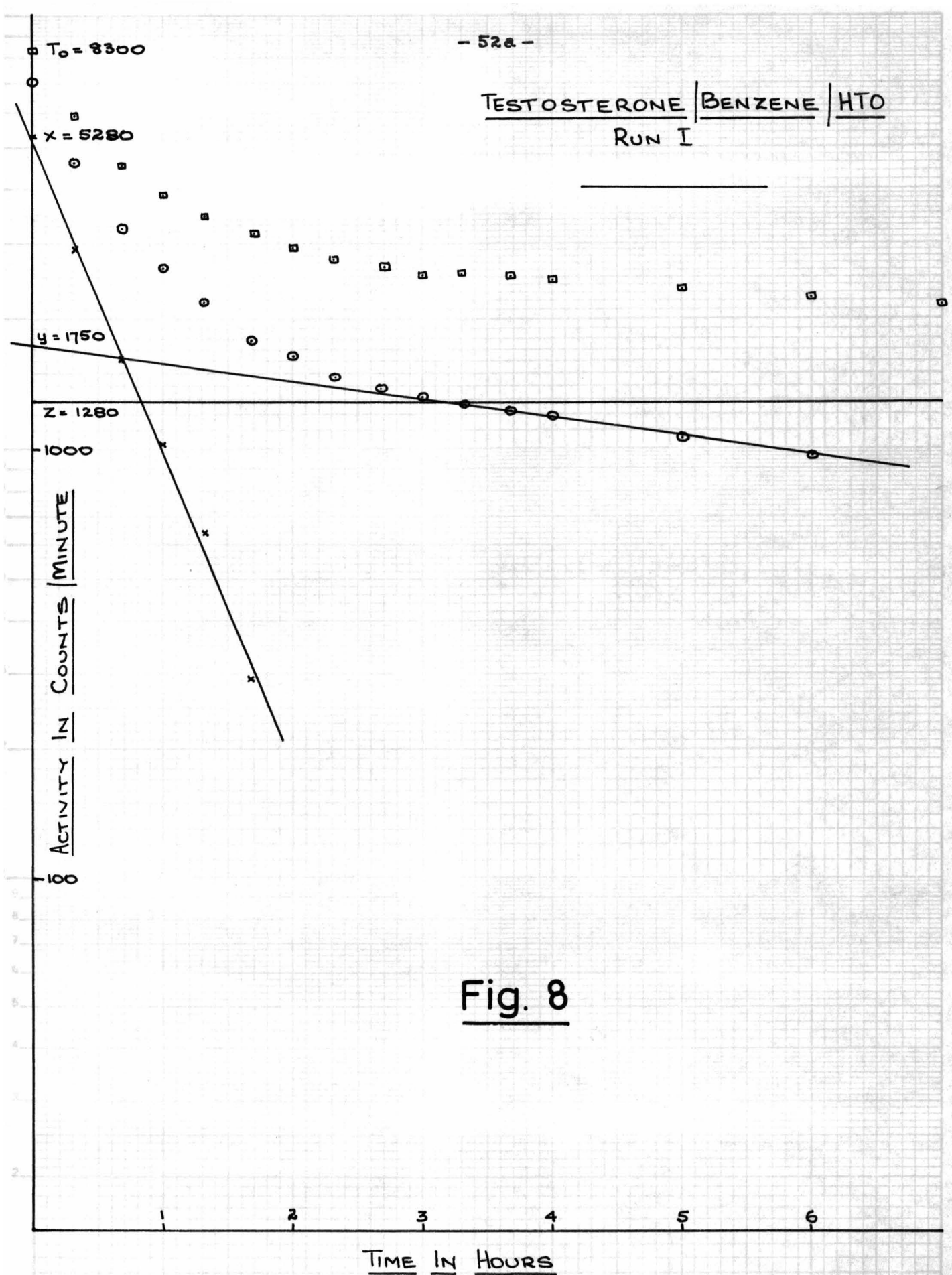
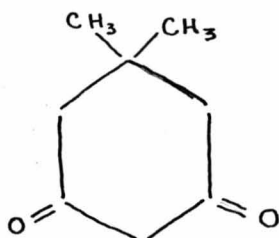


Fig. 8

PYRIDINE AS A SOLVENT

Pyridine was also tried as a solvent. A.R. Grade pyridine was redistilled twice and dried over anhydrous sodium sulphate. 1 gm was placed in the flask and a blank run done with HTO. Very little falloff due to incorporation of the activity in the pyridine was noticed and hence a further run was performed using pyridine as the solvent for dimedone



Unfortunately results and figures for this run were lost but the values obtained from the run were as follows:

Weight of dimedone = 35.2 mgm

Weight of pyridine = 1.053 gm

$$x = 5600$$

$$y = 2300$$

$$z = 1900$$

$$T_o = 9200$$

$$\frac{C}{B} = \frac{yT_o}{xz} = \frac{2300 (9200)}{5600 (1900)} = \frac{1.99}{1}$$

$$\text{or } 2 : 1 \quad \underline{\underline{\frac{C}{B} = \frac{2}{1}}}$$

The actual ratio is 4 : 2 of course but this cannot be ascertained from these experiments and is recorded as 2 : 1

The half times of exchange obtained in the two runs on ethylacetoacetate were respectively 25 minutes and 158 minutes, and 27 minutes and 175 minutes and the half times of exchange for testosterone in benzene were 24 minutes and 344 minutes respectively. In addition, although the results of the dimedone run were lost, the half time of the first exchange was in the order of 25 - 28 minutes. From the results of these runs, it appears that the half-time of exchange of the more labile hydrogen is essentially constant, although the half times of the less labile hydrogens varied considerably. This is apparently due to the fact that in each case, the more labile hydrogen was either part of a very activated methylene group, or was a phenolic or alcoholic hydroxyl hydrogen. The environment of the less labile hydrogen however, varied considerably with the various types of compound used.

However, it also seemed certain that the surface area of the exchanging medium and also possibly the rate at which the HTO vapours could diffuse from the sensitive region of the counter to the exchanging media in the flask, would be factors governing the exchange half times. The rate of diffusion of HTO was dependant on the diameter of the socket joining the flask to the counter.

