GAS CHROMATOGRAPHIC STUDIES

I. PYROLYSIS GAS CHROMATOGRAPHY OF SEVERAL PURINES, PYRIMIDINES, NUCLEOSIDES AND DEOXYRIBOSE NUCLEIC ACIDS

II. SCHIZOPHRENIC'S SWEAT

by

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To those whom I meet every day,

And to those whom I can no longer meet.

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ABSTRACT

The pyrolysis gas chromatographic technique was used to study the thermal decomposition of purines, pyrimidines, nucleosides and two types of deoxyribonucleic acids. The chromatogram of the pyrolysis products of any given compound is seen to be unique for that substance. A linear relationship is observed between the sample size and the area of any given peak on the chromatogram. An unsuccessful attempt is made to correlate the chromatogram peak areas with the pyrimidine content of the nucleic acids.

Gas chromatography was used in an unsuccessful attempt to identify the substance or substances responsible for an odour associated with schizophrenic patients.

Several aspects of the gas chromatographic technique of analysis which pertained to these two studies are discussed.

FOREWORD

This thesis attempts a dual role. It serves firstly as a record of an effort to apply the techniques of gas-liquid chromatography to two rather unrelated problems; the analysis of sweat volatiles and the study of the pyrolysis of nucleic acids. Secondly, it serves as a compilation of references and techniques pertaining to gas-liquid chromatography. The nature of the problems tackled was such that a rather extensive consideration of the available gas chromatographic techniques was necessary before any work could be attempted. Although relatively few of the techniques were actually employed in the subsequent work, it was thought that a record of the many factors to be considered for the successful use of gas chromatography would be of value. The unwieldly literature pertaining to gas chromatography could not be completely reviewed. Nevertheless enough references are given throughout the discussions so as to lead to the most recent information dealing with the subject. The aspects of gas chromatography pertaining directly to the work described are discussed in the text of the thesis. To supplement this, a brief resumé of the gas chromatographic process and of the many other aspects of this method of analysis is given in the appendix. In this manner, it is felt that the thesis will serve both a paedagogical and a nutritional purpose; paedogogical in the sense that it may possibly be used in the immediate future as a "handbook" for gas chromatography, and nutritional in the sense that it may definitely serve as nourishment for further research.

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INTRODUCTION

INTRODUCTION

GAS-LIQUID CHROMATOGRAPHY

HISTORY, PRINCIPLES AND THE PROCESS

Although the concept of chromatography is relatively old (1,2), the history of Gas-Liquid Chromatography goes back no further than the Nobel-prize-winning work of Martin and Synge on partition chromatography (3). The concept of gas-liquid chromatography envisioned by these workers underwent a long period of gestation between the planting of the idea in 1941 and the elaboration of the suggestion eleven years later (4,5). Work in the field of vapour phase chromatography then proceeded so rapidly that, as early as 1956, Martin warned those at the London Conference (6,7) that the technique was truly "getting out of hand". Keeping abreast of the expanding literature in this one field is now a computer's job. A list of references given in Table 18 of the appendix suggests one pattern of reading that can be used to follow the developments in this field.

The process of chromatography is a physical means of separating the different chemical species which may comprise some mixture. The components to be separated are distributed between two phases, one of these phases constituting a stationary bed of large surface area, while the other takes the form of a fluid that percolates through or along the stationary bed.

A judicious selection of materials and conditions will assure that each component will have a unique distribution between the two

phases and that, as a result, the percolating fluid will sweep each component along the stationary bed at a unique velocity determined essentially by the particular distribution coefficient. In this manner, some species will soon outdistance the others until finally the whole mixture is separated into the "bands" characteristic of the process. The theory of chromatographic separations has been and is being extensively dealt with. The mathematical formulations may be found in several excellent texts dealing with gas chromatography (8,9,10), and in the literature. As with any technique, gas chromatography has been given a terminology of its own. An outline of most of the recommended (11,12) terminology necessary for the understanding of this text is given in Table 19 of the appendix.

The wide range of applicability of the chromatographic method becomes at once apparent. It may be used for analytical purposes in both qualitatively and quantitatively determining the constituents of a mixture; it may be used as a basic research tool for determining certain physical quantities such as partition coefficients and adsorption isotherms (13); and it may also be used as a preparative technique for isolating pure compounds from a reaction mixture. The first mentioned application of chromatography (gas-liquid chromatography in particular) is the technique which was considered most suitable for the purpose of these investigations.

THE COMPONENTS

A gas chromatograph can be thought of as a composite of three

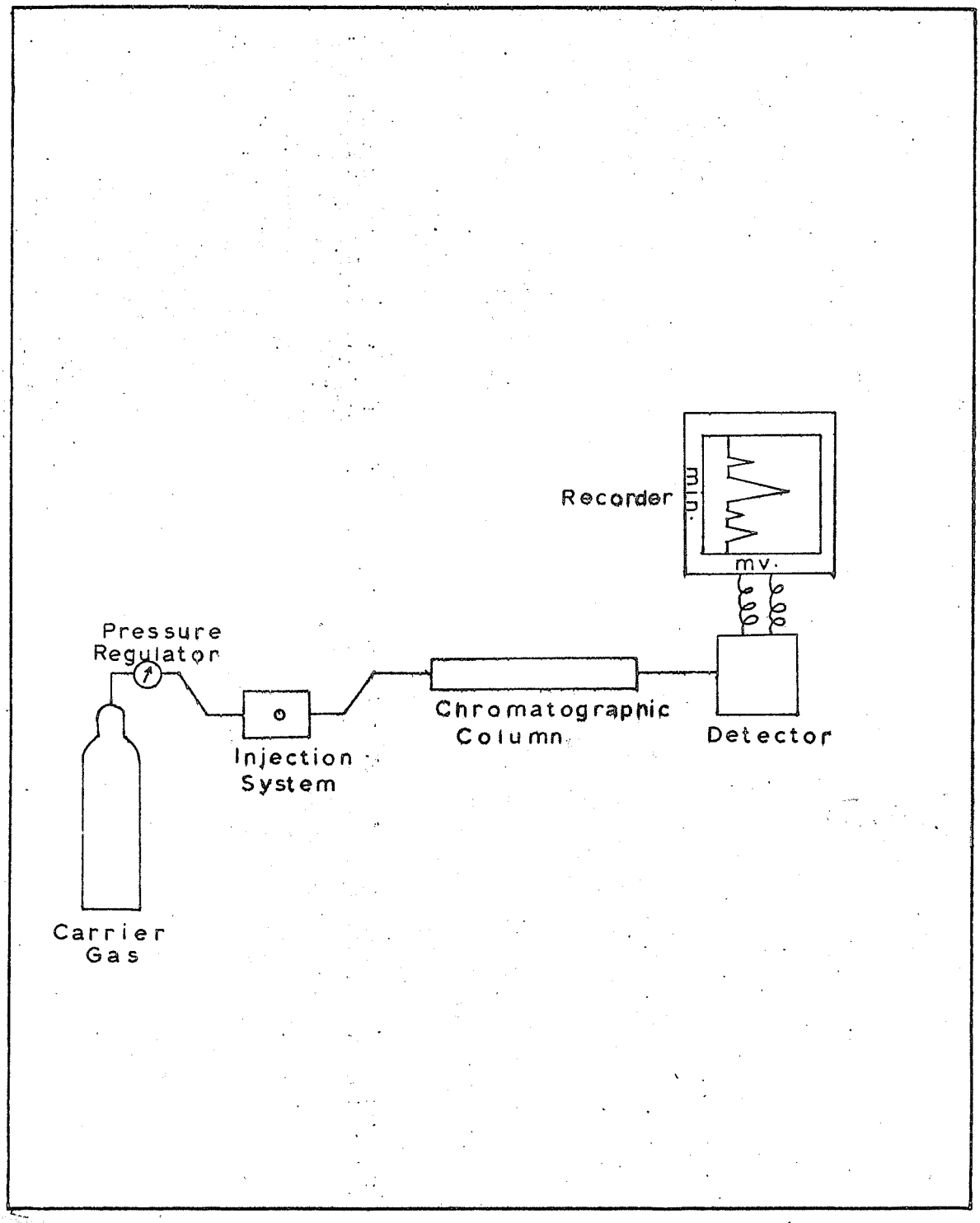
separate components all of which must function efficiently together for the useful operation of the apparatus. Figure 1 shows a schematic diagram of the apparatus. The injection system must be capable of cleanly introducing known micro-amounts of substances as a plug onto the head of the column. The chromatographic column in which the partitioning process occurs must be engineered in such a manner that the stationary liquid phase, the solid support, and the mobile phase combine to produce the best possible separation of the mixture to be analysed. Thirdly, a highly sensitive detector is required to monitor the effluent from the column. There are many considerations in the assembly of each of these components. The Appendix deals with the more important features of all three systems.

THE TECHNOLOGY OF GAS-LIQUID CHROMATOGRAPHY

ASSEMBLY OF THE INSTRUMENT

Once the most desirable components necessary to build a chromatographic system have been selected, these materials must be assembled into a workable unit. Studies such as those performed by Kirkland (14) and by Nogare and Chiu (15) have demonstrated that, in the construction of the apparatus, certain technical considerations can improve both its resolution and efficiency. The choice of the correct percentage of liquid phase (16), the elimination of void space between the column and detector, a selection of the optimum support-particle size and range (17), and the use of an optimum column length (18), all serve to improve the efficiency of the system. In addition,

FIGURE 1. A Schematic Gas Chromatograph.


Recorder
min.
mv.
Pressure
Regulator
Injection
System
Chromatographic
Column
Detector
Carrier
Gas

attention must be paid to the geometry of the column itself (19). Factors such as these are often neglected in many procedures reported in the literature.

One of the first operations that must be performed in any analysis is the selection of the column conditions. Often the flow rate will be somewhat limited by the large pressure drop required by some columns but, in most cases, an optimum flow rate (20,21) can be chosen for the separation desired. The effect of the flow rate on column efficiency is expressed theoretically by the Van Deemter equation which is discussed in the appendix. The temperature for any particular separation can be selected (providing no limitations are imposed by the liquid phase in use) by preliminary programmed temperature experiments (22). The temperature which yields the best separation in the shortest time soon becomes apparent. More extensive theoretical treatments of the effects of temperature and flow rate on any separation are available in suitable texts. However, in practice an empirical search within the recommended set of conditions is most often the approach used.

The evolution of the gas chromatographic technique has seen the use of several specific procedures which have in themselves become areas of further development. The techniques of programmed temperature gas chromatography (PTGC), of pyrolysis gas chromatography (Pyr GC), and the possible use of open tubular columns were studied for the purposes of these investigations.

PROGRAMMED TEMPERATURE GAS CHROMATOGRAPHY

For any reasonably complex mixture of compounds having widely separated boiling points, there is no optimum column temperature which will suffice for a chromatographic analysis. The extremely volatile components will remain unresolved at high temperatures, whereas the high-boiling substances will not be eluted at low temperatures. At intermediate temperatures, peaks arising from low boiling components tend to be crowded, while those of high boiling ones may be too broad to be of analytical use. The use of programmed temperature gas chromatography affords one of the best answers to this problem. Drew and McNesby (23) developed a programmed temperature system to investigate gas phase reactions. By programming the column temperature over ranges as large as -196° to $+300^{\circ}C$, (smaller ranges are usually used), it was found that the column performance was improved and that the elution rates were increased. Several arrangements have been developed to enable one to vary the column temperature continually in a linear (24) or non-linear (25) fashion between two temperature limits. Of particular importance to the problem of looking for trace components in substances such as sweat is the fact that this procedure permits the application of comparatively large samples to the columns without overloading them. Programming the temperature spreads out the early peaks and sharpens up the peaks normally having long retention times, thus improving the presentation of the chromatographic data (26).

As a result of the many factors of the chromatographic process that the temperature influences, the theory of PTGC is rather involved

(27,28). However, certain empirical correlations have been found useful in characterising the behaviour of various solute classes on specific columns. Firstly, for normal paraffins, a uniform spacing of peak maxima is obtained for various linear programming rates (29). Secondly, it is found that increasing the liquid phase polarity under identical PTGC conditions increases the retention time of each member of a polar homologous series by the same amount. The technique thus appears to be of use not only in effectively and rapidly separating the components of a wide-boiling range mixture, but also in identifying the components by their programmed temperature retention behaviour. A slight variation of this technique was adapted for the pyrolytic technique to be discussed.

PYROLYSIS GAS CHROMATOGRAPHY

Since the alchemists, pyrolysis has been employed in one way or another for the purposes of preparative organic chemistry (30). It is only recently that this method has been coupled with gas chromatography to yield a method for characterising non-volatile substances (31). The principle involved is simple. Arranging experimental conditions to eliminate secondary reactions, thermal cracking of a substance will yield certain unique degradation products. The cracking can be accomplished in several ways: by an induction element, by a heated filament, by a quartz furnace, or by any other means that can raise a small amount of material to a high temperature in a short time. In many simple cases, the kind of degradation observed can be easily

related to the structure of the parent substance (32,33). When combined with gas chromatography, this cracking technique can be used in the same manner as that developed by Zemany (34) who obtained pyrolysis "fingerprints" by feeding the fragments to a mass spectrograph. Instead of the mass spectrograph readout, by introducing the cracked sample to a chromatographic column, it is possible to obtain a chromatogram of the pyrolysis products which record is unique for any given substance. The procedures worked out originally by Zemany for the study of cracking patterns have been adapted for pyrolysis-gas chromatography to yield a method for identifying organic substances (35). The technique has been applied to the identification of various polymers (36), to the detection of the phenyl ring in organic compounds (37), to the identification of porphyrins (38) and to the identification of various purines and pyrimidines (39).[1]

The pattern of products observed for any particular compound has been found to be qualitatively insensitive to relatively large changes in pyrolysis conditions. For instance, the spectrum of products found by Jennings and Dimick for pyrolyses carried out at an estimated temperature of 800°C. was identical to that found for pyrolyses performed at an estimated temperature of 1300°C. Hence, an accurate qualitative identification of a compound can be obtained without much regard to the conditions of pyrolysis. Nevertheless, when quantitative information is desired, much more stringent limitations must be placed on the conditions of pyrolysis to yield meaningful results. Levy has found (40) that both the temperature and the pressure

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[1] These workers studied the decomposition of guanine, 2,6-dichloro-7-methyl purine, 2-amino-4-chloro-6-methyl pyrimidine, thymine, cytosine and isocytosine.

play an important role in the quantitative yields of pyrolysis products. The same variables which influence the yields of any chemical reaction, as would be expected, also alter the yield of products obtained from a pyrolysis. Unfortunately, even more factors than these can influence the quantitative results obtained. In particular, for the pyrolyses performed using a heated filament, both the rate of heating of the coil and the temperature gradient along the filament would be expected to influence the yield of products. The method of applying the sample to this coil could also influence the results. To deal with these problems, Levy delivered an initial large pulse of current to the coil and followed it with a relatively high steady current. The initial current pulse reduces the time taken for the coil to reach the desired pyrolysis temperature. The temperature gradient along the coil is presumed to be negligible once the pyrolysis temperature has been reached. The sample which might be distributed unevenly along the coil would thus all degrade at essentially the same temperature.

Since it was possible[1] to identify purines and pyrimidines separately, it was expected that their fingerprints would also be found on pyrolysing a polymer (a nucleic acid) of which they form a part. It was hoped that the peak areas attributed to these bases could be related to the relative amounts of the bases in the nucleic acid molecule.

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[1]Reference #39

OPEN TUBULAR COLUMNS

One further development which has facilitated the study of complex mixtures is that of the open tubular column. In 1957 Golay (41,42) suggested that the packing ought to be eliminated from the chromatographic column. His theoretical consideration of the chromatographic process (43) led him to experiment with well defined open tubular columns (0.010" to 0.620" I.D.) coated with a very thin film of stationary liquid. Subsequent work has revealed the high efficiency and applicability of these columns (44). The columns are of particular advantage for very fast separations and for separations requiring a large number of theoretical plates.* The limit to the number of plates attainable with these columns is governed solely by the time limit the analyst places on the separation. Columns (which may be as long as desired) exhibiting as many as 10^6 theoretical plates have been used. The main disadvantage to the widespread use of these columns lies in their inability to handle large samples. The method used to prepare these columns (45,46) leaves only a small amount of liquid phase coated on the capillary walls. This low liquid load can deal only with a small sample (47) and hence splitting devices must be used in conjunction with these columns. To overcome this sample limitation, attempts have been made to enlarge the surface area of the capillary. Using Golay's original suggestion (48,49) the inside of the tubular column has been coated with a fine inert support material (50). This procedure uses both the advantage of the large surface area

*The definition of a "theoretical plate" is found in the appendix.

of a support and the advantage of the "openness" of the tubular column as emphasised by Golay. Alternatively, the columns have been etched to effect the same sort of increase in surface area (51). Other modifications of the tubular columns are also being studied (52, 53). At the present stage of development, these columns appear to be of great assistance in the study of multi-component mixtures such as fuel oil fractions (54, 55). The columns do not, however, seem to be of particular advantage in trace analyses where the limitations of the detection system are such that the sample containing the minimum amount of detectable impurity is already too large for the tubular column to process. Hence they do not appear advantageous for the study of trace components in sweat.

METHODS OF IDENTIFYING CHROMATOGRAPHIC PEAKS

Once all the components of a mixture have been separated, the difficult task of identification can be started. Unless the technique is being used just as a process control, the identity of the substances appearing on the chromatogram will not be known. The job of identifying the separated components can often be quite laborious when no clues as to the nature of the particular fraction are known. On the other hand, identification becomes fairly routine when a good guess of the composition of the mixture can be made. The method most commonly used for identification purposes compares the behaviour of some unknown peak on a chromatogram with that of a test compound. If the behaviour of the two substances appears to be the same on at least two different columns, identity is assumed. Many other methods are used to identify

chromatographic peaks. These techniques are discussed in the appendix. In many cases a combination of the available techniques will generally be required.

Once the peaks have been identified, quantitative curves can be prepared by plotting detector response (in terms of peak area) versus the amount of sample injected. Difficulties are sometimes encountered with this procedure as a result of defects inherent in the injection system, as a result of anomalous detector response (56), or because of unpredictable column behaviour (57). With a knowledge of the problems such as these which may arise, many complications may be avoided and a suitable procedure to obtain quantitative as well as qualitative information from the chromatograms may be developed.

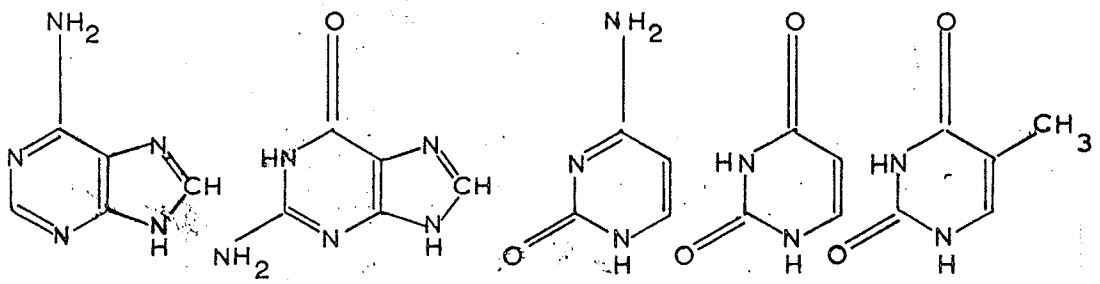
NUCLEIC ACIDS

The combination of chemical, cytological and genetic studies has led to the realisation that nucleic acids are of fundamental importance in controlling the metabolism, reproduction and growth of living systems. Since the work of Avery, Macleod and McCarty (58), experimental evidence has accumulated which confirms the fact that it is the nucleic acid which carries the genetic information in an organism. In order that the nucleic acids may convey information, a pattern or code must be incorporated into their chemical structure (59). The nucleic acids are co-polymers in which the monomers, known as nucleotides, consist of purine or pyrimidine bases linked to a sugar-phosphate (Figure 2).

FIGURE 2.[1] The Purines, Pyrimidines, Their Nucleosides, and a Schematic Deoxyribose Nucleic Acid.

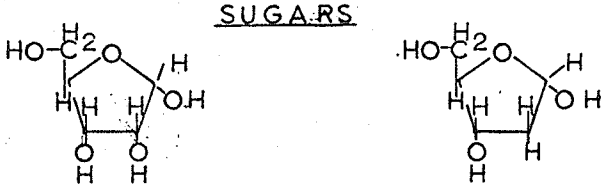
[1]Only the compounds used in the pyrolysis studies are depicted.

PURINES and PYRIMIDINES



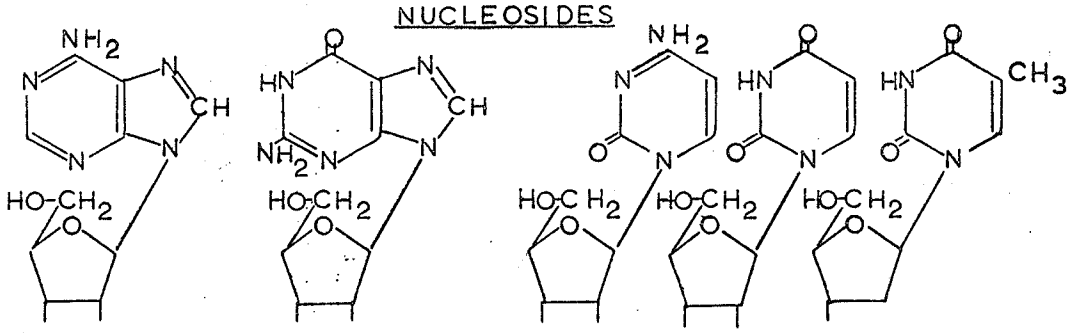
Adenine Guanine Cytosine Uracil Thymine

SUGARS



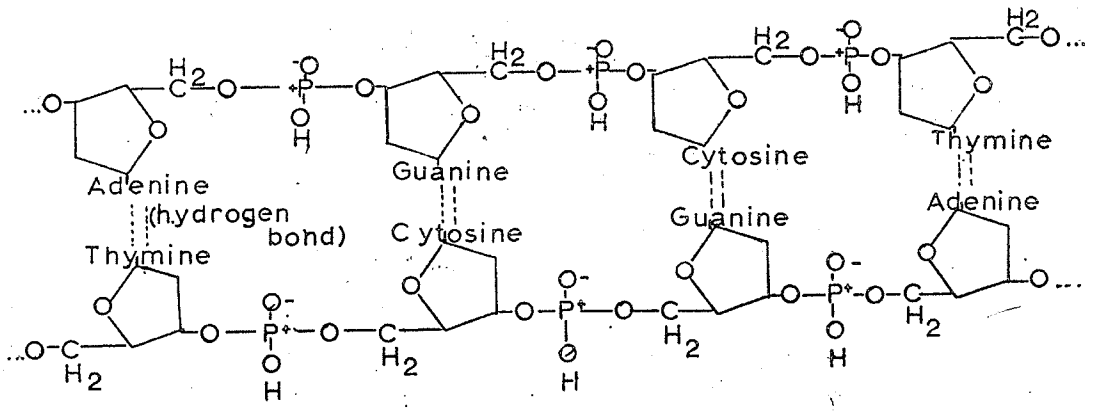
α-D-Ribofuranose α-2-Deoxy-D-Ribofuranose

NUCLEOSIDES



Adenosine Guanosine Cytidine Uridine Thymidine

A SCHEMATIC* D.N.A.



*The formula of each base is denoted by a word.

In any one nucleic acid, there is only one kind of sugar, but there are four different purines and pyrimidines. One strand of the deoxyribose nucleic acid (DNA) - (and we are here not dealing with the ribose nucleic acids (RNA)) - is formed from a backbone of deoxyribose sugar units linked by phosphate bonds from the 3' position on one furanose ring to the 5' position on the next ring. To this chain, the purine and pyrimidine bases are attatched at the 1' position. Two such complementary strands (60) are held together by hydrogen bonds to form the DNA macromolecule. The sequence of nucleotides along this strand is thought to spell out the information the molecule contains (60-A).