Runs were performed, using ethylacetoacetate in each case, with a counter and flask having a wider orifice through which the HTO vapour could diffuse. In each case the half time of exchange of the more labile hydrogen was noted.

EXCHANGE HALF TIMES

	<u>Run I</u>	<u>Run II</u>
Ordinary counter and flask	27 minutes	25 minutes
Counter and flask with enlarged orifice	26 minutes	27 minutes

From the tabulated results, it can be seen that the times of exchange were independent of the orifice size between counter and flask and hence this is not a determining factor in the exchange half time.

To determine the dependence of half time on surface area, two runs were done using tubes of different diameter to contain the ethyl acetoacetate. The surface area of the exchanging liquid in each case was calculated from the internal diameter of the tube.

	<u>Exchange Half time</u>	<u>Tube Diameter</u>	<u>Surface Area</u>
(i) Narrow tube	85 minutes	0.25 cm	0.786 sq cm
(ii) Wide tube	39 minutes	0.56 cm	1.76 sq cm
Ratio of (ii) to (i)	2.18		2.24

In each case the more labile hydrogen exchange time was studied.

DISCUSSION OF RESULTS

These results show conclusively that the exchange half times are dependant on the surface area of the exchanging media.

The slower rate of exchange (39 minutes) obtained in the run using the wider tube, compared with runs done in a flask (approximately 27 minutes) confirm that the surface area of the exchange media in the flask was greater than that of the wide tube.

The fact that in all runs performed using the flask, the exchange half times of the more labile hydrogen, were approximately the same, was due to the surface area of the liquid exchange media being almost identical in each case. Although in the case of testosterone in benzene, the volume of solvent used was far greater than in previous runs and similarly for the dimedone in pyridine run, the shape of the flask limited the surface area of the solvent to approximately that of the earlier runs, thus resulting in similar exchange half times for the more labile hydrogen.

The widely differing exchange half times for the less labile hydrogen atoms is apparently due to the widely-differing environments of these hydrogen atoms within the respective compounds. The rate of exchange of these atoms is necessarily dependant on the ease with which exchange can take place and this in turn is dependant on its position within the compound and its activation. Thus with the widely differing compounds used, it is not surprising to note the widely differing exchange half times for the less labile hydrogen atoms in these compounds.

ACTIVE HYDROGEN DETERMINATION IN ORGANIC COMPOUNDS

METHOD II

DISCUSSION

It was earlier realized that the failure of several of the solvents used in previous runs, was due to their vapours entering the sensitive region of the counter, and thus causing instability of the counter. This condition can obviously be overcome by using a solvent with extremely low vapour pressure. The first such solvent tried was medicinal paraffin and it was decided to test this solvent from the point of view of its low vapour pressure and also to ascertain its active or labile hydrogen content, which in order to make the compound suitable as a solvent in hydrogen exchange studies, must be as low as possible. It was realized at the time that this compound would be, in general, a poor solvent of most organic compounds, but it was decided to find out if enough of any compound could be dissolved in it to carry out a determination.

EXPERIMENTAL

Accordingly a blank run was performed with the counter flask containing 1.179 gm of paraffin. Activated HTO vapour and counting gas were introduced in the usual manner after determination of the counter background. It was expected that any exchange between the HTO and the paraffin would be very slow and hence a small bar magnet was placed in the bottom of the flask, and the flask placed between the poles of a magnetic stirrer, thus enabling the paraffin to be stirred throughout the run (See Plate 4). The paraffin was out-gassed before commencement of the run, and stirring also greatly facilitated this. Having out-gassed the liquid by pumping on it with a vacuum pump overnight, the background of the counter was found, and then the run was commenced and proceeded in the usual way.

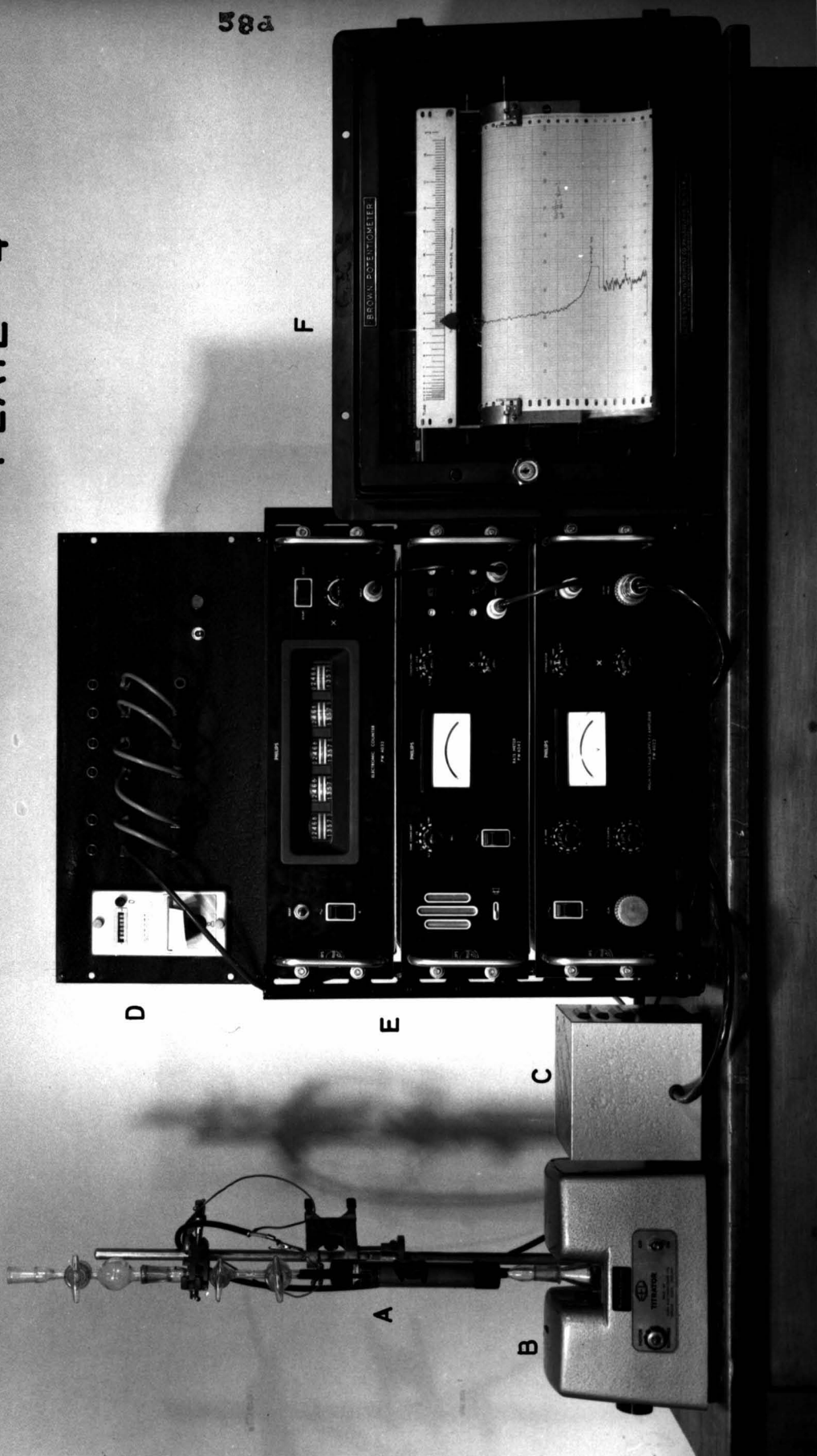
A small amount of exchange rapidly took place and then the counter became unstable and the run was discontinued.