The present methods of analysing these nucleic acids for their base composition are quite routine and very satisfactory for most purposes (61,62). After the isolation of the nucleic acid, the analysis of the components is usually effected by a hydrolysis of the chain in conjunction with some form of chromatography (ion-exchange, paper, or electrophoresis). After the separation of the components, spectrophotometric methods are used to complete the analysis. From the viewpoint of a gas chromatographer interested in pyrolysis techniques, however, the analysis of these nucleic acids poses an intriguing problem. As has been mentioned, the pyrolysis study of various purines and pyrmidines has been published concurrent with unrelated work on the application of pyrolysis techniques to the study of polymers. The extension of these techniques to the study of nucleosides, nucleotides and finally of the intact DNA molecule has not been reported.

Work has been done on the gas chromatographic separation of the intact nucleosides (63). However, this method still requires a hydrolysis of the nucleic acid followed by a reaction of the nucleosides to yield a compound suitable for the purposes of gas chromatographic analysis. It was hoped that a pyrolysis study would eventually prove useful in developing a method to measure the DNA content of single cells. Potentially the method could yield a simple, sensitive one-step procedure for this purpose. The use of pyrolysis gas chromatography to characterise the purines, pyrimidines, their corresponding nucleosides and the nucleic acids (DNA) from two different sources was thus investigated.

PERSPIRATION

Mention of the reluctance to use sweat as a routine sampling fluid to indicate the body's state of affairs has recently appeared in the Lancet (64). Foster suggests that biological changes in the more important but less accessable body secretions should be reflected by similar changes in the composition of sweat. The only routinely used test using sweat correlates an abnormal sodium chloride level in sweat with the incidence of cystic fibrosis (65). In 1960, Smith and coworkers (66, 67) reported the presence of a smelly component in the sweat of chronic schizophrenic patients which was not present in the sweat of non-schizophrenic patients. Smith has found that the odour disappears when an extract of the sweat is made alkaline. These workers

have been trying with little success to identify this substance with the aid of a gas chromatograph (68). To collect the sweat, a stripped subject is placed in a polyethylene bag which is sealed at the patient's neck. After a rest period in a warm room, the subject is helped out of the bag and the sweat is poured off. Up to 400 cc. of sweat have been obtained in one sitting by this method. The sweat collected is subjected to an extraction and concentration procedure. An ether extract is finally injected into a gas chromatograph.

Work by Posner and coworkers (69) places the cause for the odour on the action of an organism, pseudomonas aeruginosa. Presumably some substance is degraded on the skin surface to yield the smelly compound (70). This rather indirect source of the odour suggests that there is little chance of a correlation between the smell observed and a possible primary metabolic defect common to schizophrenics. Perhaps a good analogy to this situation is found in the observed presence of acetone in the breath of diabetic patients. Here, the knowledge that it is acetone which is eliminated does not aid significantly in the elucidation of the rather complex disease.

The odiferous substance peculiar to some schizophrenics, however, has not been identified as yet. A gas chromatograph, when used to analyse the sweat volatiles from both "normal"* and schizophrenic patients, should reveal this compound as an anomalous peak in the set of chromatograms obtained from the schizophrenics. Smith has apparently found

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* here "normal" refers to non-schizophrenic subjects.

trouble in obtaining a "standard" set of chromatograms with which to compare those of schizophrenic sweat. It was felt that the search for the odorous substance in sweat would be made easier if contaminant-free sweat could be collected. The method of collection used by Smith provides no means of excluding skin contaminants from the sweat obtained. To eliminate contaminants from the sweat, Hurley and Shelley have shown (70) that a cleansing of the skin surface to be sampled must be performed. It was hoped that "pure" sweat samples collected by a method similar to that developed by Dubowski (71) would yield a consistent set of chromatograms in which any anomalous peak would be apparent. The subsequent use of the identification procedures available (see appendix) would ascertain the identity of the odd peak.

In a wider sense, the development of a method for the gas chromatographic analysis of the components of sweat would be of use not only for the "difference detector" purposes required for these investigations, but also for the development of other routine "sweat tests" for clinical use. Sweat contains many constituents but all of these appear in such small amounts that the total solid components comprise only 0.3 to 0.8% of the total mass (72). It is thus at once the most dilute and perhaps the least interesting (from an analytical point of view) of human secretions. The literature dealing with the chemistry of sweat is very large and quite contradictory in many places, suggestive of some inadequacy of technique coupled with a multiplicity of factors.

In his monograph, Kuno (72) gives an extensive review of the many aspects of perspiration that have been investigated. Tables 1-A to 1-C list the common organic and inorganic constituents to be found in sweat. The low levels of concentration of organic substances in this saline aqueous medium are immediately apparent. Even a relatively high-level component such as lactic acid appears only at concentrations ranging from one to three micrograms per microlitre. The analysis of very dilute aqueous solutions is not an easy task for the gas chromatograph (73,74,75), but special techniques may be devised to overcome this problem.

TABLE[1] I-A. Inorganic Constituents of Sweat

Constituent	Range of Amounts Reported (Mgm. percent)[2]
Cl	36 - 995
Na	17 - 400
K	7 - 145
Ca	0.3 - 11.8
Mg	0.02 - 3.32
P	None - 4.8
S	Trace - 7.37
SO_4	4 - 17
I	0.0007 - 0.00095
Cu	0.006
Mn	0.006
Fe	0.024 - 0.2

[1]From Kuno (72) p.224.

[2]Mgm. percent:milligrams of compound per 100 cm^3 of sweat

TABLE[3] 1-B. Nitrogen Compounds Found in Sweat

Constituent	Range of Amounts Reported (Mgm. percent)
Total Nitrogen	17 - 145
Non-Protein-N	66 - 108
Amino acid N	1 - 8
Ammonia N	10 - 35
Urea N	7.5 - 128
Uric acid N	0 - 1.2
Creatinine N	0.2 - 8.6
Creatine N	trace

TABLE[4] 1-C. Sugar and Its Metabolites Found in Sweat.

Constituent	Range of Amounts Reported (Mgm. percent)
Glucose	1 - 25.6
Lactic Acid	33 - 300
Pyruvic acid	0.90- 6.90

[3] From Kuno (72) p.235.

[4] From Kuno (72) p.241.

EXPERIMENTAL

INITIAL PREPARATIONS

A gas chromatographic study of the sort attempted necessitates a rather complete supply of equipment and materials. Any difficult problem of separation and identification which may arise must be met with a variety of columns and techniques. The first work on this project was thus concerned with the preparation of a variety of columns and column packings (see Tables 2, 3 and 6 and the section dealing with "materials"). Three detection systems were readied for use, and a fourth apparatus employing a gas density balance was designed. The materials for the latter apparatus were purchased so that it could be rapidly constructed when needed. More specialised equipment for the pyrolysis study and for the analysis of sweat was either purchased or constructed according to the requirements of these projects.

APPARATUS FOR GAS CHROMATOGRAPHY

The use of three, and possibly four, types of detectors was anticipated:

THE AEROGRAPH A-600-B HY-FI GAS CHROMATOGRAPH

The "Hy-Fi" gas chromatograph (Wilkins Instrument and Research Inc., Walnut Creek, Calif.) is equipped with a hydrogen flame ionisation detector, an electron capture detector, a sample stream splitter, a sample port pre-heater, and an oven temperature control. The temperature of the pre-heater and oven is determined by the setting of two

variable transformers and by the rate of heat loss to the surroundings. No provision is made for accurate temperature control or for reproducible temperature programming. A fan is mounted inside the electrically heated oven to minimise any temperature inhomogenies that may affect the detector and column.

The nitrogen and hydrogen used as carrier gas and combustion fuel respectively were taken from cylinders fitted with conventional pressure regulators. Both cylinders were fitted with home-made molecular sieve (Linde 5A) filters and the hydrogen line was provided with a restrictor (Wilkens) to stabilise the fuel supply. Air was supplied to the detector head at a rate of about 300 ml/minute by an Oscar "55" Aerator supplied with the chromatograph. In several experiments commercial oxygen purified over copper oxide at 800°C. was supplied to the detector instead of air. This procedure reduced the background noise level of the instrument. A molecular sieve supplied with the chromatograph was installed between the detector and the air or oxygen supply. Flow rates were measured in all cases with a soap bubble flowmeter.

Columns for the "Hy-Fi" were made either from 1/8" O.D. (0.06" I.D.) refrigerator tubing or from 1/8" O.D. #12 thin-walled teflon spaghetti tubing. Column lengths of one, two and three metres were used. A vibrating apparatus was adapted (76) to aid in the packing of the copper columns. Open tubular columns, coated according to the method described by Condon (77), had been prepared (78) and were available for use.

For the purposes of a technique to be described for use in conjunc-

tion with pyrolysis gas chromatography, the 1/8" copper columns were provided with an aluminum foil envelope which enclosed the first two coils of the column. The small column jacket was formed with an opening at the top in order that it might be filled with liquid nitrogen.

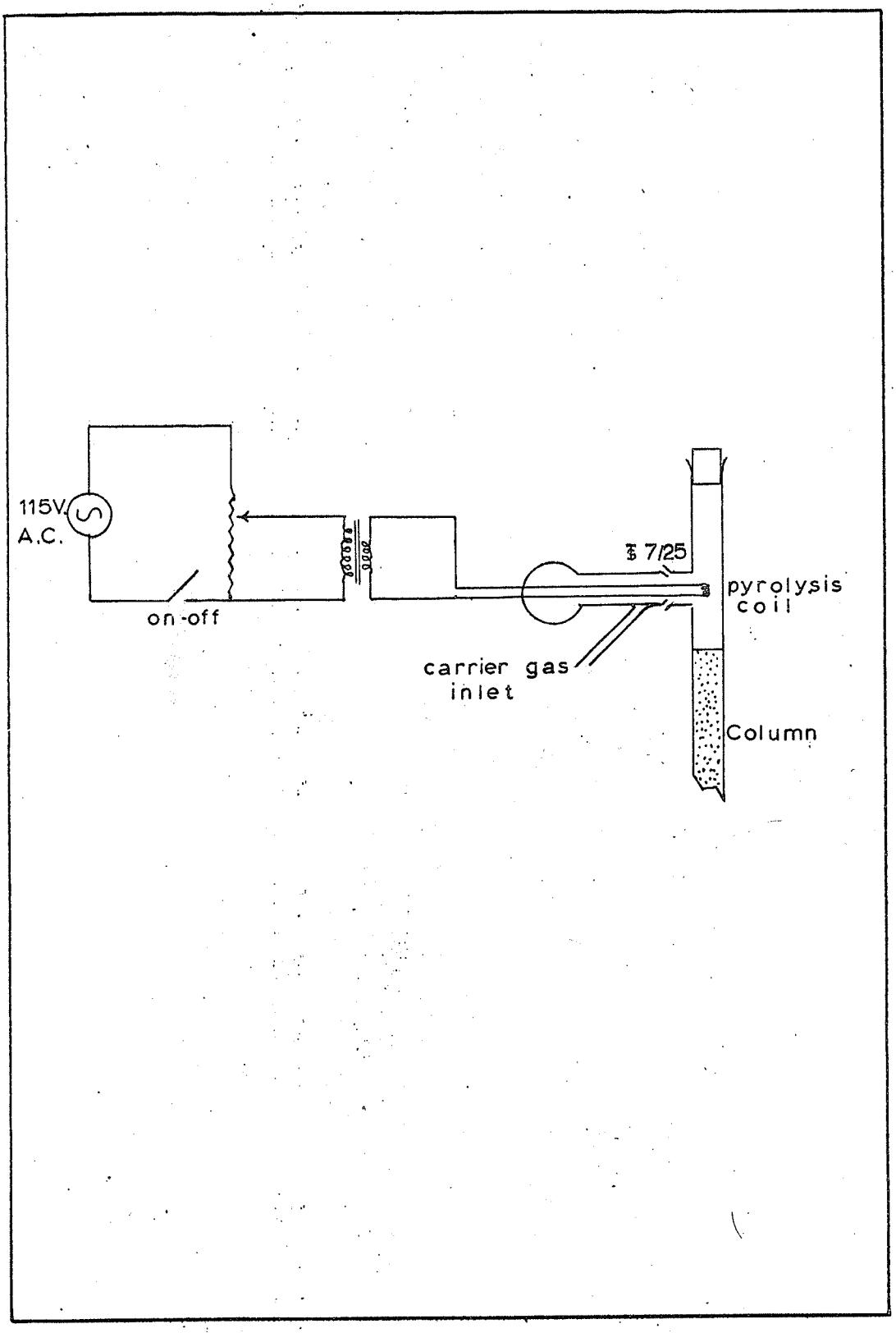
THE PYE ARGON CHROMATOGRAPH

The Pye Argon Chromatograph (W.G. Pye & Co., Cambridge, Eng.) is equipped with a large volume ionisation detector of the Lovelock type (79). Both Radium D and Tritium activated detectors were available. The latter offers the lowest background noise. The chromatograph is provided with a rough ($\pm$ 2°C.) mercury actuated thermostat which permits isothermal operation at 25°C. intervals between 25 and 250°C. The detector voltage supply permits settings between 750 and 2000 volts, which arrangement allows the instrument to be used in a versatile manner (80). Only three signal attenuation settings are provided.

The Argon (Linde) carrier gas was used directly and was metered with reducing valve fitted with a needle valve. Flow rates for this apparatus were also measured with a soap bubble flow meter.

The 4 mm. I.D. Pyrex columns for this chromatograph come in 4.5 to 5' lengths. They are packed by plugging the bottom with a piece of glass rope and sifting the packing material through the top. These columns were modified so that the chromatograph could be used for pyrolysis studies. The carrier gas inlet was altered so that the platinum pyrolysis coil could be inserted in the gas stream. The tip of the coil was situated directly above the column packing as shown in Figure 3.

FIGURE 3. A Pyrolysis Accessory for the Pye Argon Chromatograph.


115V.
A.C.
on-off
7/25
pyrolysis
coil
carrier gas
inlet
Column

Current was delivered to the coil via a step-down transformer and a variable autotransformer. The pyrolysis temperature is determined by the variac setting and the time of the pyrolysis is controlled with the on-off switch on the variac. The glass columns permitted a visual estimate of the pyrolysis temperature. A good feature of this apparatus was the provision for the introduction of samples onto the column through the usual injection septum without removing the coil from the column.

THE THERMAL CONDUCTIVITY DETECTOR

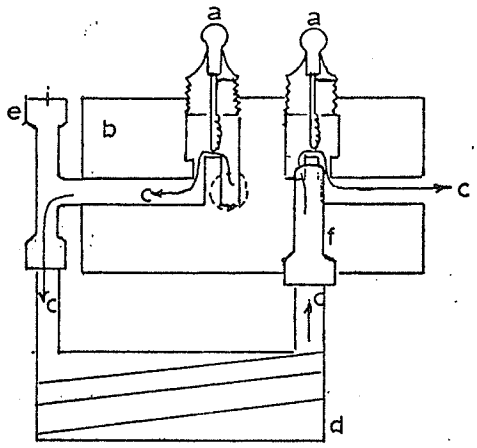
A home-made thermal conductivity apparatus using model airplane engine glow plugs (81) as detecting elements was constructed. The detector head is provided with a 50 watt finger heating element that can be controlled by a variac. The geometry of the apparatus is such (Figure 4) that the column can be provided with an immersion-type temperature control. Columns are made of 1/4" O.D. copper tubing and are packed in the same manner as the glass columns. The helium used as carrier gas is regulated by the same apparatus used for the Pye Chromatograph. The detector bridge can use the full six volt range of the battery supply. Several signal attenuations are provided.

THE GAS DENSITY BALANCE DETECTOR

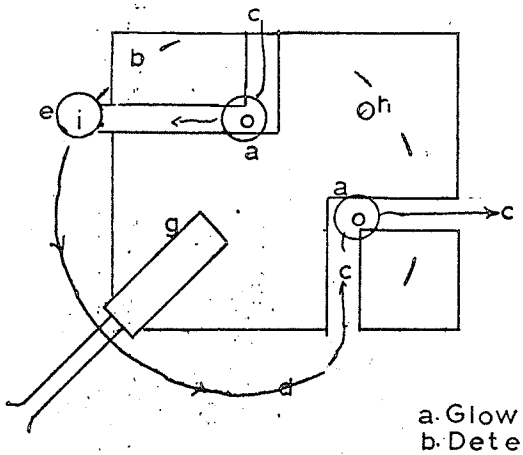
A Gow-Mac Gas density detector was purchased from the Gow-Mac Instrument Company (Madison, N.J.). Plans were drawn up (see Figure 5) for an apparatus in which provision was made for separate column and detector

FIGURE 4. A Thermal Conductivity Detector
Using Model Airplane Glow Plugs.

SIDE VIEW



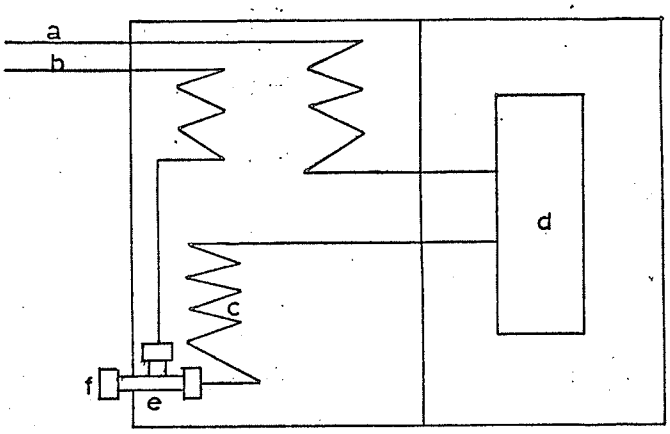
TOP VIEW



a. Glow Plugs
b. Detector Block
c. Carrier Gas Flow
d. Column
e. Swagelok Tee
f. Elbow Union
g. Heater
h. Thermometer Hole
i. Injection Septum

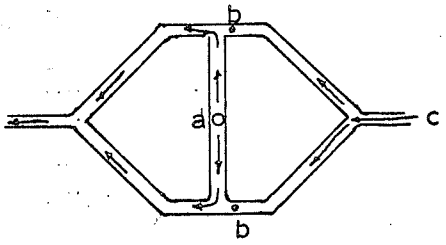
FIGURE 5. A Gas Chromatograph Using a Gas Density Balance Detector.

TOP VIEW



a. Reference Stream
b. Carrier Stream
c. Column
d. Detector
e. Swagelok Tee
f. Injection Septum

GAS DENSITY DETECTOR



a. Carrier Stream
b. Detector Element
c. Reference Stream

temperature control and for the use of 1/8" or 1/4" O.D.columns. Preheating of the reference stream is also required. The bridge circuit used is similar to that employed in the thermal conductivity apparatus.

SAMPLE INJECTION EQUIPMENT

In most cases microlitre syringes provided by the Hamilton Co. were used to dispense the liquid samples. Samples were either injected directly onto the column through a silicone rubber seal or applied to the platinum coil of the pyrolysis apparatus. For the retention time studies on known compounds, the "nano-jector" syringes purchased from the Scientific Kit Company (P.O. Box 244, Washington, Pa.) were used.

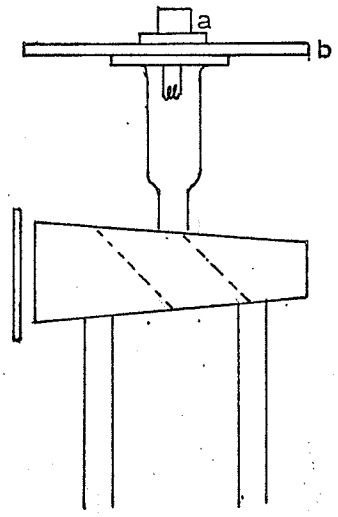
COLUMN CONDITIONER

To precondition the columns for use, a transite "prep" box with heating elements and Swagelok column fittings was constructed. Two columns could be conditioned simultaneously.

STEAM GENERATOR

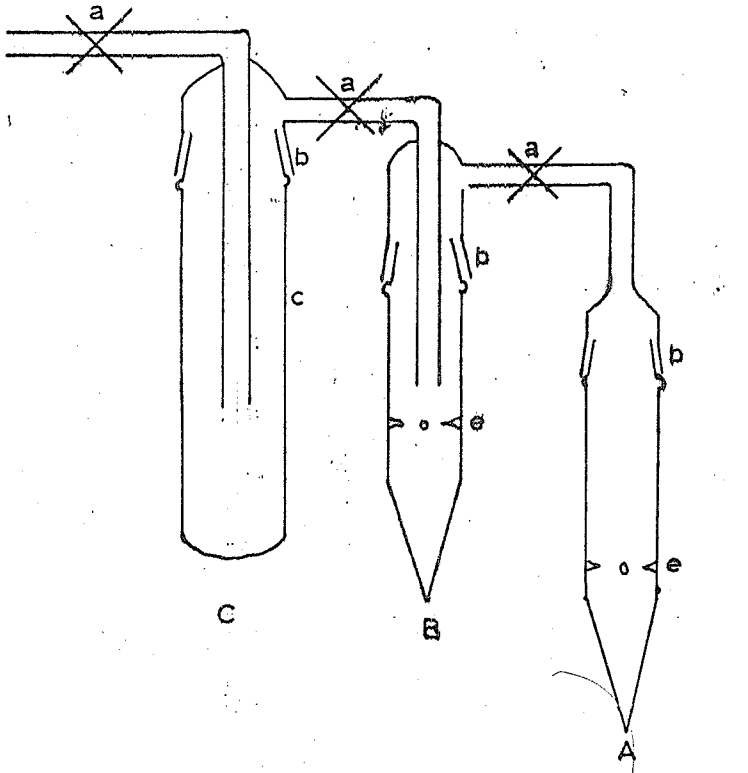
A model #A-675 Steam Generator was purchased from Wilkins Instrument and Research Inc. so that steam could be used as a carrier gas for the chromatography of polar compounds (82).

FIGURE 6. A Vacuum Apparatus for Removing Solvent From a Pyrolysis Coil.

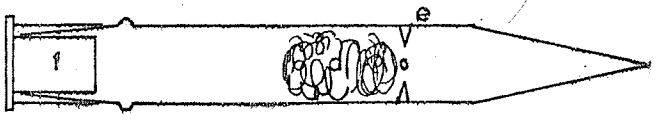


a. Pyrolysis Coil
b. Silicone Rubber Washer

FIGURE 7.-A and 7-B. Diagrams Showing the Apparatus for the Fractionation of Sweat and the Centrifuge Tubes Modified for Sweat Collection.



SIDE VIEW



END VIEW

a. Stopcocks
b. ⅀ 24/40
c. Liquid Nitrogen Trap
d. Gauze Sponge
e. Indentations
f. Capall, Size D

drawn out so that they might function as centrifuge tubes. Four indentations were made around the side to hold up the material (gauze or otherwise) from which the sweat was centrifugally squeezed out. After removal of the collection pad, the tubes could be connected into the vacuum apparatus for processing,[1] or they could be stoppered in a convenient manner and stored. Larger centrifuge tubes were adapted in a similar manner (Figure 7-B) to be used for the collection of greater volumes of sweat. For these larger volumes, the vacuum rack could be used to fractionate the sweat by fitting it with bigger ground glass joint collection flasks.

The centrifuge used to squeeze out the pads was a precision universal centrifuge (Precision Scientific Catalogue #67310). The maximum rotor speed (2200 R.P.M.) was used at all times.

Sweat samples were stored in a freezer at -36°C. This temperature would arrest the growth of any organisms present in the sweat collected.

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[1] See p.46

MATERIALS FOR GAS CHROMATOGRAPHY

LIQUID PHASES

A list of the available liquid phases, along with their specifications, is given in Table 2. The source of the materials is also indicated.