It was assumed that this instability was caused by dissolution of the ethylene of the counting gas, in the paraffin. In order to try and overcome this, the next run was not commenced until counting gas had been introduced into the counter and the paraffin stirred for three hours. The counting gas was then evacuated and replaced and the run proceeded with in the usual sequence. From the plot of the resulting run two straight lines were obtained with half times of 7 minutes and 130 minutes and the total activity fall off was 8930 c/m.

In order to determine whether the rate of this fall off was dependant on the stirring rate of the paraffin, the run was repeated using the same amount of paraffin and the same technique. However, this time the

PLATE 4

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KEY TO PLATE 4

- A Geiger counter tube (no shielding)
 - B Stirring assembly
 - C Paralysis unit
 - D Printing unit
 - E Counting apparatus bank
 - F Strip chart recorder
-

rate of stirring was advanced from approximately 25 r.p.m. of the stirrer to approximately 270 r.p.m. From the resulting plot two straight lines were again obtained this time with half times of exchange of 6 minutes and 54 minutes. Thus it was apparent that while the first type of exchange is unaffected by the rate of stirring, the second type is in some manner dependant on the rate of stirring. This second type of exchange could be either due to HTO dissolving in paraffin or to exchange between labile hydrogen in the paraffin and the HTO, while it was suggested that the faster exchange was some kind of equilibration within the counter.

In order to establish the origin of the slower exchange, a further run was carried out under conditions similar to the two previous ones. However, instead of using the usual amount of HTO of medium activity for the exchange, a very small amount of high-specific-activity HTO was used. If the slower exchange is due to dissolution of the HTO in the paraffin, then using a smaller amount of HTO should result in a slower rate of activity fall off, as there is less HTO present and hence less tendency for the HTO to dissolve in the paraffin. If, however, the slower exchange is due to exchange between the HTO and labile hydrogen in the paraffin, the rate should be relatively unaltered by alteration of the amount of the HTO present, provided the activities are similar.

The stirring rate remained approximately the same as in the previous run, i.e. 270 r.p.m. and the run was carried out with the same counter and flask and similar quantities of paraffin. Again two straight lines were obtained, with exchange half times of 6 minutes and 57 minutes. From these results, it was assumed that in these runs there are two modes

of activity decrease going on within the counter flask. The first, with a half time of exchange of 6-7 minutes has not had its source positively identified but appears to be independant of stirring rate and of amount of HTO present and is probably due to some type of equilibration process on the counter walls etc. or to the rate meter overshooting the true activity value when being set at the commencement of a run.

The second mode of exchange is dependant on the rate of stirring but independant of the amount of HTO present and is apparently due to exchange of activity between the HTO in the counter and labile hydrogen in the paraffin solvent.

The results just obtained immediately suggested another method for the determination of the number of exchangeable hydrogen atoms in an organic compound. The paraffin results show two types of exchange, one of which is due to the labile or exchangeable hydrogen present as impurities in the paraffin. The amount of this type of exchange may be obtained from the usual graph of the run, at time equal to zero, plotting activity against time on a logarithmic plot. If an organic compound were dissolved in the paraffin, the amount of fall off due to the compound plus the paraffin could be obtained. That due to the paraffin could be obtained from a blank run on paraffin alone, and subtracted, giving the amount of exchange due solely to the exchange of the organic compound with HTO. The amount of this exchange is dependant on two factors, namely the number of moles of organic compound present and the number of active or labile hydrogen atoms it contains. Having obtained the amount of exchange per mole of compound for an organic compound, this could then be compared with the exchange for a similar

number of moles of organic compound having a known number of exchangeable hydrogen atoms. These should be in the simple arithmetic ratio of their respective number of exchangeable hydrogen atoms.

As it is only the second mode of exchange which is concerned with the labile hydrogen exchange, the determinations must be made using only these results and thus ignoring phenomena concerned with the first type of exchange.

From the previously obtained formula $\frac{C}{B} = \frac{yT_o}{xz}$ a value for C in terms of B can be obtained as x, y, z and T_o can be found from the graph of the run.

Now B + C in terms of A can be calculated from data also obtained in each run

$$\frac{T_o}{z} = \frac{A+(B+C)}{A} \quad T_o \text{ and } z \text{ both being known from each run.}$$

In this expression one can substitute the value for B in terms of C and finally one obtains an expression for C in terms of A

In the above discussion:

A = active hydrogen in the counting space of the counter

B = the active hydrogen in the fast exchange of unknown origin

C = the active hydrogen in the labile hydrogen with the solvent and/or test compound.

As very little HTO is added, A can be assumed constant in all runs. The actual value of A is dependant on the volume of the counter and the relative humidity of the entrant HTO. Although the actual value can be calculated, in all the runs carried out, A is retained as a constant and the fall off of active hydrogen C is obtained in terms of this constant A.

In order to obtain the amount of fall off due to exchange with the paraffin, a blank run was performed using 1.5519 gms paraffin. This was outgassed by pumping on it for two hours and the background determined. The run was then performed as usual and plotted on the usual graph.