TABLE 2. Stationary Phases for Gas Chromatography

	Stationary Phase	Maximum temperature (°C)	Application	Supplier[1]
1.	Apiezon L	300	polar & non-polar compounds	
2.	Armeen S.D.	100	Amines	M.
3.	Benzyl Diphenyl*[2]	120	Aromatic compounds & halogen compounds in general	M.&B.
4.	1,4 Butanediol Succinate	225	polar compounds (fatty acid methyl esters)	M.& B.
5.	Carbowax 400	100	polar compounds	W.
6.	Carbowax 600	125	polar compounds	W.
7.	Carbowax 1540	150	polar compounds	W.
8.	Carbowax 6000	175	polar compounds	W.
9.	Carbowax 20M	250	polar compounds	W.
10.	Diglycerol*	150	phenols, alcohols, aliphatic amines	M.& B.
11.	Diethylene glycol Adipate (LAC-446)	225	Fatty acid methyl esters.	W.

continued.

TABLE 2 CONTINUED/

Stationary Phase	Maximum temperature (°C)	Application	Supplier[1]
12. Hallcomid	150	polar compounds (alcohols,ketones)	W.
13. Hallcomid-M-18	150	"	W.
14. Paraffin wax	200	hydrocarbons	
15. Paraffin oil (Nujol)	100	"	
16. Poly (M) phenyl ether	250	polar compounds	W.
17. Polyethylene glycol succinate	180	polar compounds (e.g. fatty acid esters).	W.
18. Reoplex 400 (polypropylene glycol adipate)	190	polar compounds	W.
19. Silicone SE-30 (gum rubber)	300+	polar and non-polar materials	W.
20. Silicone DC-710 (fluid)	300	some aromatic selectivity	W.
21. Silicone GE-SF-96 (fluid)	300	polar and non-polar	W.
22. Silicone SE-52 (gum rubber)	300+	some aromatic selectivity	W.
23. Silicone DC-11 (fluid)	300	polynuclear compounds	W.
24. Silicone QF-1 (FS-1265) (fluid)	250	halogen compounds	W.
25. Squalane*	160	hydrocarbons	W.
26. Tetraethylene glycol dimethyl ether	80	polar compounds	E.
27. Tetrahydroxyethyl-ethylenediamine (THEED)	135	nitrogen containing compounds and glycols.	W.
28. Ucon polar	200	alcohols and other polar compounds.	W.

continued

TABLE 2 CONTINUED/

Stationary Phase	Maximum temperature (°C)	Application	Supplier[1]
29. Versamid 900	450	nitrogen containing compounds.	W.

[1] Key to list of suppliers: E. - Eastman - Kodak
M. - Microtek - Distanal
M.& B.- May and Baker
W. - Wilkins Inst. & Research.

2. Stationary phases marked with an asterisk are recommended by the London Symposium Committee (1956).

SOLID SUPPORTS

A number of solid supports were prepared for use:

Wilkins Firebrick,Regular.

This size-graded (60-80 ASTM mesh) pink support was first flotated and dried in order to remove any fine particles. According to the purposes in mind, the support was either acid washed, base washed, or treated with hexamethyl disilazane (84), to improve the surface characteristics of the material. A similar commercial support, Neutraport (Distanal Research) was also used to prepare various column packings.

Teflon 6.

The fluorocarbon support (60/70 mesh) was used directly as supplied by the manufacturer (Analytical Engineering Laboratories). Special precautions are required for the use of this support (85).

COLUMN PACKINGS

The column packings (Table 3) for these investigations were prepared in the following manner:

A weighed amount of liquid phase was dissolved in a suitable solvent. To this solution, the desired amount of support material was added and the mixture was allowed to stand for fifteen minutes with occasional stirring. The slurry was then poured into a flat pyrex utility dish from which the solvent was allowed to evaporate. The dry packing was then heated in a drying oven at 110°C. for several hours to remove any remaining volatiles, and was stored for subsequent use in air tight containers.

The percent of liquid phase was calculated:

$$\% \text{ liquid phase, } w/w = \frac{\text{Weight of liquid used}}{\text{Weight of solid support}} \times 100$$

TABLE 3. Column Packings for Gas Chromatography

Liquid Phase	Support and Mesh Size	Support Treatment	Composition w/w
1. Carbowax 6000	60/80 Wilkins Firebrick regular	None	10%
2. Carbowax 1540	60/80 Neutraport -S (Microtek-Distanal)	None	2%
3. Diethylene glycol adipate-H_3PO_4	60/80 Wilkins Firebrick Regular	hexamethyl-disilazane (HMDS)	25%-2%
4. Diethylene glycol adipate -H_3PO_4	"	HMDS	2%-0.2%

continued

TABLE 3 CONTINUED/

Liquid Phase	Support and Mesh Size	Support Treatment	Composition w/w
5. Hallcomid - KOH	60/80 Wilkins Firebrick Regular	acid washed base washed	15% - 10%
6. Hallcomid-M 18-Carbowax 600[1]	Johns-Manville Chromosorb W, 60/80 mesh	HMDS	3.8% - 0.5%
7. Polyethylene glycol succinate-H_3PO_4	60/80 Wilkins Firebrick Regular	HMDS	2% - 0.5%
8. Polyethylene glycol succinate -H_3PO_4	60/70 teflon "6" (Analabs)	None	1% - 0.4%
9. Silicone SE-30	60/80 Wilkins Firebrick, Regular	None	10%
10. "	"	HMDS	2%
11. Silicone SE-30	60/80 Neutraport (Microtek-Distanal)	None	10%
12. Silicone DC-QF-1	60/80 Wilkins Firebrick Regular	HMDS	10%
13. Tetraethylene glycol dimethyl ether	60/80 Wilkins Firebrick Regular	Base washed	10%
14. Ucon Polar	60/80 Wilkins Firebrick, Regular	HMDS	2%
15. Versamid 900	60/80 Wilkins Firebrick, Regular	HMDS	10%

[1]Graciously provided by R. Levy of the Dept. of Plant Science, University of Manitoba.

MATERIALS FOR SWEAT COLLECTION

A list of the materials used for sweat collection is given in Table 4. The gauze sponges were treated prior to their use for collecting sweat. An extraction of the pads with ether and acetone followed by an oven drying of the pads was first tried. This procedure left too many volatile materials on the pads for the purposes of the gas chromatographic analysis. In the second instance, the sponges were first boiled in several portions of distilled water and then squeezed out and vacuum dried at 80°C. This treatment seemed adequate to remove any volatile impurities from the pads.

TABLE 4. Materials For Sweat Collection

Item	Supplier
Capalls (Size D)	Fisher Scientific
Centrifuge tubes (15 and 100 ml capacity)	"
Collection bottles (2 oz., wide-mouth)	"
Gauze sponges ("Softloom" 3"x3", 12-ply)	The Stevens Companies (Winnipeg)
Micropore Surgical Tape (3-M brand 3"x10 yd)	"
pHisoHex skin cleanser	Winthrop Laboratories of Canada Ltd., Aurora, Ont.
Teflon sheeting (2 mil)	Johnson Plastics (Winnipeg)

The teflon sheeting was cut into 4-inch squares and cleaned for subsequent use. The collection bottles were also cleaned before use.

CHEMICALS

A list of the chemicals used in the pyrolysis studies is given in Table 5. Except for the quaternary ammonium salt, these compounds were graciously provided by Dr. L.H.Cohen of the University of Manitoba Biochemistry Department. The quaternary salt was prepared in the laboratory.

An aqueous 1% phosphoric acid solvent was used to prepare solutions of the purines, pyrimidines, and nucleosides, of the ribose sugar and of the nucleic acids. Unfortunately no deoxyribose sugar was available for these studies. The quarternary salt was dissolved in ethanol.

For identification purposes, compounds were taken from the sample storeroom. These substances were not always chromatographically pure, but there was never any doubt as to which peak belonged to the compound in question.

The stock solutions of acids and bases used in these studies were prepared from reagent grade chemicals using distilled water from the laboratory supply. The distilled water gave several peaks when injected into the chromatograph. However, water distilled from an alkaline, aqueous potassium permanganate solution gave the same peaks. It is thought that these peaks were not from impurities in the water but were

caused by organic compounds that the water may have displaced from the chromatographic column. The solvents used for the pyrolysis studies were checked for dissolved, non-volatile impurities by using them as blanks in the pyrolysis procedure.

TABLE 5. Chemicals Used For Pyrolysis Studies.

Compound	Source[1]
Adenine	Calbiochem.
Adenosine	"
Cytosine	"
Cytidine	"
DNA-Calf thymus	Courtesy of Dr.L.H. Cohen
DNA-aerobacter arogenes	"
Guanine	Calbiochem.
Guanosine	"
D-ribose	"
Thymine	"
Thymidine	"
Triethyl methyl ammonium iodide	Prepared
Uracil	Calbiochem.
Uridine	"

[1]Key to source: "Calbiochem": Biochemicals purchased from Calbiochem (1962) and graciously provided by Dr. L.H. Cohen of the Biochemistry Department at the University of Manitoba Medical School.
"Prepared": The quarternary salt was prepared in the laboratory from stock chemicals.

PROCEDURE

COLUMN PREPARATION

Table 6 lists the columns prepared for these studies. The 1/8" copper columns were made by sifting the prepared packing through a funnel into the top of the tubing, the bottom of which was plugged by a piece of glass rope. A motor driven hex-nut was rotated against the side of the tube to speed up the operation and to render the packing more uniform. Subsequently the top of the column was plugged with a piece of glass rope and the tubing was wound on a 2" diameter cardboard mandril, fashioned from an old mailing tube. The teflon columns were filled by suspending the length of tubing in a stairwell. A weight was attached to the bottom to keep the column taught during the packing procedure. The packing was introduced through a funnel at the top. Constant tapping of the clamp holding the column while it was being filled served to settle the packing.

TABLE 6. Columns For Gas Chromatography[1]

Packing	Number in Table 3	Column Dimensions	Column Material
1. 2% Carbowax 1540	#2	1/8" O.D. x 6'6 3/4" (0.33 cm x 2 meters)	Copper refrigerator tubing
2. 15% Hallcomid -10% KOH	#5	1/8"O.D. x 6'6 3/4" (0.33 cm x 2 meters)	"
3. 3.8% Hallcomid-M-18 0.5% Carbowax 600	#6	1/8"O.D. x 9'10 1/8" (0.33 x 3 meters)	thin walled #12 teflon spaghetti tubing
4. 2% Polyethylene glycol succinate-0.5% H_3PO_4	#7	1/8"O.D. x 6'6 3/4" (0.33 cm x 2 meters)	copper refrigerator tubing

continued

TABLE 6 CONTINUED

Packing	Number in Table 3	Column Dimensions	Column material
5. 1% Polyethylene glycol succinate - 0.4% H_3PO_4	#8	1/8"O.D. x 6'6$\frac{3}{4}$" (0.33 cm x 2 meters)	Thin-walled #12 teflon spaghetti tubing
6. 2% Silicone SE-30	#10	1/8"O.D. x 6'6$\frac{3}{4}$" (0.33 cm x 2 meters)	Copper refrigerator tubing
7. 10% Silicone SE-30 (on Neutraport-S)	#11	1/8"O.D. x 5'3" (0.33 cm x 1.6 meters)	"
8. 10% Silicone DC-QF-I	#12	1/8"O.D. x 6'6$\frac{3}{4}$" (0.33 cm x 2 meters)	"
9. 10% tetraethylene glycol dimethyl ether	#13	1/8" O.D.x 6'6$\frac{3}{4}$" (0.33 cm x 2 meters)	"
10. 2% Ucon polar	#14	1/8"O.D. x 5'3" (0.33 cm x 1.6 meters)	"

[1]See Table 3 for details of packing material.

PYROLYSIS STUDIES USING THE HY-FI CHROMATOGRAPH

A solution of the compound to be pyrolysed (usually containing from 10 to 50 µg) was applied to the platinum coil with a microlitre syringe. Two methods were used to remove the solvent:

(a) The solvent was driven off thermally by carefully heating the coil in the open laboratory.

(b) The vacuum chamber was used to evaporate the solvent. A low coil heat could be used to aid in this operation.

After the solvent was removed, the coil was replaced in the column head and the substance was pyrolysed from the coil. The pyrolysis temperature was determined by the powerstat setting, whereas the time of pyrolysis was measured by a stop watch. A seven second pyrolysis period was used in most cases, with a powerstat setting of #80.

For this procedure, care had to be taken to avoid contaminating the chromatographic system with vapours from the laboratory. The carrier gas valve was thus closed before the coil was removed from the column head. However, the valve to the pyrolysis chamber was left open to maintain a small positive pressure of carrier gas at the opening. A loose silicone rubber plug was placed in the opening as a further precaution to reduce the chance of contaminating the column. After the coil had been replaced in the socket, the carrier gas valve was reopened and the column was allowed to purge itself before the pyrolysis was performed. The temperature of the pyrolysis chamber was maintained about fifty degrees above the temperature of the column. Between each pyrolysis the coil was cleaned by firing it either in the air or in a stream of oxygen.

After the pyrolyses of the individual compounds were performed, pyrograms of mixtures of the bases and mixtures of the nucleosides were obtained. The mixed samples were made by withdrawing a certain volume of one solution (say containing x micrograms of component A) into a microlitre syringe and then withdrawing a volume of another solution (say containing y micrograms of component B) into the same syringe. The whole sample was then applied to the coil and pyrolysed.

The composition of the pyrolysed sample was expressed:

$$\%A = \frac{x}{x+y} + 100$$

This procedure was used to prepare samples containing from zero to one hundred percent of any one component. A constant total weight of sample was used for the mixtures of various compositions.

For most of the pyrolyses, the column was kept at a constant temperature. However, one procedure was developed which uses the principle of the programmed temperature technique to improve the presentation of the chromatographic analysis. Instead of programming the whole column, only the first portion (approximately 5 percent of the total length) was programmed. This was done immediately before a pyrolysis by lifting the oven lid just long enough to pour a small amount of liquid nitrogen into the jacket around the first coil of the column. The pyrolysis products would then issue onto a frozen column head. The small amount of liquid nitrogen (about 25 ml) would soon boil off and permit the first part of the column to warm up to the original oven temperature. By using the oven temperature control to apply a "heat pulse" to the column just as the nitrogen was added to the aluminum foil envelope, the overall oven temperature could be maintained within $\pm$ 2°C. of the starting column temperature.

PYROLYSIS STUDIES USING THE PYE CHROMATOGRAPH

Samples of tri-ethyl methyl ammonium iodide were pyrolysed using this apparatus in an attempt to evaluate the system for further use. An ethanol solution of the salt was applied to the coil and the

solvent was allowed to evaporate. After the coil was reinserted, the system was allowed to purge itself before pyrolysing the sample. Directly after the pyrolysis experiment, a synthetic mixture of compounds expected to result from the pyrolysis was injected. A comparison of the pyrogram and the chromatogram of the synthetic mixture could be made immediately.

SWEAT STUDIES

The use of the chromatograph to sense body odours was first demonstrated by injecting several mls of vapour withdrawn near the armpit of an unwashed volunteer onto a SE-30 column (Column #7). The peaks obtained suggested that the analysis of sweat for similar compounds would be feasible.

Before work could be done on the analysis of sweat, a clean, convenient method had to be developed for collecting the sweat. Furthermore, the procedure had to be standardised so that both groups of sweat samples (schizophrenic and normal) could be collected and analysed under identical conditions. In this manner, the chances of finding a significant difference in the two types of sweat samples would be optimised.

A method of sweat collection developed by Dubowski (86) was adapted to collect sweat from the various body areas. The area from which the sweat was to be collected was first washed well with pHisoHex soap and rinsed thoroughly with distilled water. The 3" x 3" gauze pads were used for this purpose. The area was dried and a clean gauze sponge was applied. The teflon cover was then placed on the pad with

the edges folded over so as to completely envelop the pad. The encased pad was then taped down securely to the skin surface. After a time sufficient to allow a desired amount of sweat to collect in the pad (usually 20-30 minutes in a warm environment resulted in 3-4 cm^3 of sweat depending on the subject's sweating rate), one end of the teflon envelope was lifted, and the pad was transferred using forceps to a collection bottle or to a centrifuge tube. The top of the container was first covered by taping the teflon sheet over the mouth and then secured with the screw-top or Capall stopper. The containers were then stored in a freezer. (On the field trips to Selkirk a picnic basket cooled with dry ice was used for this purpose). Subsequently, the sponges could be transferred to the specially prepared centrifuge tubes in which the sweat was wrung out.

Sweat collected by this procedure was obtained on two occasions from male patients under treatment at the Selkirk Mental Hospital. Both schizophrenic and non-schizophrenic patients living in the same ward were selected by the doctors at the hospital for this study. The patients were given a ten minute bath (the temperature of the water was about $39^{\circ}C$). Ivory soap was used in all cases for the bath. The body area to be sampled was then washed with pHisoHex and rinsed with distilled water. The collection bandage was taped on, and the patient was allowed to rest under blankets in a warm room for about an hour. Samples were taken from the armpit of each patient (this is the major source of the odour according to Smith) and also from the small of the

back in several cases. In addition to these samples, sweat was also collected in a similar manner from normal male volunteers interested in these analyses.

The sweat samples were treated in several ways. In all cases an attempt was made to handle the sweat as little as possible. It was felt that the normal concentration and extraction techniques used would introduce too many complicating impurities for the purposes of the gas chromatographic analysis. An analysis was first attempted on the vapours above the pads in the bottle. A small vapour sample (0.5 - 1.00 ml) was withdrawn from the sealed containers with a syringe and injected into the chromatograph. Secondly, the pads were transferred to the centrifuge tubes and were wrung out for several minutes at the maximum rotor speed of the centrifuge. The pad was removed from the tube which was capped with a Capall size D stopper. Several microlitres of this "raw" sweat were injected directly into the chromatograph. Finally the sweat was treated with the fractionating apparatus. The volatile acidic, basic and neutral fractions could be selectively vacuum distilled from the sweat in the collection tube. For example, to remove the volatile acid and neutral components, the sweat was first made acidic with a dilute phosphoric acid solution. The solution[1] was then frozen in liquid nitrogen and the system was evacuated[2] with a vacuum pump. As the frozen material warmed up, the volatiles distilled over into trap "B" of the apparatus (Figure 7). Liquid nitrogen was used in preference to other coolants to eliminate the chance of introducing impurities to the sweat sample at this point. The residue was then taken up in a dilute aqueous

[1] In trap "A".

[2] At trap "C".

(or other, if desired) potassium hydroxide solution. Both the distillate and the base solution were injected directly into the chromatograph without further treatment.

Various columns, conditions and techniques were used for the sweat analyses. The Silicone columns (#6, 7 and 8 of Table 6), the Phosphoric Acid-PEGS columns (#4 and 5 of Table 6) and the Carbowax 1540 and Ucon Polar columns (#1 and 10 of Table 6) were all used. In most cases a programmed temperature procedure was used to reduce the possibility of leaving non-volatile components on the column. In several cases a sub-ambient programmed temperature technique was tried by subjecting the whole column to a liquid nitrogen bath before injecting the sample. An aluminum foil envelope encasing the whole column was used for this method. After the nitrogen had boiled off, the column was heated up to a relatively high temperature (as high as 300°C.) depending on the liquid phase in use. The SE-30 and QF-1 columns (# 7 and 8 of Table 6) recommended for this type of procedure were used (87).

To test the chromatograph response to aqueous solutions, dilute samples (about 1 percent) of fatty acids, pyruvic acid and lactic acid were prepared and injected onto the Phosphoric Acid-PEGS column. The "Nano-Jector" syringes were used for this purpose.

At one point, the steam generator was used in an attempt to try water as a carrier gas for the aqueous sample. The apparatus was put together according to the instructions in the Wilkins instrument manual and the generator was turned on. The steam pressure gauge was kept between 19-20 lbs. and the temperature of the steam was maintained at

124°C. The column temperature was set at 150°C. "Raw" sweat samples were injected directly into the chromatograph with steam being used as the carrier gas. The noise level of this system was too high for trace analysis work.

COMPOUND IDENTIFICATION

The several attempts at compound identification were made using the relative retention time technique. For the pyrolysis studies, synthetic mixtures of the products expected on the basis of the work by Jennings and Dimick (39) were prepared. Using nanolitre syringes, small amounts of these mixtures were injected into the chromatograph. The needle capillary was first evacuated by forcing it through the silicone rubber septum (Figure 8) into the vacuum chamber. A drop of sample was then placed on top of the septum and the needle drawn slowly up out of the chamber and through the sample. The needle was then inserted directly into the heated injection port of the chromatograph where the sample vaporised onto the column. The chromatograms obtained in this manner were compared with the pyrograms[1] to sort out the identity of the pyrolysis products.

PEAK AREA MEASUREMENTS

The areas for symmetrical peaks were measured by triangulation. Non-symmetrical peaks were measured by the method recommended by Condal-Bosch (88).

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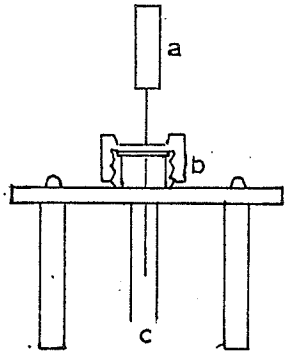
[1] See footnote p.50.

FIGURE 8. Apparatus for Using Nano-jector Syringes.



a. Nano-Jector
b. Cutaway Showing Septum
c. To Vacuum

RESULTS

RESULTS OF PYROLYSIS STUDIES

The first attempts to analyse the pyrolysis products of several purines and pyrimidines indicated that columns #2 and #3 of Table 6 would be best suited for these studies. Subsequently, column #9 (Table 6) was also found to give a good separation of the pyrolysis products of the nucleic acids. However, only the first two columns were used to analyse the pyrolysis products of the purines, pyrimidines and their corresponding nucleosides. Columns #1, #6, #10 and a combined column formed by joining columns #1 and #2 did not give good separations of the pyrolysis products. (A list of the compounds pyrolysed appeared in Table 5).

The two series of chromatograms shown in Figures 9 to 32A indicate how the pyrolysis products of the bases differ from those of the corresponding nucleosides. The differences between the pyrograms[1] of the various bases, and between the pyrograms of the various nucleosides, is also apparent. The pyrolysis of the ribose sugar yielded only one major peak at the coil temperature used. The absence of this peak is seen to be a major difference between the pattern for the bases and the pattern for the corresponding nucleosides. For the purines,only several pyrolysis products were obtained. The products from these purines had retention times similar to the products of the pyrimidine pyrolyses. However, a

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[1] A "Pyrogram" is the chromatogram obtained from the mixture of pyrolysis products.

PYROGRAMS[1]

First Series: On Hallcomid-KOH Column (#2, Table 6) pp 52-66.

Second Series: On Hallcomid M18/Carbowax 600 Column (#3, Table 6) pp67-81.

[1]Peaks believed to result from identical pyrolysis products are indicated numerically. #1 refers in all cases to the first group of peaks.

FIGURE 9.[1] Pyrogram of Guanine.

Detector Sensitivity: Impedance 10^9; Output 10x; Attenuator x 1.

Sample Size: Approx. 40 µg.

Column: #2

Conditions: Preheater Temperature: 108°C
Column Temperature: 30°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting[2]: #80
Pyrolysis Time: 6 seconds.

[1] Figures 9 to 21 of Pyrograms on Column #2.

[2] This gives an estimated coil temperature of 1000°C.

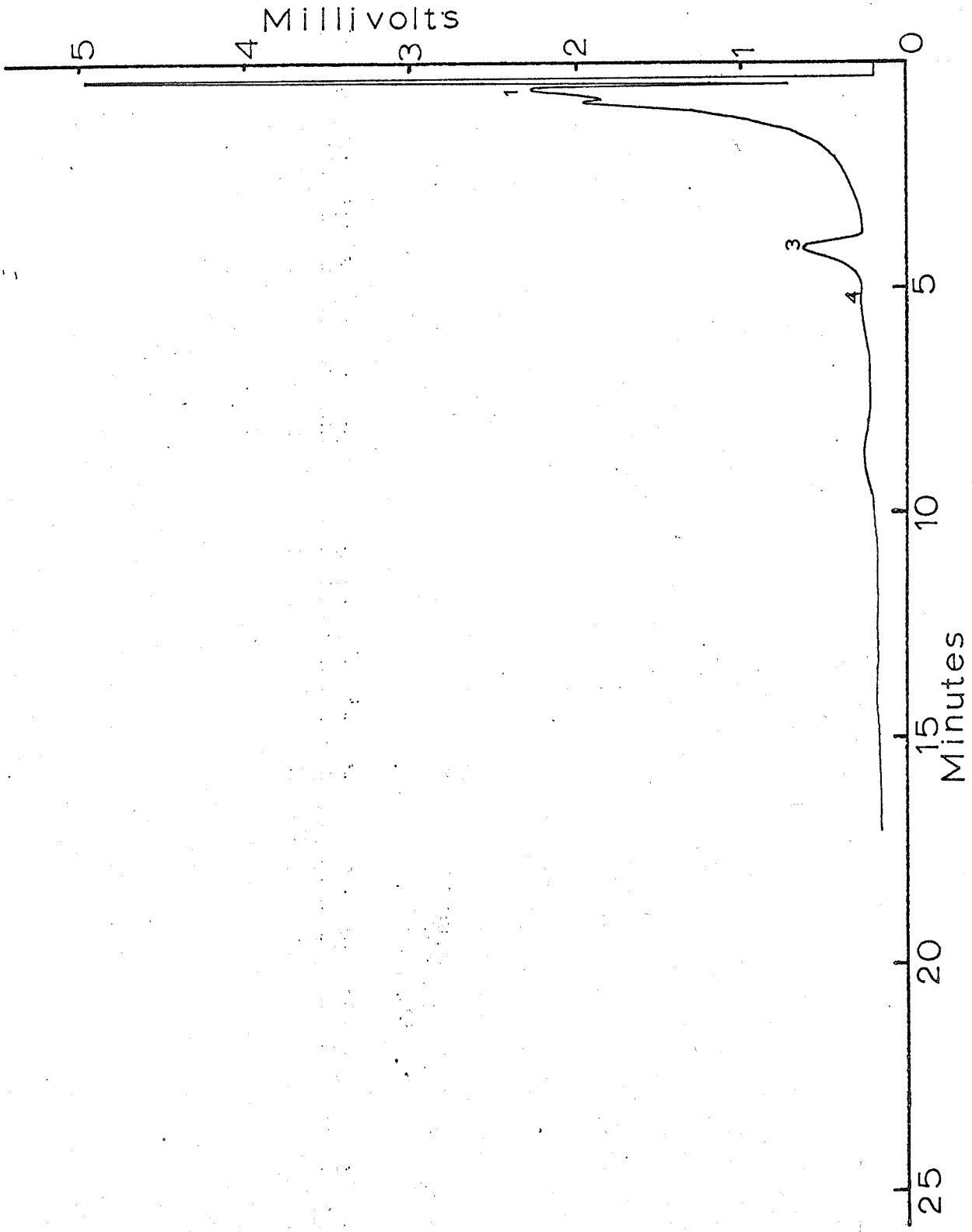

Millivolts
5
4
3
2
1
0
1
3
4
5
10
15
20
25
Minutes

FIGURE 10. Pyrogram of Guanosine.

Detector Sensitivity: Impedance 10^9; Output 10x;
Attenuator x 1.

Sample Size : 25 µg.
Column : #2
Conditions: Preheater Temperature: 108°C.
Column Temperature: 31°C.
Nitrogen Flow: 23 ml/min at 14 P.S.I.G.
Hydgrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat setting: #80.
Pyrolysis Time: 6 seconds.

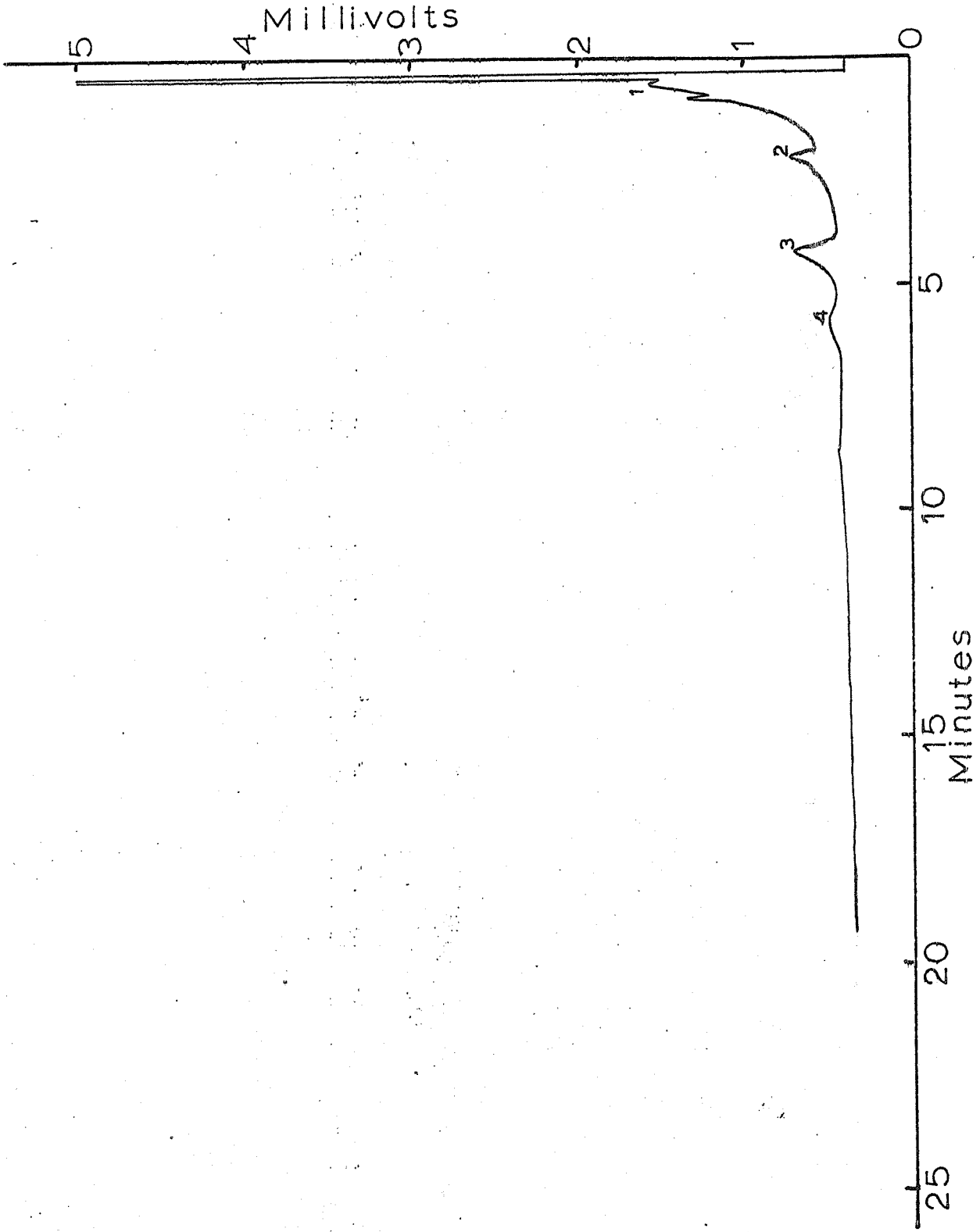

Millivolts
5
4
3
2
1
0
1
2
3
4
5
10
15
20
25
Minutes

FIGURE 11. Pyrogram of Adenine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x 1.

Sample Size : 50 µg.
Column: #2
Conditions: Preheater Temperature: 108°C.
Column Temperature : 32°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 6 seconds.

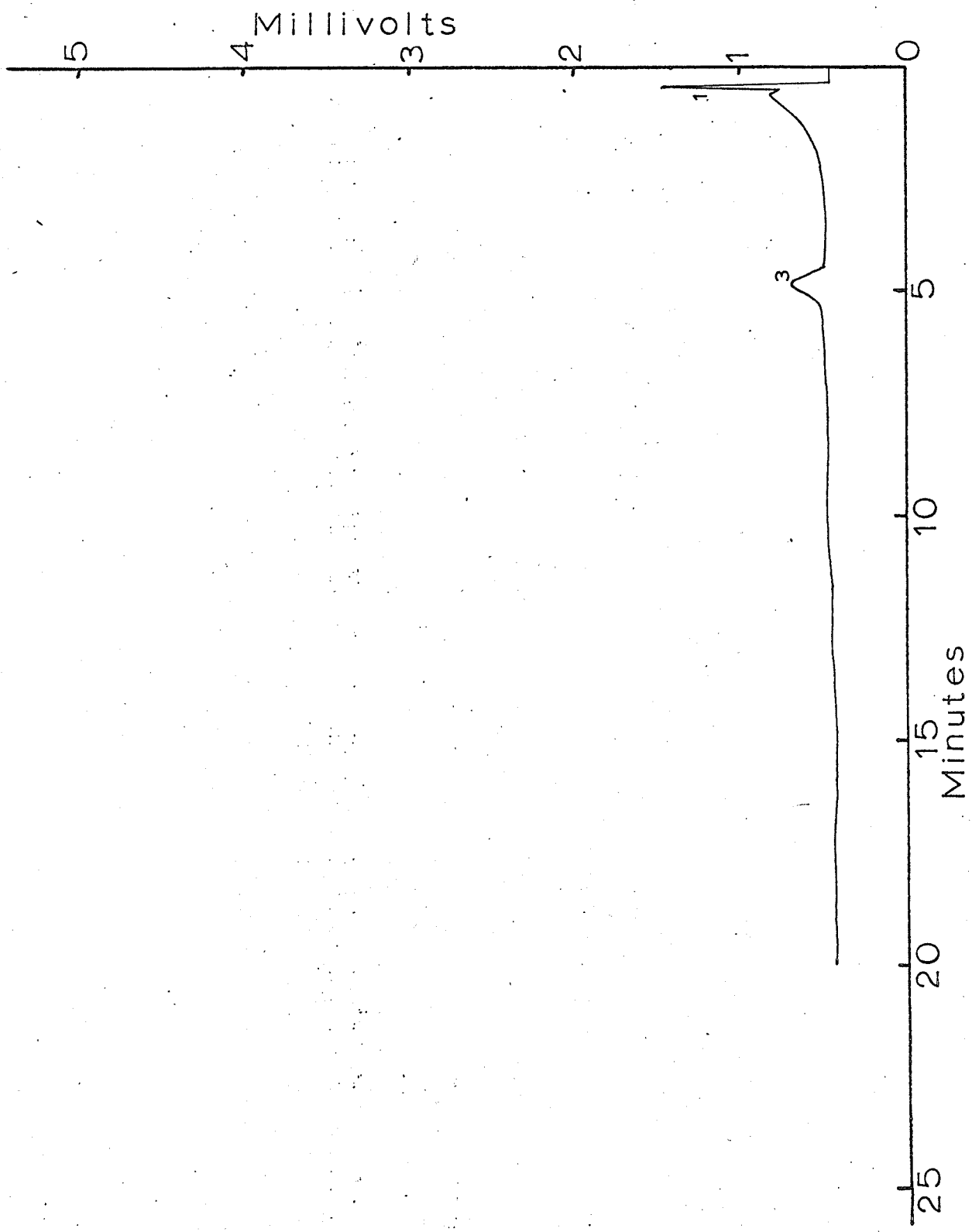

Millivolts
5
4
3
2
1
0
1
3
5
10
15
20
25
Minutes

FIGURE 12: Pyrogram of Adenosine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x 1.
Sample Size: 25 µg.
Column: #2.
Conditions: Preheater Temperature: 108°C.
Column Temperature: 30°C.
Nitrogen Flow : 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow : 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 6 seconds.

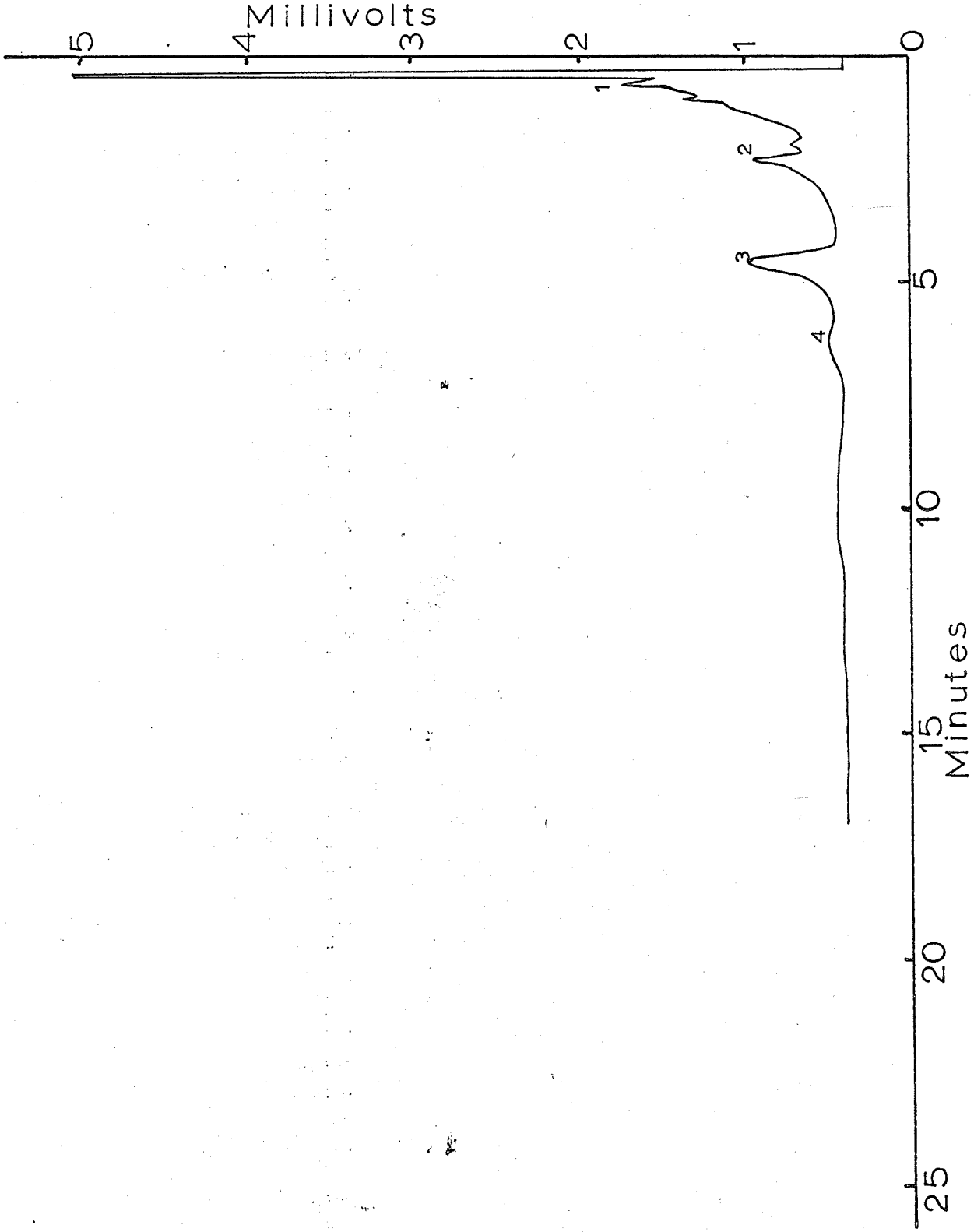

Millivolts
5
4
3
2
1
0
1
2
3
4
5
10
15
20
25
Minutes

FIGURE 13. Pyrogram of Cytosine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x 1.
Sample Size: 25 µg.
Column : #2.
Conditions: Preheater Temperature: 108°C.
Column Temperature: 33°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 5 seconds.

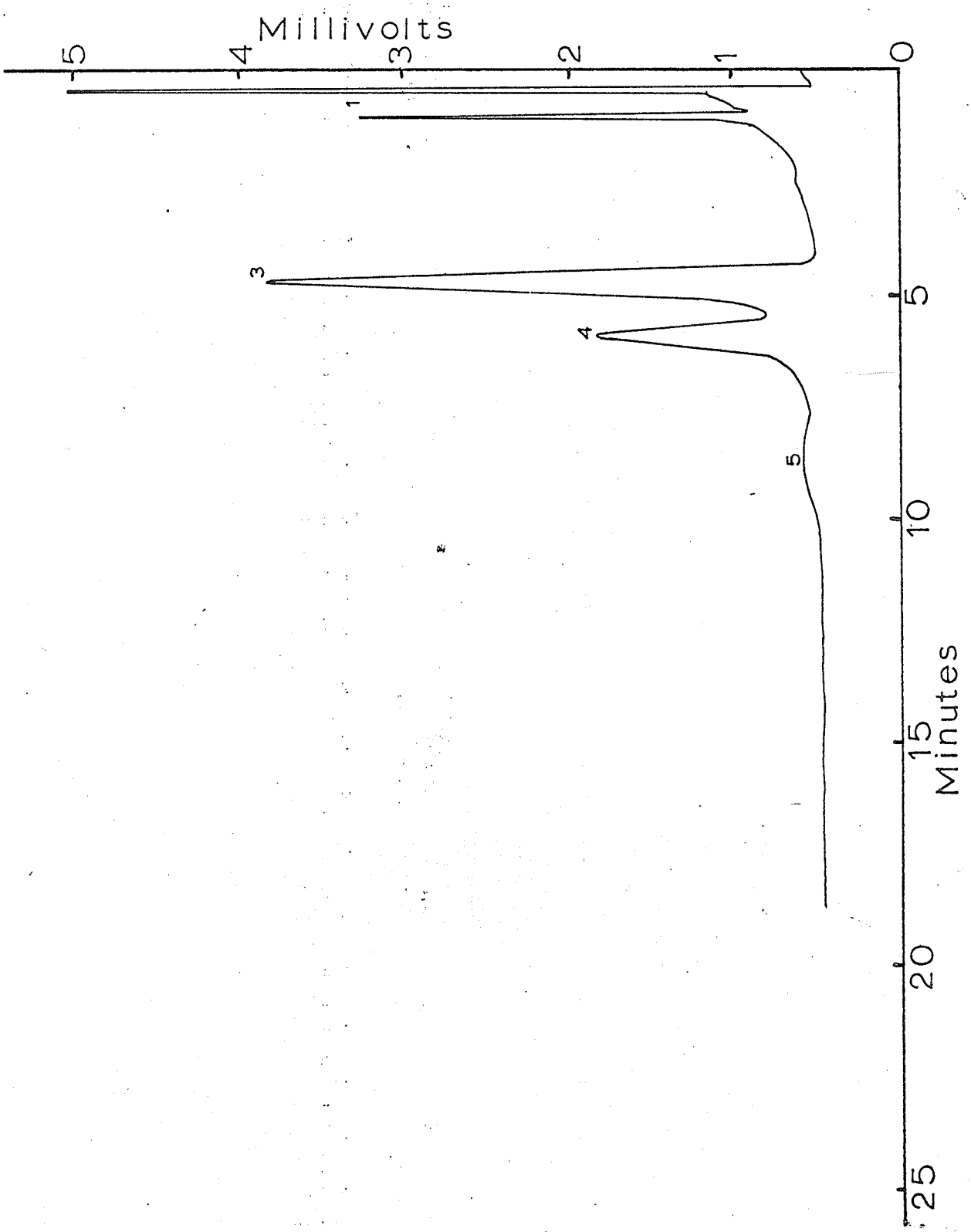

Millivolts
5
4
3
2
1
0
1
3
4
5
5
10
15
20
25
Minutes

FIGURE 14. Pyrogram of Cytidine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x 1, x4
(as shown)

Sample Size: 25 µg.
Column: #2
Conditions: Preheater Temperature: 108°C.
Column Temperature: 31°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 6 seconds.

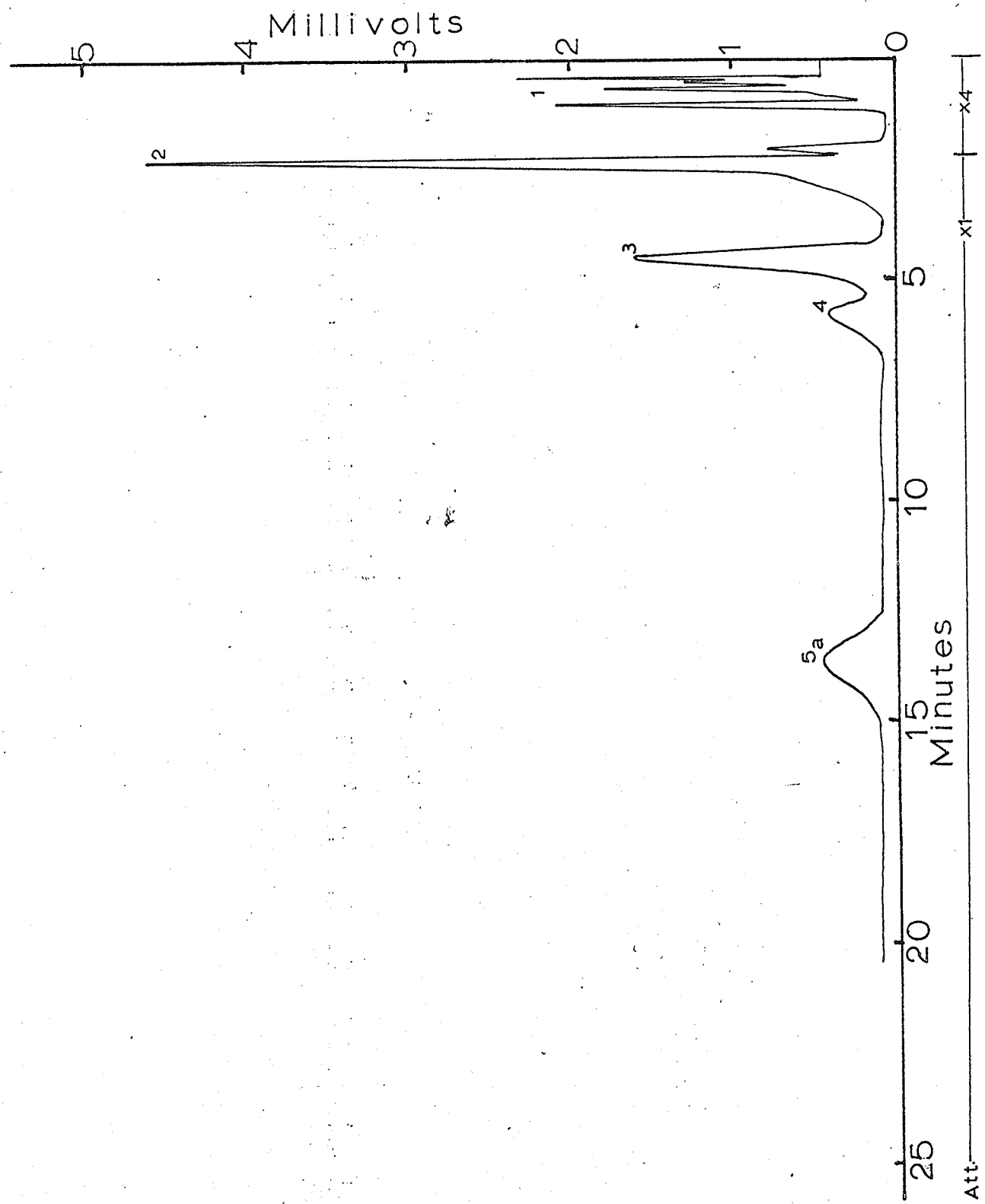

Millivolts
5
4
3
2
1
0
1
2
3
4
5a
5
10
15
20
25
Minutes
x4
x1
Att.

FIGURE 15. Pyrogram of Uracil.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuation x1, x2,
x 1 (as shown)

Sample Size: 40 µg.

Column: #2.

Conditions: Preheater Temperature : 108°C.
Column Temperature: 31°C.
Nitrogen Flow: 24 ml/min. at 14.5 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 6 seconds.