EXPERIMENTAL DATA

PARAFFIN/HTO BLANK RUN I

Weight of paraffin - 1.5519 gms

Background 416 c/m

Two straight lines were obtained, half time of exchange being 7 minutes and 154 minutes.

Values obtained from this run were:

x 1170 c/m

y 875 c/m

z 3065 c/m

To 5105 c/m

$$\frac{C}{B} = \frac{875 (5105)}{1170 (3065)} = \frac{yTo}{xz}$$

$$\underline{B = 0.86C}$$

$$\frac{A+(B+C)}{A} = \frac{To}{z} = \frac{5105}{3065}$$

$$\underline{B+C = 0.73A}$$

$$\text{Substituting for B:} \quad 1.86C = 0.73A$$

$$\underline{C = 0.392A}$$

Paraffin fall off was 0.392A on 1.5519 gms

PARAFFIN/HTO RUN I

Weight of paraffin used - 1.5519gm

Stirring and outgassing time - 2 hours

Background - 416 c/m

<u>Time</u>	<u>Activity and Paralysis</u> <u>Correction</u>	<u>Total - z</u>	<u>Second Exchange</u>
0	5105	2035	1160
10 mins	4490	1425	655
20	4095	1030	350
30	3860	795	200
40	3735	670	150
50	3600	535	75
60	3490	425	
80	3390	325	
100	3305	240	
120	3283	218	
130	3260	165	
140	3236	151	
150	3185	120	
160	3174	109	
170	3177	112	
180	3160	85	
200	3119	54	
240	3087	22	
Final	3065		

BENZOIC ACID/PARAFFIN RUN I

A run was next performed using benzoic acid as a test compound. The paraffin was outgassed again for two hours, this time using 2.9992 gm paraffin, in which were dissolved 7.2 milligrams (6.42×10^{-2} millimoles) of benzoic acid A.R. grade. Two straight lines were obtained, with half times of 6 minutes and 183 minutes. Values obtained from the resulting graph were:

$$x = 3200$$

$$y = 8300$$

$$z = 2472$$

$$T_o = 13972$$

$$\frac{C}{B} = \frac{13972 (8300)}{3200 (2472)}$$

$$B = 0.069C$$

$$\text{Now } \frac{A+(B+C)}{A} = \frac{13972}{2472}$$

$$1.069C = 8.62A$$

$$C = \frac{8.62}{1.069}A$$

$$C = 8.06A$$

But this is the total fall off from benzoic acid and paraffin and the fall off of paraffin must be subtracted from it.

Now from the blank run the fall off due to the paraffin is as follows:

$$C = \frac{0.392 \times 2.9992}{1.6619} \quad A = 0.755A$$

$$\text{Total fall off} = 8.06A \quad \text{and fall off due to paraffin} = 0.755A$$

$$\text{Fall off of benzoic acid} = 7.305A \text{ on } 6.43 \times 10^{-2} \text{ millimoles}$$

A further run, using a smaller amount of benzoic acid, was carried out to see if a comparable answer to the previous one could be obtained. However, in spite of the run proceeding apparently normally, anomalous results were obtained, the fall off due to the benzoic acid being too low compared with that of the previous run. The weight of benzoic acid used was 6.7 mgm which, based on results obtained from Run I should have given a fall off of 6.80A. But it gave a fall off of 6.02A. However, it was realized that this run had been outgassed overnight instead of two hours as previously. From ensuing work it was found that the amount of exchangeable hydrogen within the paraffin decreased with longer outgassing, thus causing the discrepancy noticed above. As it was convenient to outgas the paraffin overnight this procedure was standardized and followed in all ensuing runs except the next.

The previous anomalous run was however repeated, this time using the outgassing time of two hours.

BENZOIC ACID/PARAFFIN RUN III

Weight of benzoic acid used - 6.7 mgm

Weight of paraffin - 3.0338 gm

Background 174 c/m

Two straight lines were obtained with exchange half times of
8 minutes and 173 minutes.

Values obtained from the resulting graph were:

$$x = 5070$$

$$y = 12300$$

$$z = 2367$$

$$T_o = 19673$$

$$\frac{C}{B} = \frac{12300 (19673)}{5070 (2367)}$$

$$B = 0.049C$$

$$\frac{T_o}{z} = \frac{A+(B+C)}{A} = \frac{19673}{2367}$$

$$B+C = 7.98A$$

$$1.049C = 7.98A$$

$$C = 7.61A$$

$$\text{Now fall off due to paraffin } C = \frac{0.392 \times 3.0338}{1.5519} A$$

$$C = 0.765A$$

$$\text{Fall off due to 6.7 mgm benzoic acid} = (7.61 - .765) A$$

$$C = 6.845A$$

In order to enable paraffin solutions to be degassed overnight (approximately 17 hours) a blank run was performed on paraffin treated in this manner. Stirring was used, as in all previous runs, throughout the outgassing procedure.

BENZOIC ACID/PARAFFIN RUN III

Weight of benzoic acid used - 6.7 mgm

Weight of paraffin used - 3.0338 gm

Stirring and outgassing time - 2 hours

Background - 174 c/m

<u>Time</u>	<u>Activity and Paralysis</u> <u>Correction</u>	<u>Total - z</u>	<u>Second Exchange</u>
0 hours	19750	17383	5083
0.3	14140	11773	562
0.6	12790	10423	
1.0	11850	9483	
1.3	11170	8803	
1.6	10500	8133	
2.0	9780	7413	
3.0	8550	6183	
4.0	7005	4638	
5.0	6110	3743	
6.0	5310	2943	
7.0	4710	2343	
8.0	4198	1831	
9.0	3755	1388	
10.0	3450	1083	
11.0	3215	848	
12.0	3035	668	
13.0	2880	513	
14.0	2765	398	
z = Final	2367	-	

PARAFFIN/HTO RUN II

Weight of paraffin - 0.5991 gm

Background 975 c/m

Outgassing time - approximately 17 hours

Values obtained from the ensuing graph were (See Fig 9)

x 3000 c/m

y 1780 c/m

x 9225 c/m

To 14005 c/m

$$\frac{C}{B} = \frac{1780 (14005)}{3000 (9225)}$$

$$\underline{B = 1.112C}$$

$$\frac{To}{z} = \frac{A+(B+C)}{A} = \frac{14005}{9225}$$

$$B + C = 0.588A$$

$$2.112C = 0.588A$$

$$\underline{C = 0.279A \text{ on } 0.5991 \text{ gm}}$$

Four further runs were then carried out on benzoic acid using in each case different amounts of benzoic acid. The fall off was calculated in each case, and after all runs had been calculated, a graph was plotted of fall off in terms of A versus millimoles of benzoic acid. If the runs were in agreement with each other, this graph would be a straight line passing through all points.

In all runs, the usual stirring was employed and outgassing times in all cases were the same to within half an hour.

Run VII was done to see to just what limits this method was capable of extension. The benzoic acid was added to the flask by making up a solution of 13 mgm of benzoic acid in 6.1595 gm of paraffin and 0.0853 gms of the solution was placed in the flask.

From the small amounts used in the experiment it follows that the sources of error must be very high and at this concentration it is difficult to determine accurately the number of active hydrogen atoms from the graph of concentration versus fall off.

A run was also performed using stearic acid to see if the results obtained agreed with those obtained for benzoic acid. This was carried out in the same way as those on benzoic acid.

BENZOIC ACID/PARAFFIN RUN IV

Weight of benzoic acid - 9.45 mgm

Weight of paraffin - 2.9858 gm

Background 247 c/m

Two straight lines half times of exchange 6 minutes and 120 minutes

Values obtained from the graph were (See Fig 10)

$$x = 4300 \text{ c/m}$$

$$y = 8000 \text{ c/m}$$

$$z = 1100 \text{ c/m}$$

$$T_o = 13400 \text{ c/m}$$

$$\frac{C}{B} = \frac{13400 (8000)}{4300 (1100)} = \frac{yT_o}{xz}$$

$$B = 0.0442C$$

$$\frac{A+(B+C)}{A} = \frac{T_o}{z} = \frac{13400}{1100}$$

$$B+C = 10.79A$$

$$C = \frac{10.79}{1.0442} A = 10.31A$$

$$\text{Falloff due to paraffin} = \frac{2.9858}{0.5911} \times \frac{0.279}{1} A$$

$$= 1.41A$$

$$\text{Fall off due to benzoic acid} = (1031 - 1.41) A$$

$$\underline{\text{Benzoic Acid}} = 8.90A \text{ on } 9.45 \text{ mgm}$$

BENZOIC ACID/PARAFFIN RUN V

Weight of benzoic acid = 4.1 mgm

Weight of paraffin - 1.9837 gm

Background 144 c/m

Two straight lines half times 7 minutes 96 minutes

Values obtained from the graph were:

x 2527

y 5160

z 1943

To 12510

$$\frac{C}{B} = \frac{12510 (5160)}{2527 (1943)} = \frac{yTo}{xz}$$

$$\underline{B = 0.146C}$$

$$\frac{A+(B+C)}{A} = \frac{To}{z} = \frac{12510}{1943}$$

$$B+C = 5.43A$$

$$\underline{C = \frac{5.43}{1.146} A = 4.74A}$$

$$\text{Fall off due to paraffin} = \frac{1.9837 \times 0.279}{0.5919} A$$

$$= \underline{0.935A}$$

$$\text{Fall off due to benzoic acid} = (4.74 - .935) A$$

$$\underline{\text{Benzoic Acid} = 3.605A \text{ on } 4.1 \text{ mgm}}$$

BENZOIC ACID/PARAFFIN RUN VI

Weight of benzoic acid - 1.2 mgm

Weight of paraffin - 0.5038 gm

Back ground - 455 c/m

Two straight lines, half times of exchange 5 minutes, 82 minutes.

Values obtained from the graph were:

x 5650 c/m

y 7950 c/m

z 9060 c/m

To 22660 c/m

$$\frac{C}{B} = \frac{7950 (22660)}{5650 (9060)} = \frac{yTo}{xz}$$

$$\underline{B = 0.284C}$$

$$\frac{A+(B+C)}{A} = \frac{To}{z} = \frac{22660}{9060}$$

$$\underline{B+C = 1.58A}$$

$$\underline{C = \frac{1.58}{1.284} A = 1.23A}$$

$$\text{Fall off due to paraffin} = \frac{0.279 \times 0.5038}{0.5911}$$

$$\underline{= 0.238A}$$

$$\text{Fall off due to benzoic acid} = (1.23 - 0.238)A$$

$$\underline{\text{Benzoic Acid} = 0.992A \text{ on } 1.2 \text{ mgm}}$$

VII

acid - 0.18 mgm

n - 0.0851 gm

7 c/m

s, half times of exchange 5 minutes, 30 minutes

from the graph were:

$$\frac{y}{x} = \frac{yTo}{xz}$$

$$\frac{24630}{14690}$$

$$= 0.255A$$

$$\text{paraffin} = \frac{0.0851 \times 0.279}{0.5919} A$$

$$\text{benzoic acid} = (0.255 - 0.0402)A$$

$$0.215A \text{ on } 0.18 \text{ mgm}$$

BENZOIC ACID/PARAFFIN RUN

Weight of benzoic

Weight of paraffin

Background - 10

Two straight lines

Values obtained from

$$x = 7300 \text{ c/m}$$

$$y = 2640 \text{ c/m}$$

$$z = 14690 \text{ c/m}$$

$$To = 24630 \text{ c/m}$$

$$\frac{C}{B} = \frac{24630 (2640)}{7300 (14690)}$$

$$B = 1.648C$$

$$\frac{A+(B+C)}{A} = \frac{To}{z}$$

$$B+C = 0.676A$$

$$C = \frac{.676}{2.648} A$$

Fall off due to

$$C = 0.0402A$$

Fall off due to

$$\text{Benzoic acid} =$$

BENZOIC ACID/PARAFFIN RUN VII

Weight of benzoic acid used - 0.18 mgm

Weight of paraffin used - 0.0851 gm

Outgassing time - 17 hours

Background 107 c/m

<u>Time</u>	<u>Activity and Paralysis</u> <u>Correction</u>	<u>Total - z</u>	<u>Second Exchange</u>
0 minutes	24710	10020	7380
10	18000	3310	1210
20	16960	2270	580
30	16070	1380	
40	15750	1060	
50	15550	860	
60	15375	685	
70	15230	540	
80	15110	420	
90	15030	340	
100	14990	280	
z = Final	14690	-	