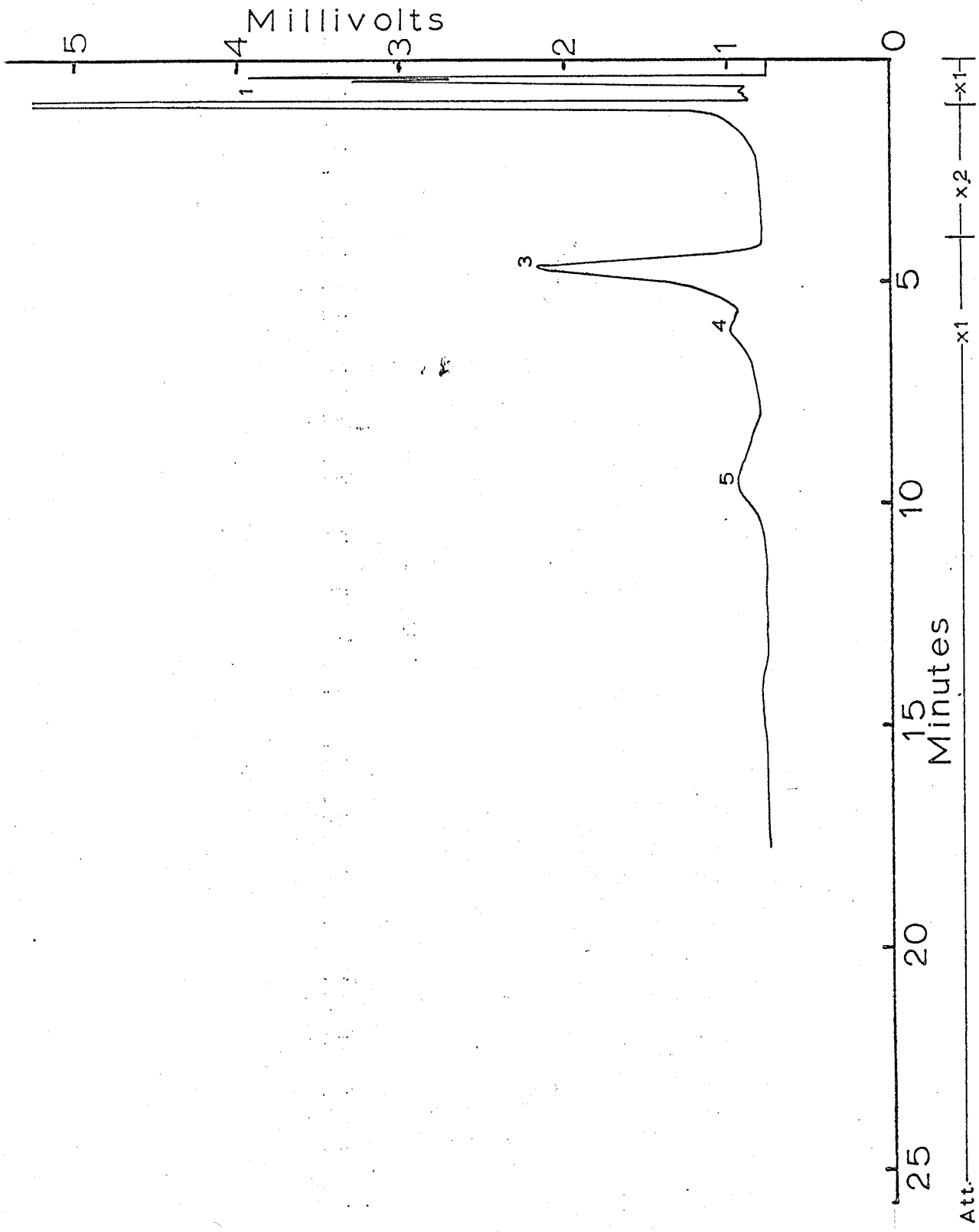

Millivolts
5
4
3
2
1
0
1
3
4
5
0
5
10
15
20
25
Minutes
x1
x2
x1
Att.

FIGURE 16. Pyrogram of Uridine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1,x2,x1
(as shown)

Sample Size: 25 µg.
Column: #2
Conditions: Preheater Temperature: 105°C.
Column Temperature: 29°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300ml/min.
Powerstat Setting: #80
Pyrolysis Time: 5 seconds.

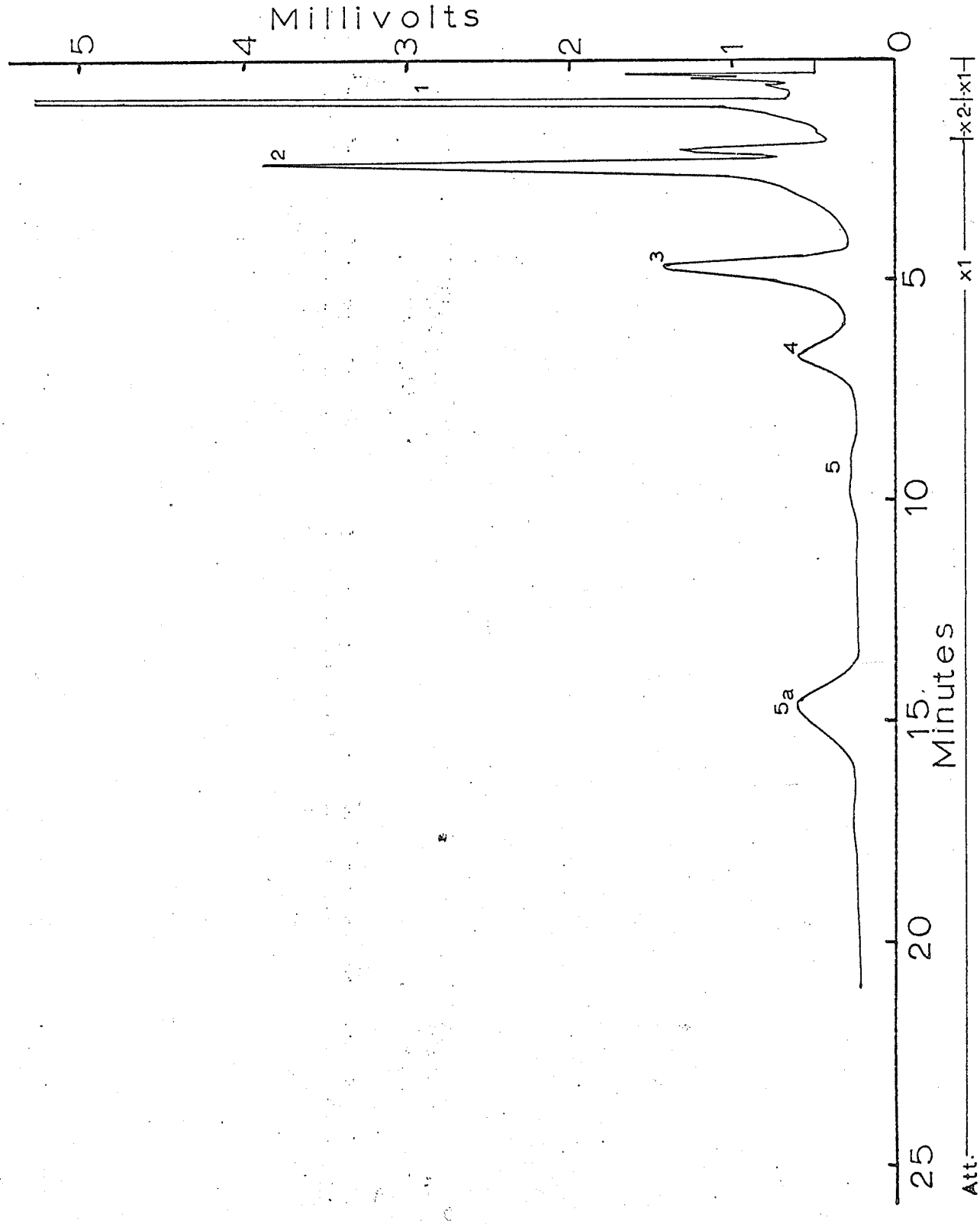

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5a
5
10
15
20
25
Minutes
Att.
x1
x2
x1

FIGURE 16-A. Repeat Pyrogram of Uridine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x4,x1 (as shown)

Sample Size: 25 µg.

Column: #2.

Conditions: Preheater Temperature: 115°C.
Column Temperature: 30°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 5 seconds.

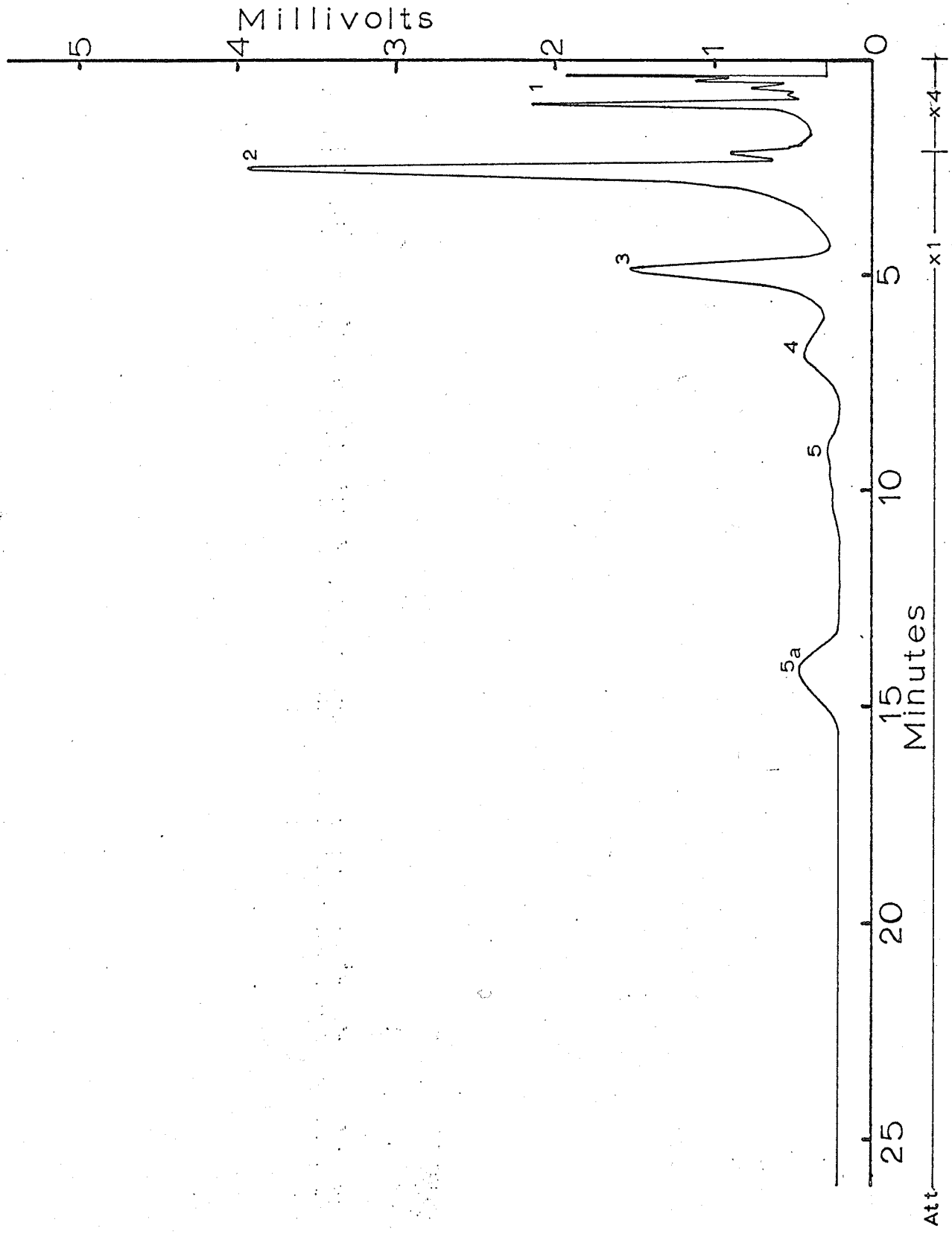

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5a
x4
x1
5
10
15
20
25
Minutes
Att

FIGURE 16-B. Pyrogram of Uridine Using "Frozen Head Technique".

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1,
(as shown)

Sample Size: 25 µg
Column: #2.
Conditions: Preheater Temperature: 115°C.
Column Temperature: 25° to 52°C.
(non-linear program)
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Powerstat Setting: #80.
Pyrolysis Time: 5 seconds.

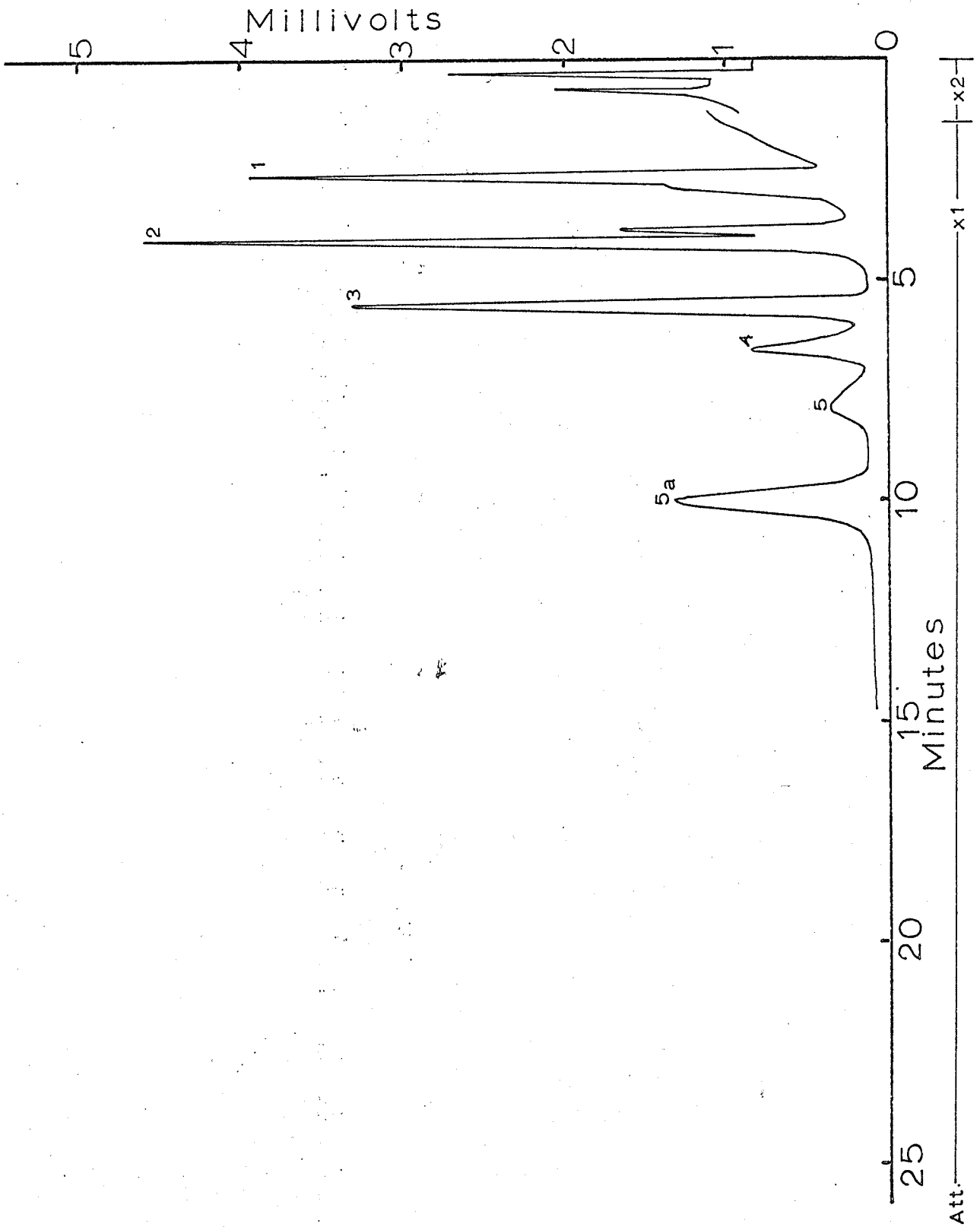

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5a
5
10
15
20
25
Minutes
x2
x1
Att.

FIGURE 17. Pyrogram of Thymine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 25 µg.
Column #2.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 30°C.
Nitrogen Flow: 24 ml/min at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

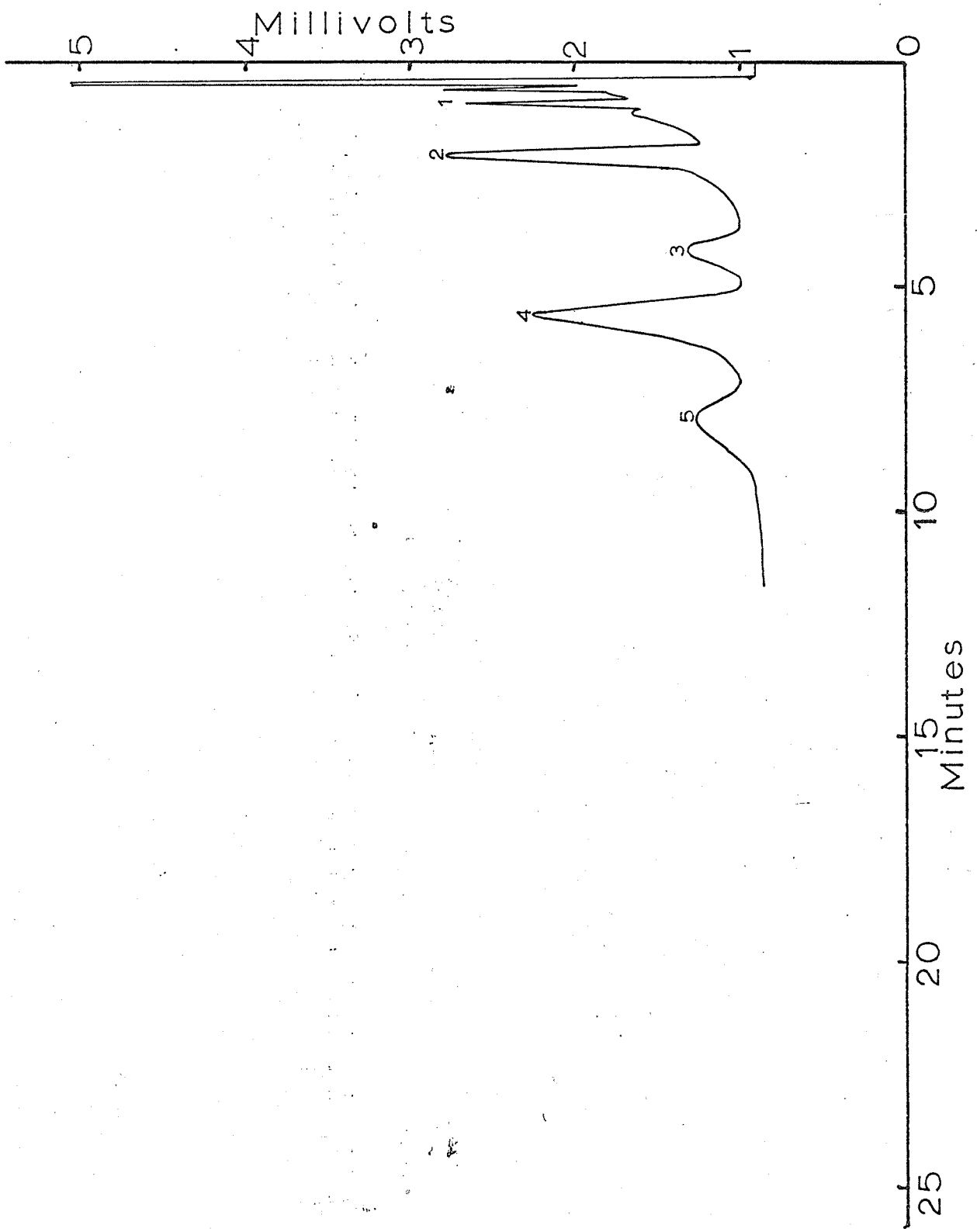

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5
10
15
20
25
Minutes

FIGURE 18. Pyrogram of Thymidine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1
Sample Size: 50 µg.
Column: #2.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 31°C.
Nitrogen Flow: 23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

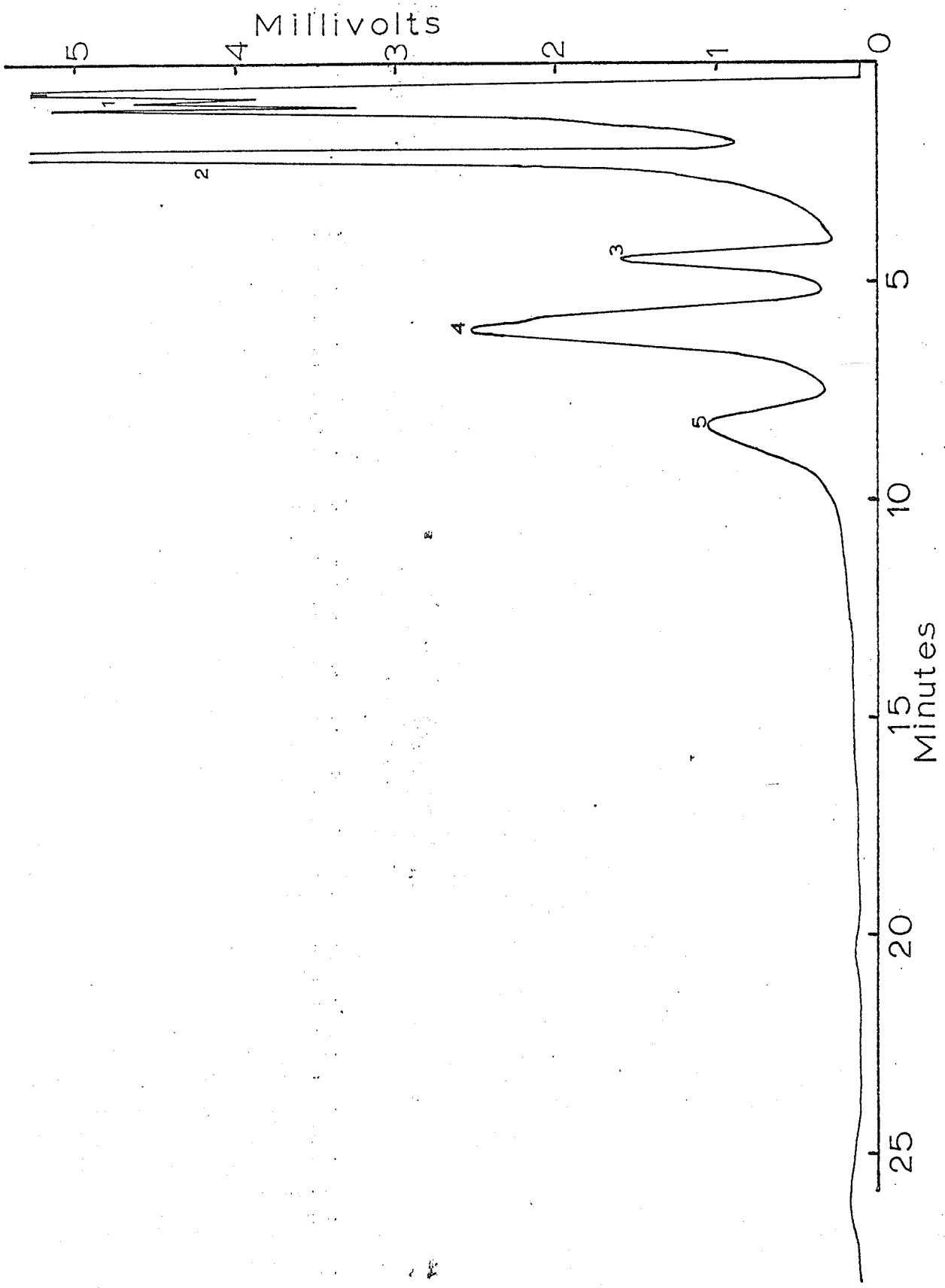

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5
10
15
20
25
Minutes

FIGURE 19. Pyrogram of D(-) Ribose

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1

Sample Size: 20 µg.

Column: #2.