BENZOIC ACID | PARAFFIN | HTO
RUN VII

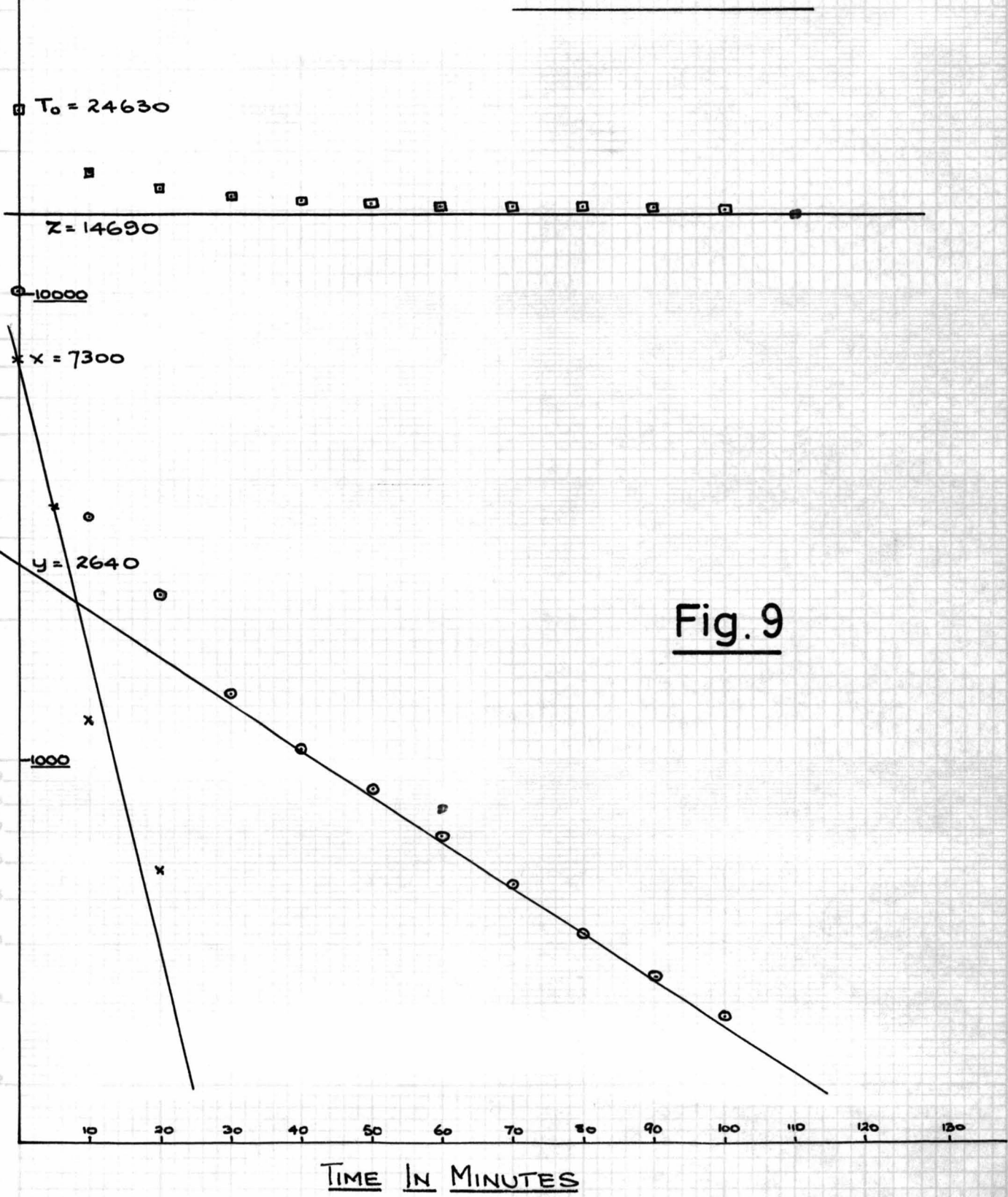


Fig. 9

TIME IN MINUTES

STEARIC ACID/PARAFFIN RUN I

Weight of stearic acid - 3.0 mgm

Weight of paraffin - 2.7277 gm

Background -375 c/m

Two straight lines were obtained with half times of exchange
6 minutes and 114 minutes

Values obtained from the graph were (See Fig 11).

$$x = 3000$$

$$y = 4300$$

$$z = 3280$$

$$T_o = 10580$$

$$\frac{C}{B} = \frac{4300 (10580)}{3000 (3280)} = \frac{yT_o}{xz}$$

$$\underline{B = 0.216C}$$

$$\frac{A+(B+C)}{A} = \frac{T_o}{z} = \frac{10580}{3280}$$

$$\underline{B + C = 2.72A}$$

$$\underline{C = \frac{2.72}{1.216} A = 2.24A}$$

$$\text{Fall off due to paraffin} = \frac{2.7277 \times 0.279}{0.5911} A$$

$$\underline{C = 1.276A}$$

$$\text{Fall off due to benzoic acid} = (2.24 - 1.276) A$$

$$\text{Stearic Acid} = 0.91 A \text{ on } 3.0 \text{ mgm}$$

STEARIC ACID/PARAFFIN RUN I

Weight of stearic acid used - 3.0 mgm

Weight of paraffin used - 2.727 gm

Outgassing time - 17 hours

Background - 375 c/m

<u>Time</u>	<u>Activity and Paralysis</u> <u>Correction</u>	<u>Total - z</u>	<u>Second Exchange</u>
0 Hours	10850	7570	3270
0.3	7440	4160	360
0.6	6780	3500	-
1.0	6330	3050	
2.0	5375	2095	
3.0	4710	1430	
4.0	4270	990	
5.0	3990	710	
6.0	3755	475	
7.0	3595	315	
8.0	3515	235	
9.0	3418	138	
10.0	3366	86	
11.0	3334	54	
z = Final	3280	-	