Conditions: Preheater Temperature: 115°C.
Column Temperature: 31°C.
Nitrogen Flow:23 ml/min. at 14 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 5 seconds.

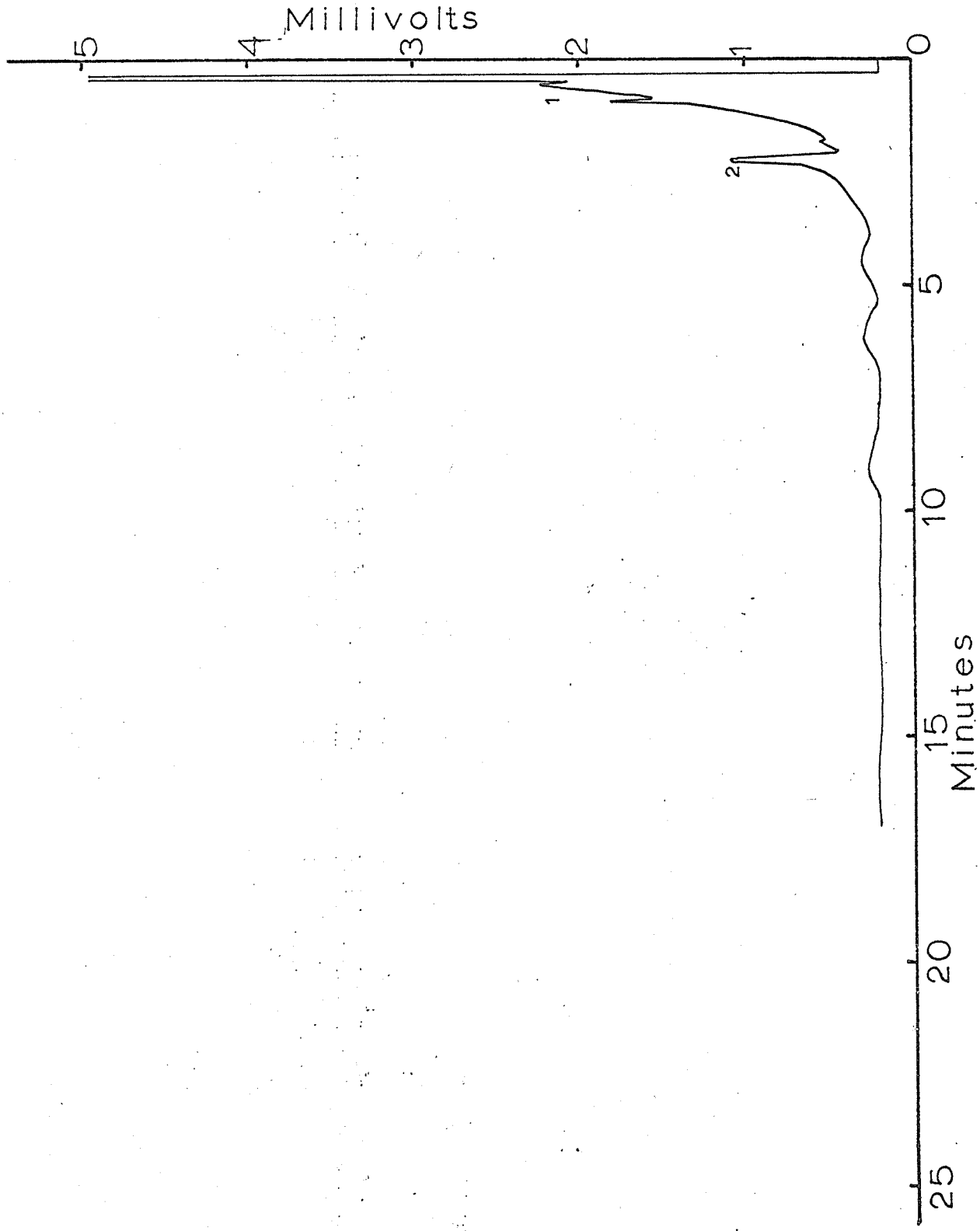

Millivolts
5
4
3
2
1
0
1
2
5
10
15
20
25
Minutes

FIGURE 20. Pyrogram of Calf Thymus DNA.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1

Sample Size: 30 µg.

Column: #2.

Conditions: Preheater Temperature: 100°C.
Column Temperature: 31°C.
Nitrogen Flow: 25 ml/min. at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 second.

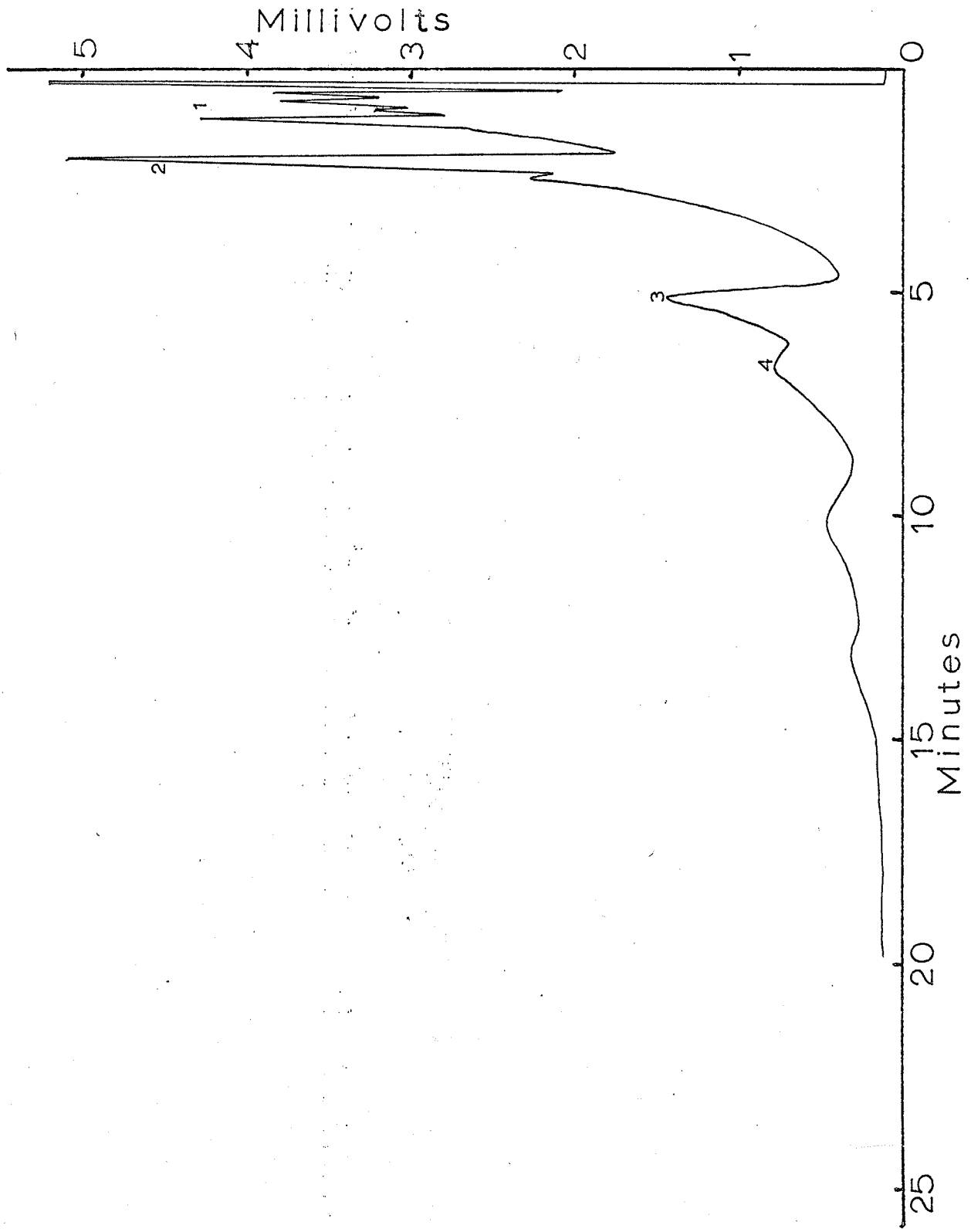

Millivolts
5
4
3
2
1
0
1
2
3
4
5
10
15
20
25
Minutes

FIGURE 21. Repeat Pyrogram of Calf Thymus DNA.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 20 µg.
Column: #2.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 31°C.
Nitrogen Flow: 25 ml/min. at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

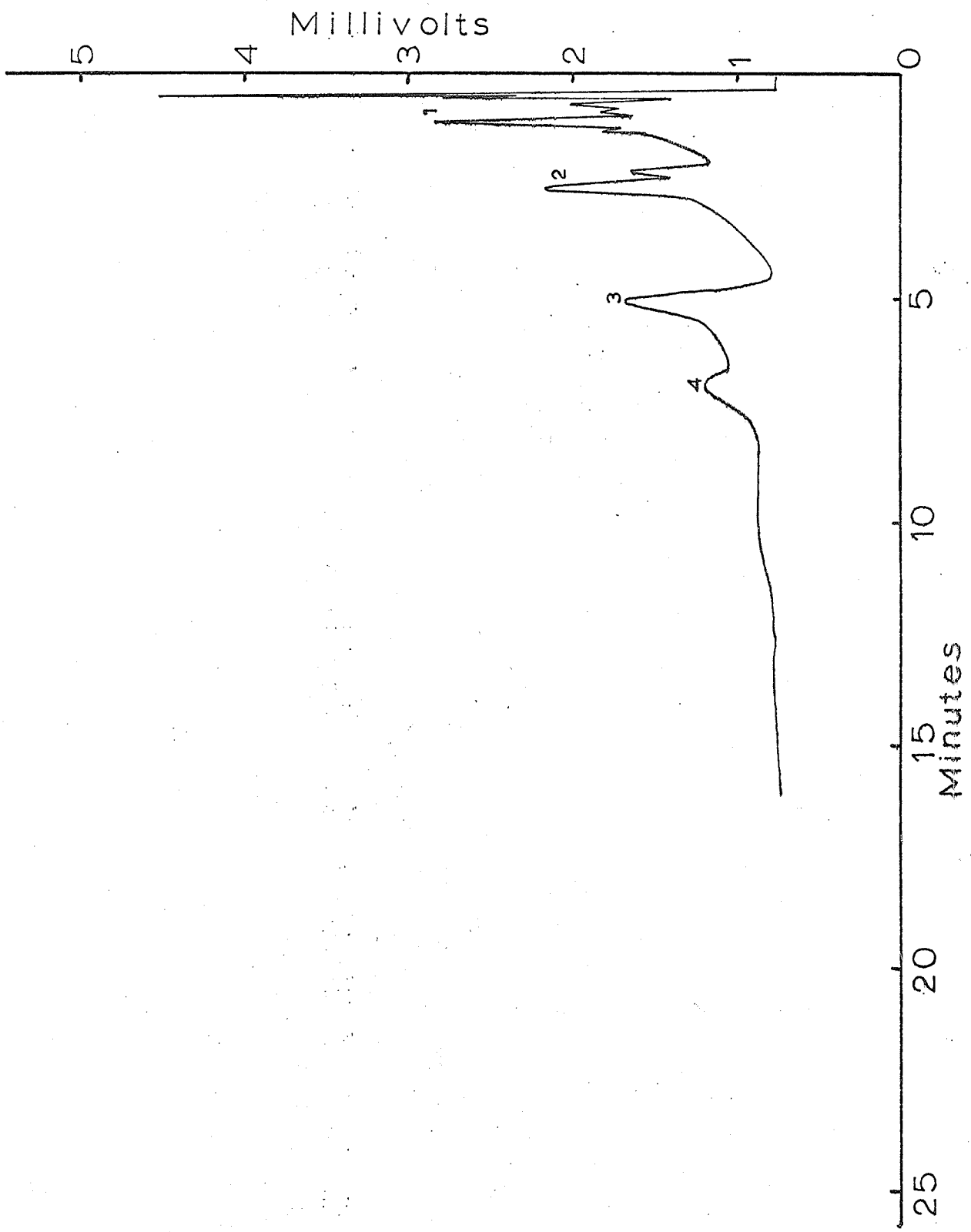

Millivolts
5
4
3
2
1
0
1
2
3
4
5
10
15
20
25
Minutes

FIGURE 22.[1] Pyrogram of Guanosine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1 (as shown)
Sample Size: 50 µg.
Column: #3
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

[1]Figures 22 to 32-A of Pyrograms using column #3.

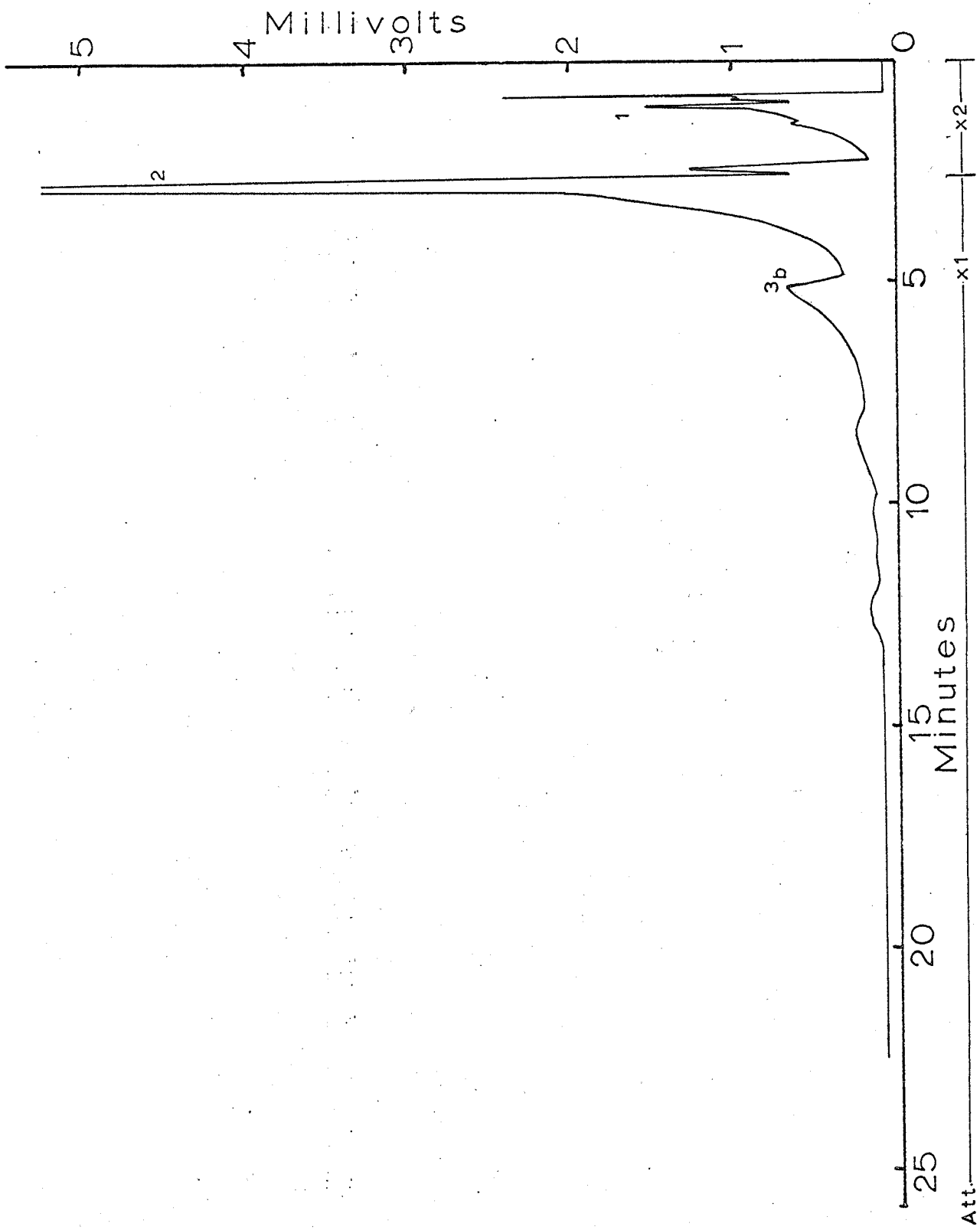

Millivolts
5
4
3
2
1
0
1
2
3b
x2
x1
5
10
15
20
25
Minutes
Att.

FIGURE 22-A. Pyrogram of Guanosine Using "Frozen Head Technique".

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2

Sample Size: 25 µg.

Column: #3.

Conditions: Preheater Temperature: 100°C.
Column Temperature: 53 ± 2°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

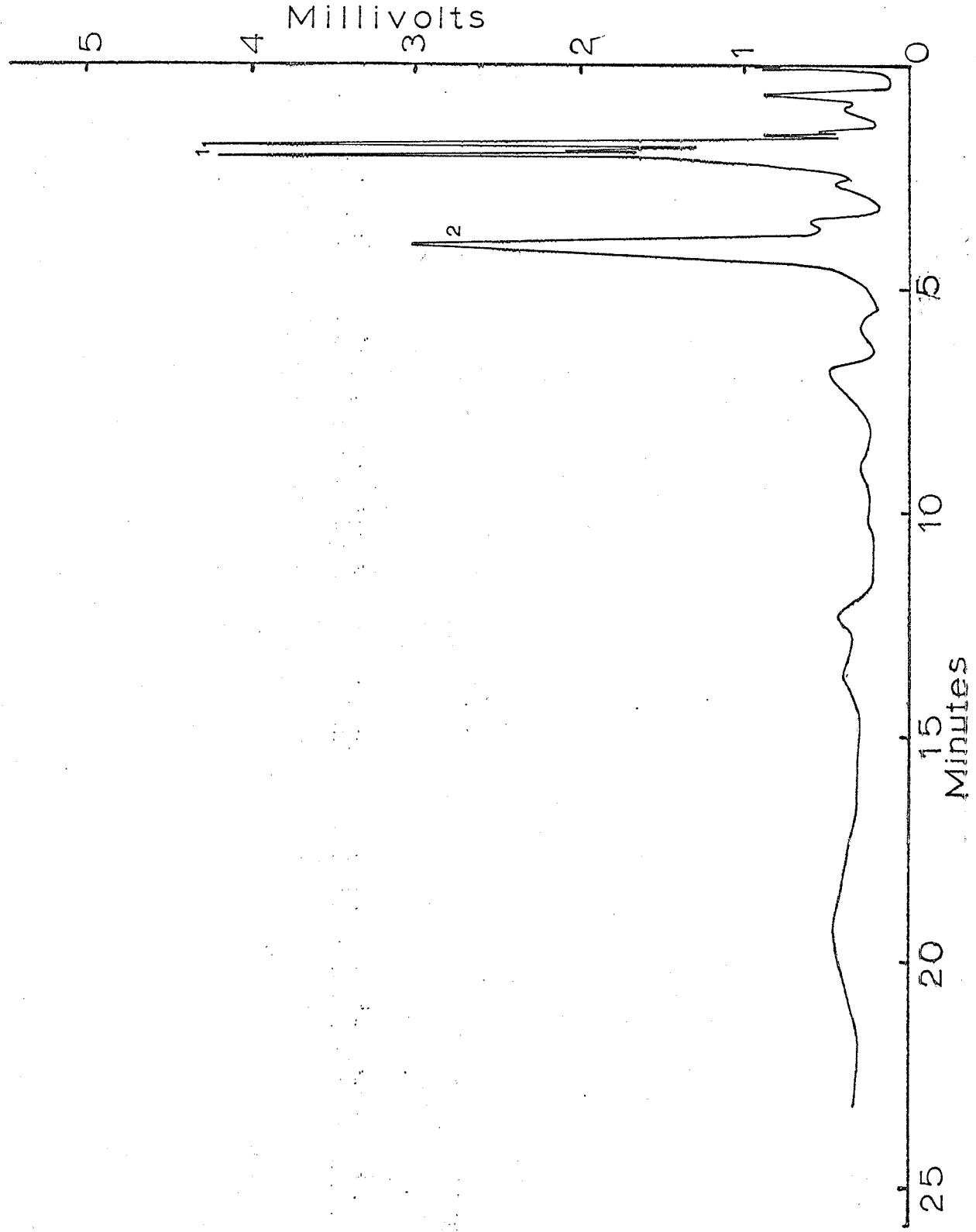

Millivolts
5
4
3
2
1
0
1
2
5
10
15
20
25
Minutes

FIGURE 23. Pyrogram of Adenosine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1 (as shown).
Sample Size: 50 µg.
Column: #3
Conditions: Preheater Temperature: 100°C.
Column Temperature: 52°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

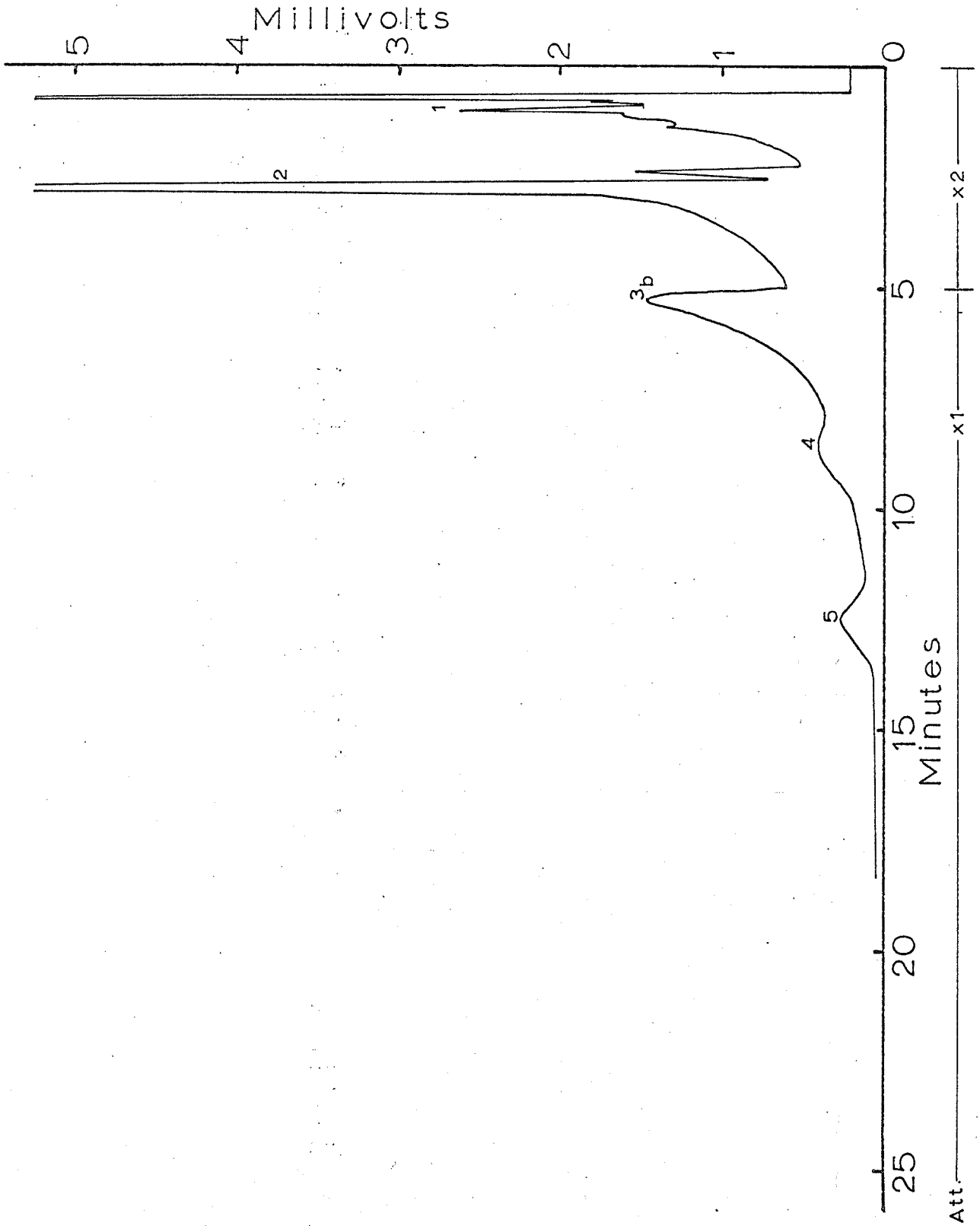

Millivolts
5
4
3
2
1
0
1
2
3b
4
5
0
5
10
15
20
25
Minutes
x2
x1
Att.