$T_0 = 10580$

- 77a -

STEARIC ACID / PARAFFIN / HTO

RUN I

$y = 4300$

$x = 3000$

$z = 3280$

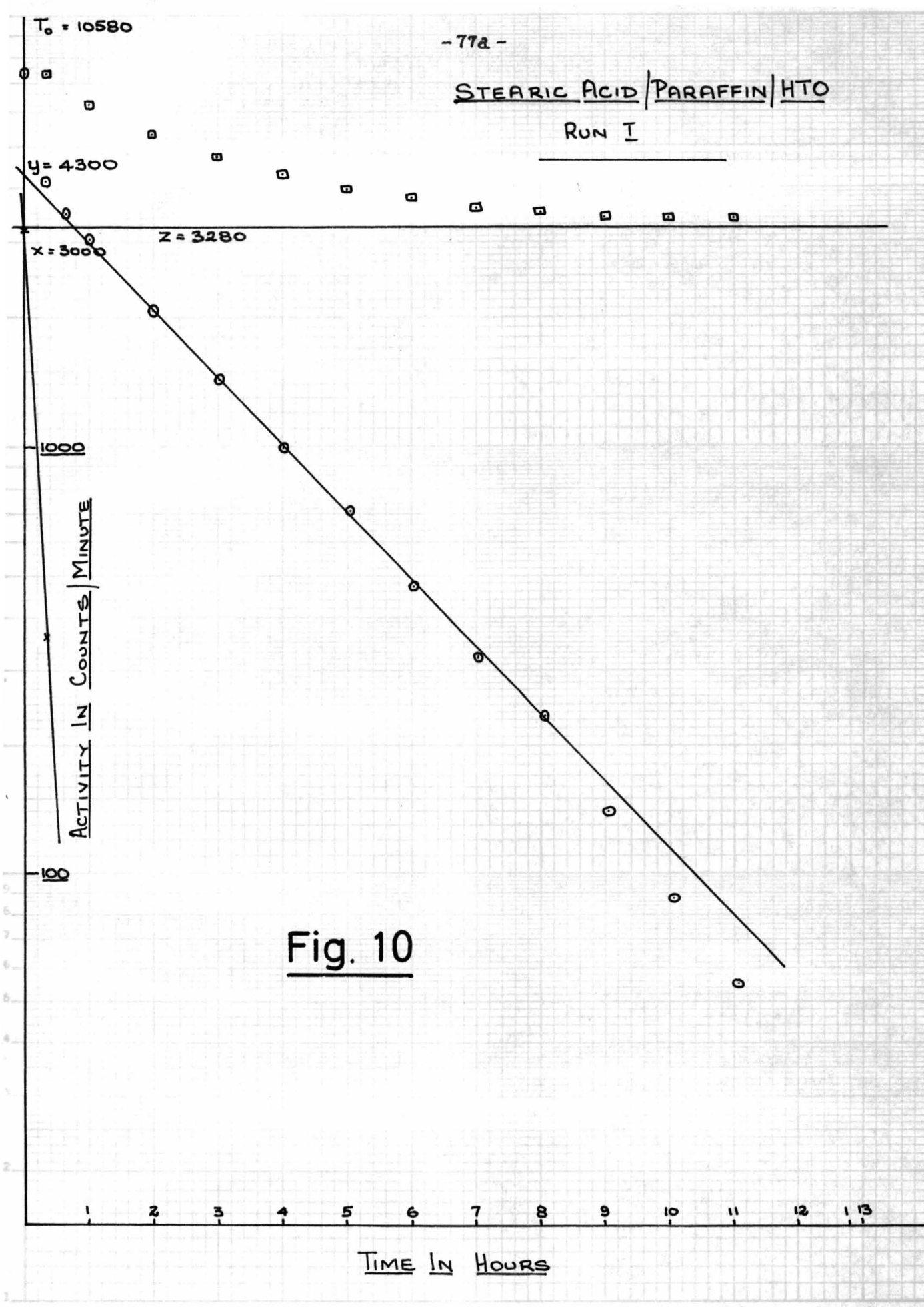
1000

ACTIVITY IN COUNTS / MINUTE

10

Fig. 10

TIME IN HOURS



Having completed the previous runs, it was then possible to plot a graph of fall off in terms of A versus number of millimoles of compound used. From theory, it is predicted that this graph should be a straight line. The results obtained during the preceding runs confirm this theory and all lay very close to the theoretical line. See Fig 12.

As theory predicts that compounds containing two exchangeable hydrogens should have twice the fall off value, in terms of A, of compounds containing only active hydrogen atoms, it was possible from the results obtained, to plot a theoretical line for fall off of compounds containing two active hydrogen atoms.

Having plotted this theoretical line, two runs were carried out using azeleic acid as a trial compound containing two active hydrogen atoms.

Both these runs gave results which agreed closely with the theoretical results:

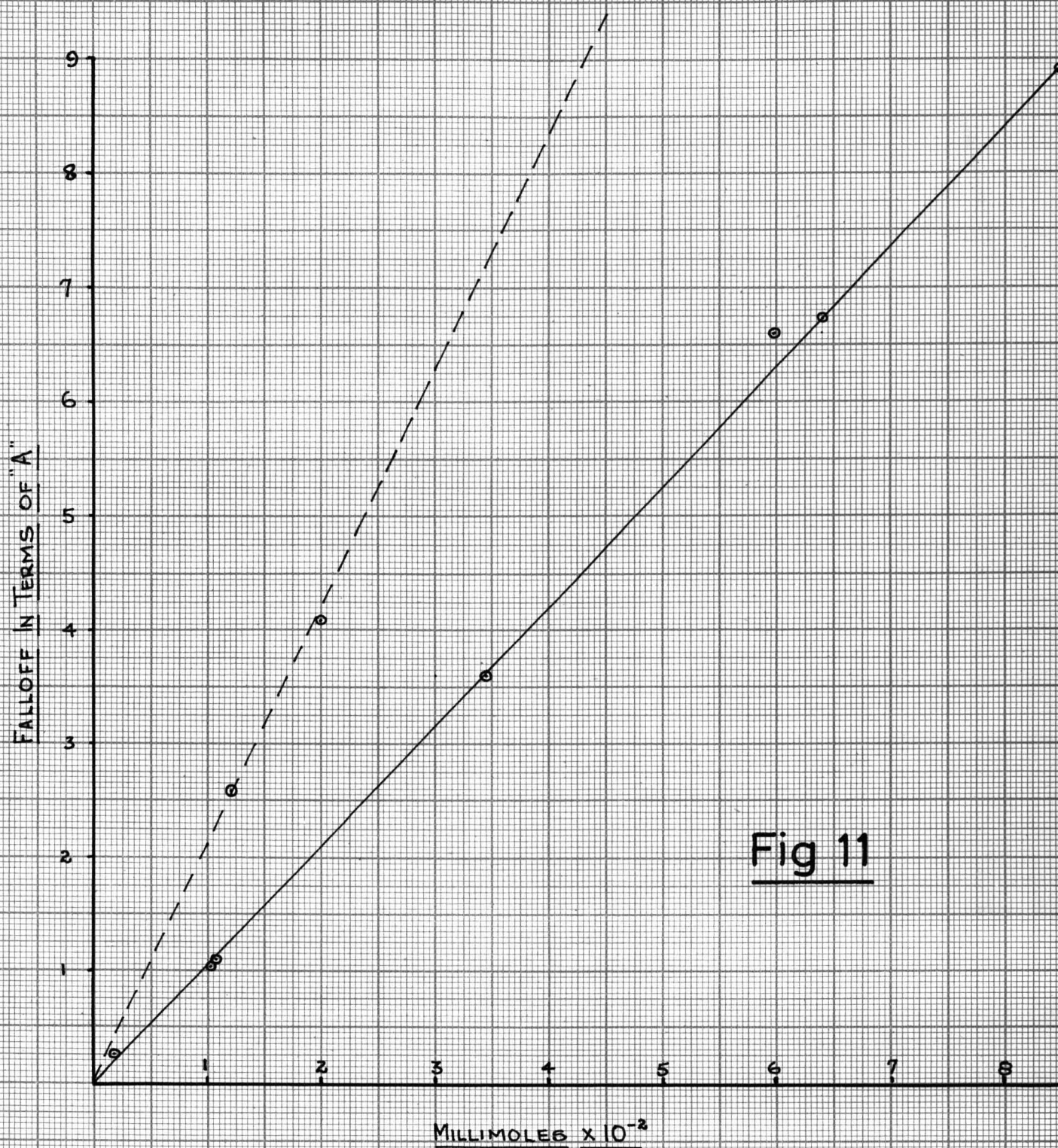


Fig 11

AZELEIC ACID/PARAFFIN RUN I

Weight of azeleic acid used - 2.4 mgm

Weight of paraffin used - 3.1873 mgm

Outgassing time - 17 hours

Background - 293 c/m

Two straight lines were obtained with half times of exchange
7 minutes and 120 minutes. Values obtained from the graph were:

$$x = 3260 \text{ c/m}$$

$$y = 9600 \text{ c/m}$$

$$z = 3000 \text{ c/m}$$

$$T_o = 15860 \text{ c/m}$$

$$\frac{C}{B} = \frac{yT_o}{xz} = \frac{9600 (15860)}{3000 (3280)}$$

$$B = 0.064C$$

$$\frac{A+(B+C)}{A} = \frac{T_o}{z} = \frac{15860}{3000}$$

$$B+C = 4.3A$$

$$C = \frac{4.3}{1.064} A = 4.04A$$

$$\text{But fall off due to paraffin} = \frac{.279}{.5914} \times \frac{3.1873}{1} A$$

$$C = 1.50A$$

$$\text{Fall off due to azeleic acid} = (4.04 - 1.50) A$$

$$\underline{\text{Azeleic Acid} = 2.54A \text{ on } 2.4 \text{ mgm}}$$

AZELEIC ACID/PARAFFIN RUN II

Weight of azeleic acid used 3.48 mgm

Weight of paraffin used 5.2860 gm

Outgassing time 17 hours

Background 523 c/m

Two straight lines were obtained with half tims of exchange
6 minutes and 130 minutes. Values obtained from the graph were:

$$x \quad 3590 \text{ c/m}$$

$$y \quad 11000 \text{ c/m}$$

$$z \quad 2280 \text{ c/m}$$

$$T_o \quad 18050 \text{ c/m}$$

$$\frac{C}{B} = \frac{yT_o}{xz} = \frac{18050 (11000)}{(2280) 3590}$$

$$\underline{B = 0.0411C}$$

$$\frac{A+(B+C)}{A} = \frac{T_o}{z} = \frac{18050}{2280}$$

$$\underline{B+C = 6.92A}$$

$$\underline{C = \frac{6.92}{1.0411} A = 6.56A}$$

$$\text{But fall off due to paraffin} = \frac{0.279}{0.5911} \times \frac{5.2860}{1} A$$

$$C = 2.49A$$

$$\text{Fall off due to azeleic acid} = (6.56 - 2.49) A$$

$$\underline{\text{Azeleic acid} = 4.07A \text{ on } 3.48 \text{ mgms}}$$

SUMMARY

A method has been developed for the determination of exchangeable hydrogen on only approximately a milligram of unknown compound. This method is better and more sensitive than existing techniques and is non destructive.

This method has been briefly studied in an extended form, applied to exchangeable hydrogen atoms adjacent to an activating group, such as the carbonyl group, within the molecule.

Another method has been briefly examined and found suitable for the determination of exchangeable hydrogen, on a sample of approximately 10 milligrams. This method also has the advantage of being non destructive, but is less sensitive than the first method.

It is suggested that this work has opened a field for more extensive study in exchange of labile atoms within organic compounds, and also in the study of surface exchange phenomena. It is possible to study the adsorption of water and other compounds on various surfaces, studies which up till now has been difficult because of the problem of examining the exchanges under equilibrium conditions. This is now possible by techniques similar to those discussed in this work, where exchanges have been carried out at all times under conditions of equilibrium.

SUGGESTIONS FOR FURTHER WORK

It is realized that this work has been largely of an exploratory nature and much work is still possible to perfect these methods.

Obviously paraffin, in spite of its favourable characteristics of low active hydrogen content and very low vapour pressure, is a poor solvent for most organic compounds, especially those containing a large number of active hydrogen atoms, i.e. the more polar compounds. Thus further work is possible to find a more suitable solvent for this work.

Work is also possible in the study of exchange of hydrogen atoms activated by groups other than the ketone group.

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