FIGURE 24. Pyrogram of Cytosine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 50 µg.
Column: #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

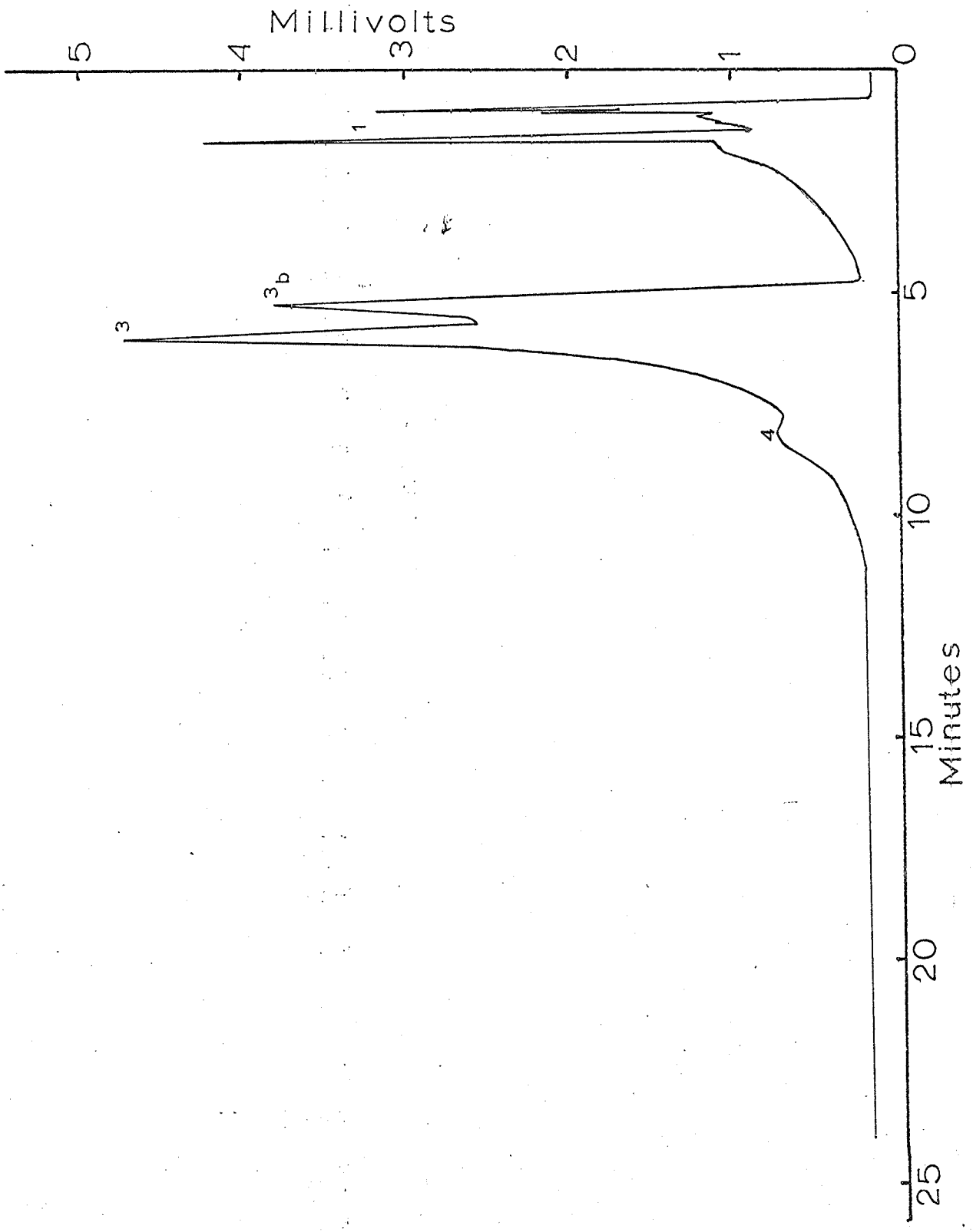

Millivolts
5
4
3
2
1
0
1
3b
3
4
5
10
15
20
25
Minutes

FIGURE 25. Pyrogram of Cytidine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1, (as shown)
Sample Size: 50 µg.
Column: #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 52°C.
Nitrogen Flow: 30.8 ml/min at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

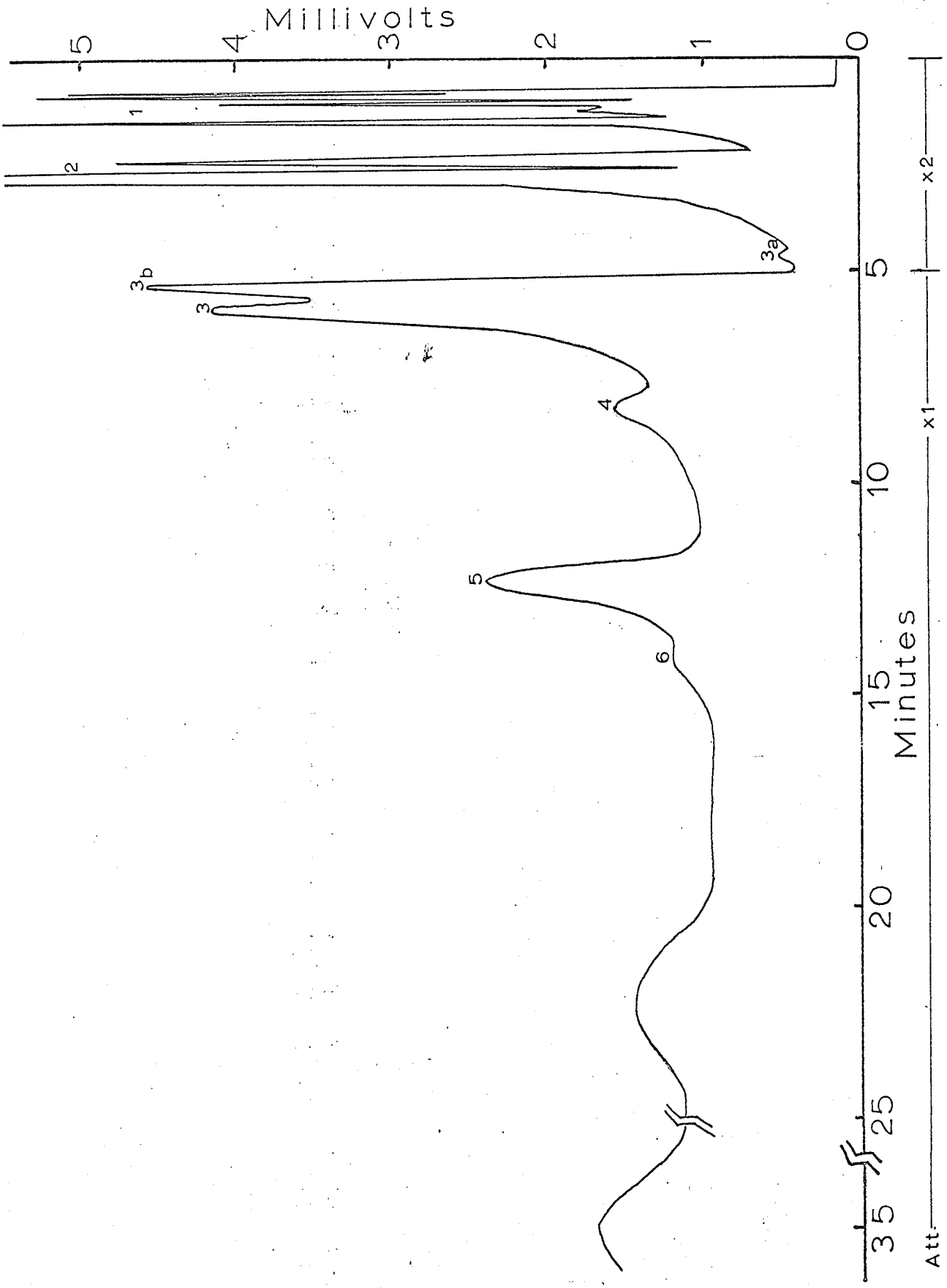

Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
6
5
10
15
20
25
35
Minutes
x2
x1
Att.

FIGURE 26. Repeat Pyrogram of Cytidine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x4,x1 (as shown)
Sample Size: 50 µg.
Column : #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7seconds.

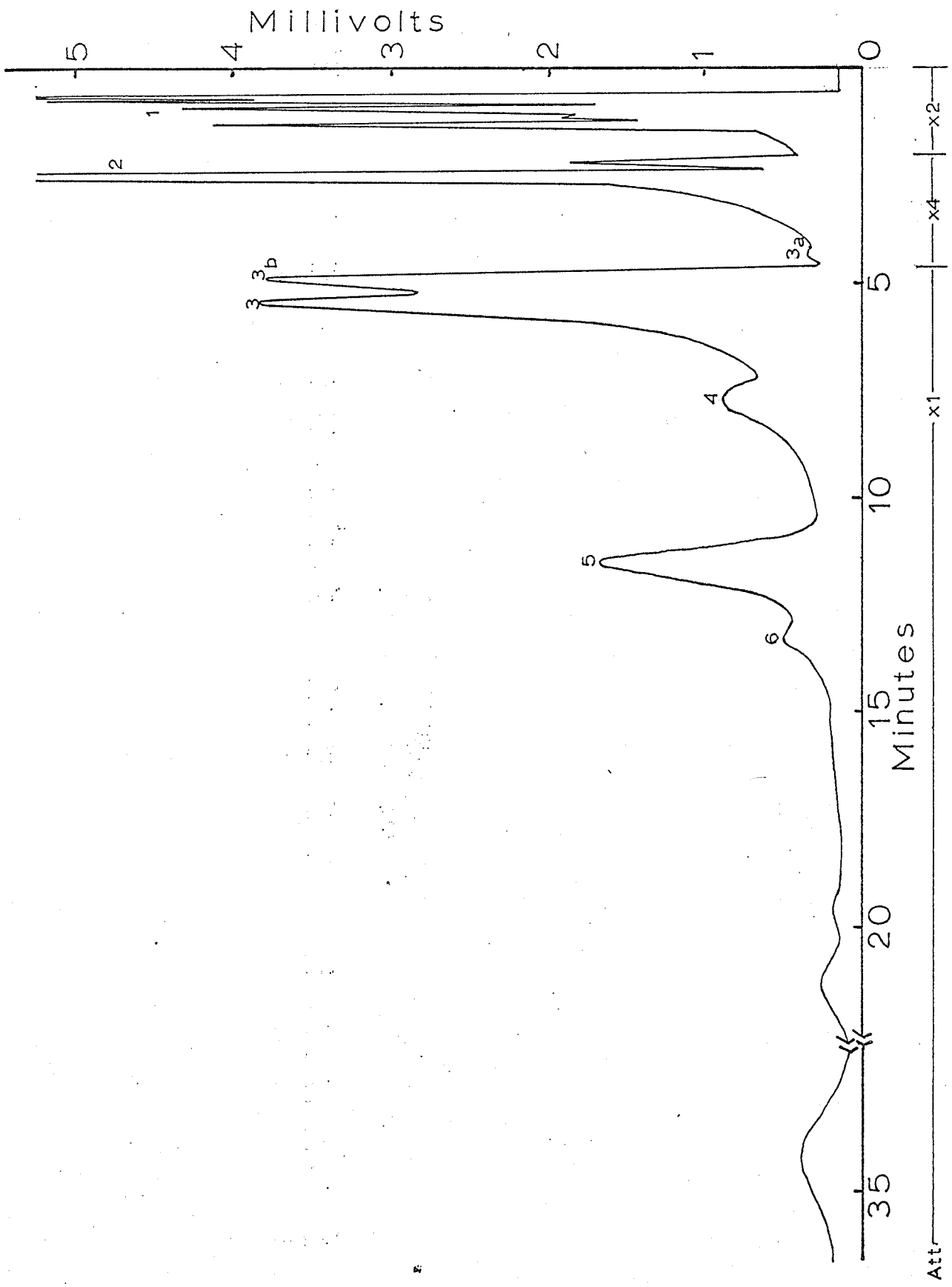

Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
6
5
10
15
20
35
Minutes
x2
x4
x1
Att

FIGURE 27. Pyrogram of Uracil.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x 1
Sample Size: 50 µg.
Column: #3.
Conditions: Preheater Temperature: 110°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

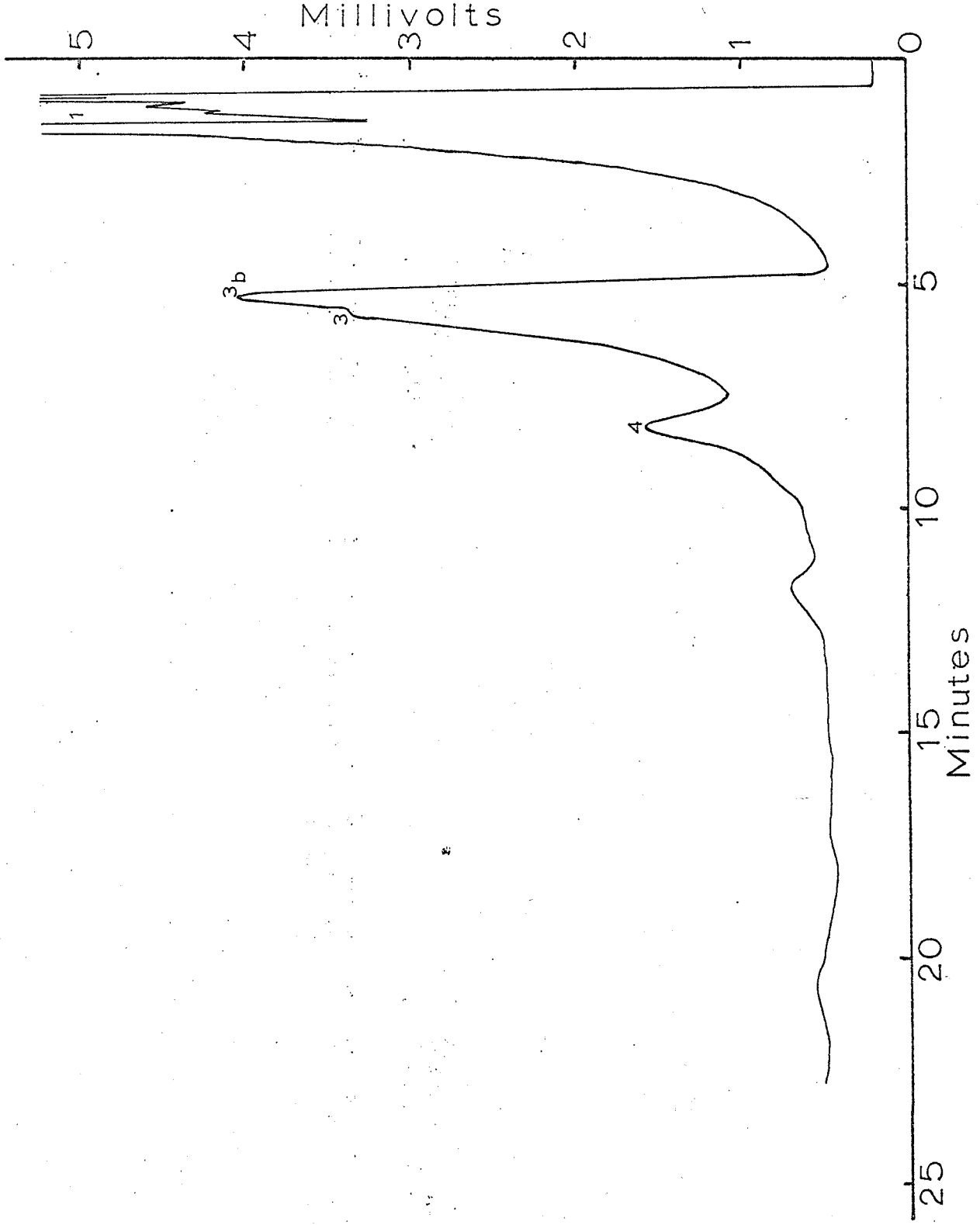

Millivolts
5
4
3
2
1
0
1
3b
3
4
5
10
15
20
25
Minutes

FIGURE 28. Pyrogram of Uridine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x4,x2,x1 (as shown)
Sample Size: 50 µg.
Column #3.
Conditions: Preheater Temperature: 110°C.
Column Temperature: 52°C.
Nitrogen Flow: 30.8 ml/min.at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

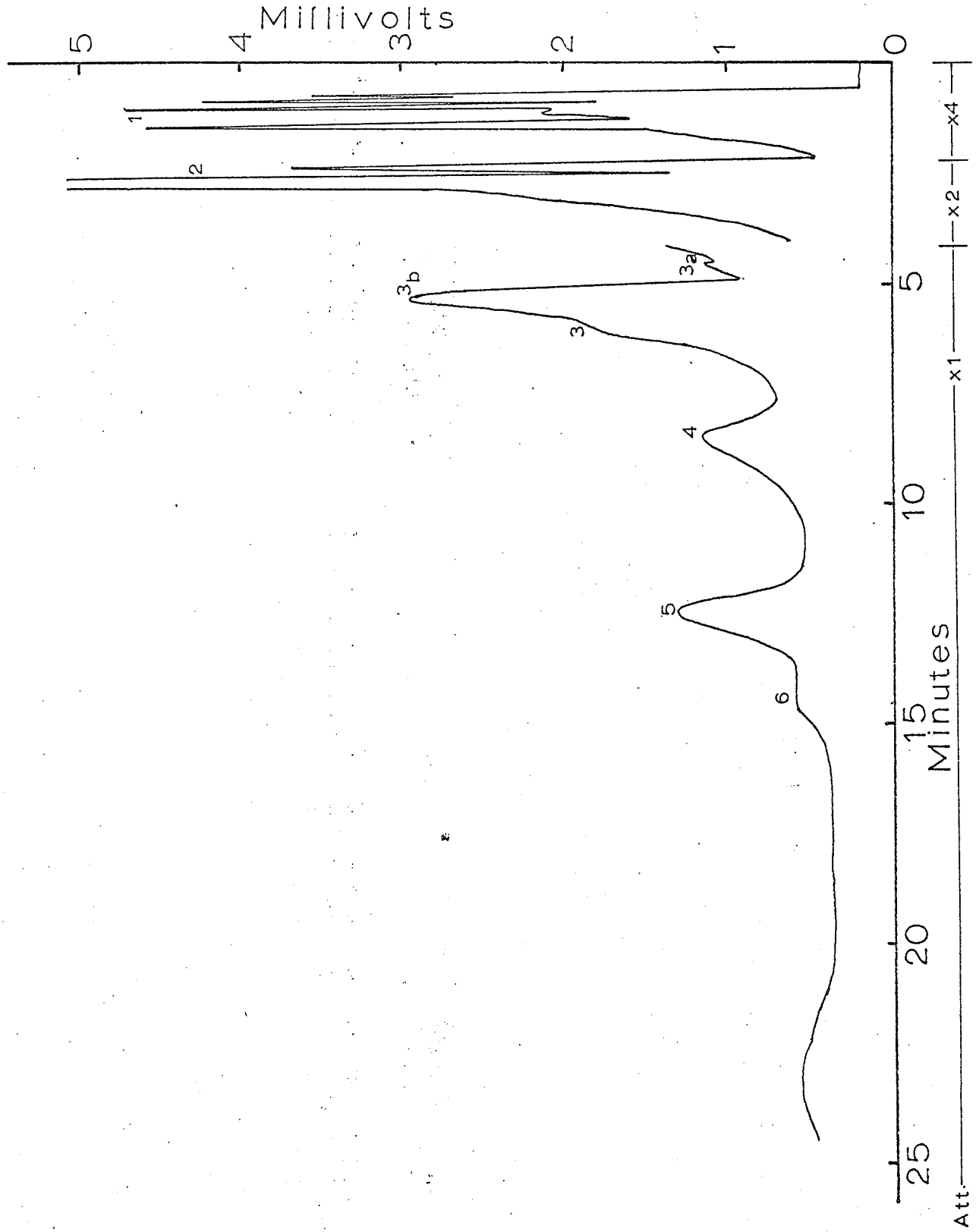

Millivolts
5
4
3
2
1
0
1
2
2
3b
3a
3
4
5
6
5
10
15
20
25
Minutes
x4
x2
x1
Att.

FIGURE 29. Pyrogram of Thymine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 50 µg.
Column: #3.
Conditions: Preheater Temperature: 110°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

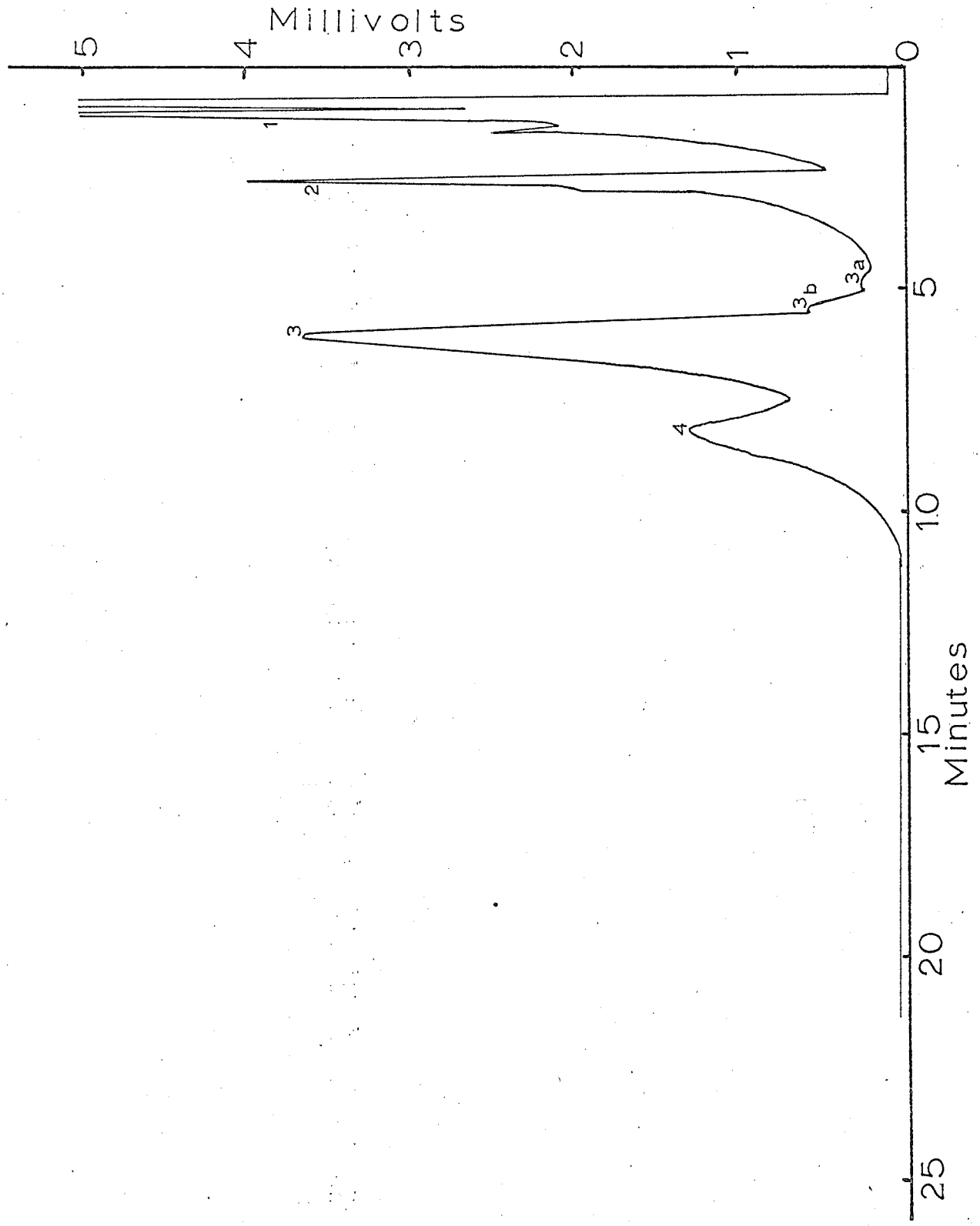

Millivolts
5
4
3
2
1
0
1
2
3
3a
3b
4
5
10
15
20
25
Minutes

FIGURE 29.A. Pyrogram of Thymine Using "Frozen Head Technique".

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1 (as shown)
Sample Size: 25 µg.
Column: #3.
Conditions: Preheater Temperature: 110°C.
Column Temperature: 54-55°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

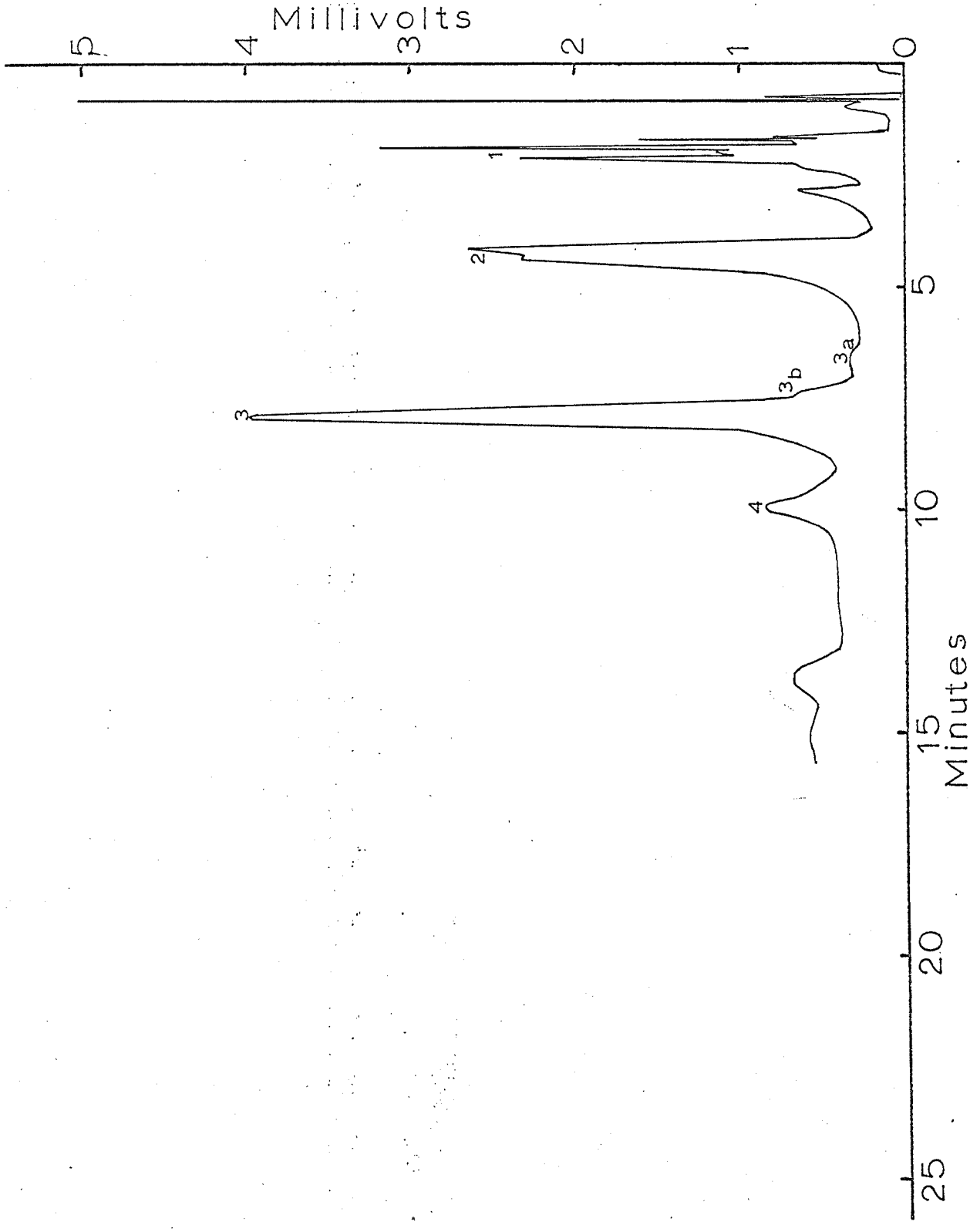

Millivolts
5
4
3
2
1
0
1
2
3
3a
3b
4
5
10
15
20
25
Minutes

FIGURE 30. Pyrogram of Thymidine.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1 (as shown)
Sample Size: 50 µg.
Column: #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 50°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml./min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

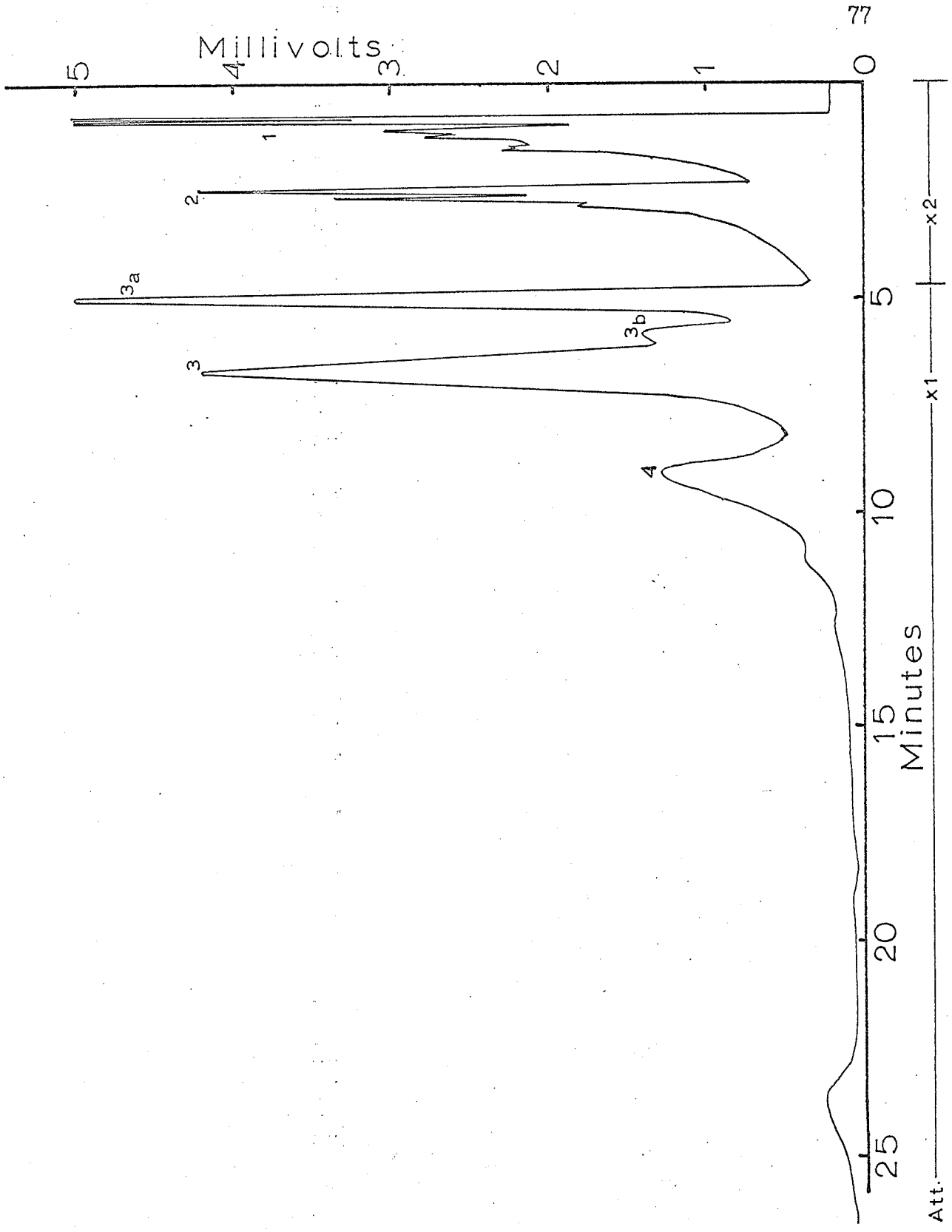

Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
x2
x1
5
10
15
20
25
Minutes
Att.

FIGURE 30-A. Pyrogram of Thymidine Using "Frozen Head Technique".

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x4

Sample Size: 20 µg.

Column : #3.

Conditions: Preheater Temperature: 100°C.
Column Temperature: Approx. 54°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

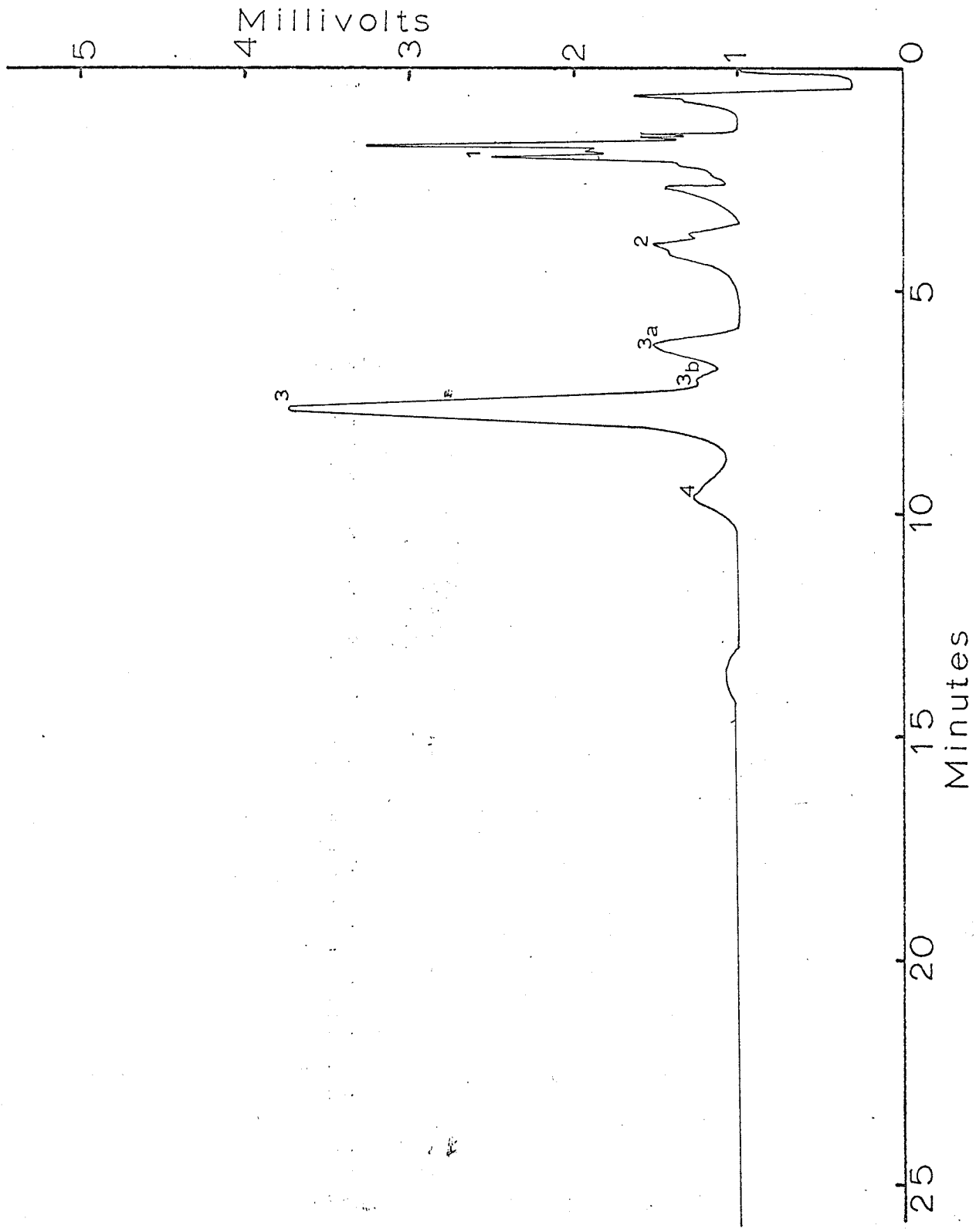

Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
10
15
20
25
Minutes

FIGURE 31. Pyrogram of D(-) Ribose.

Detector Sensitivity: Impedance 10^9;
output: 10x;
Attenuator x2,x1.
Sample Size: 12 µg.
Column: #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.

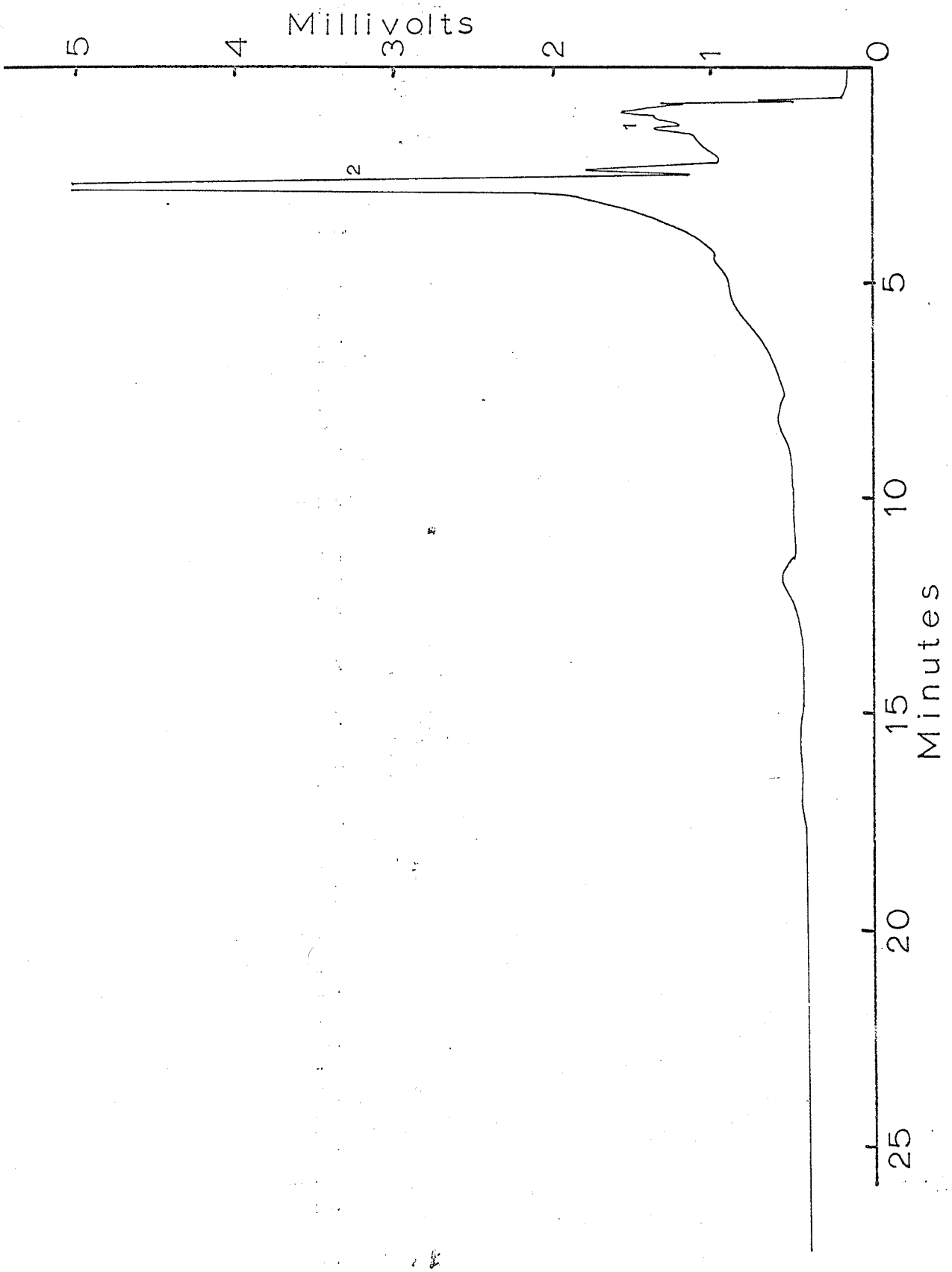
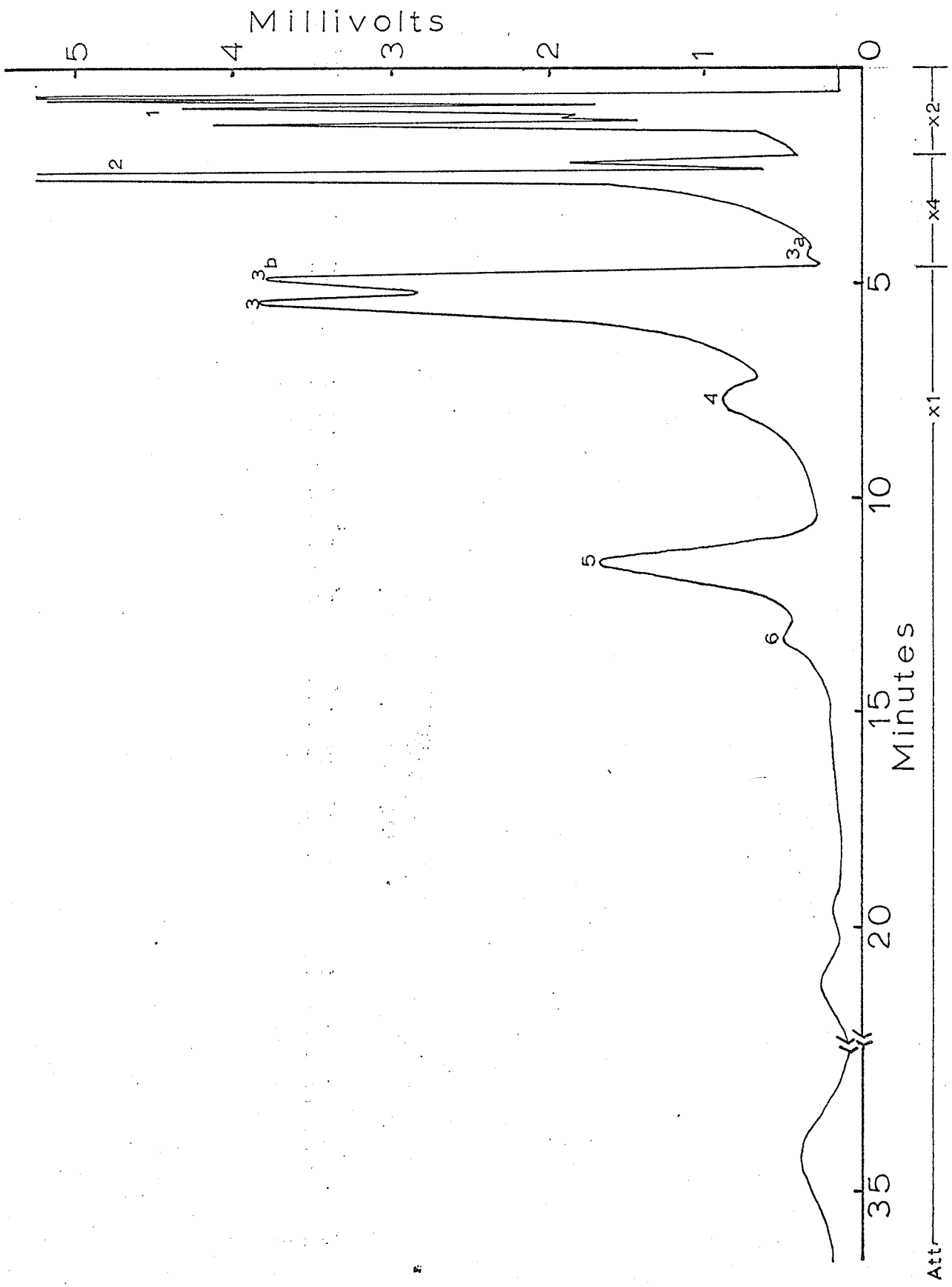
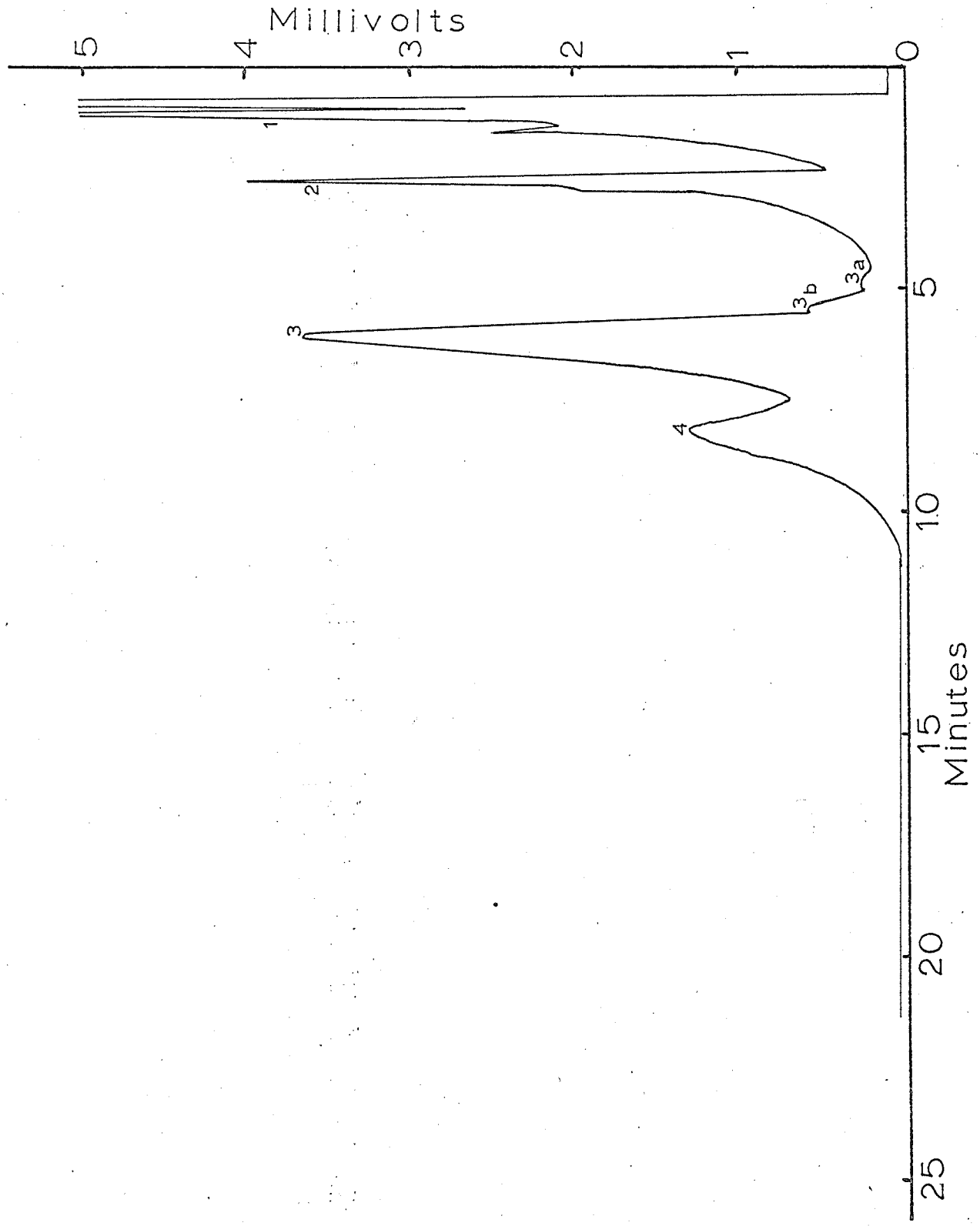
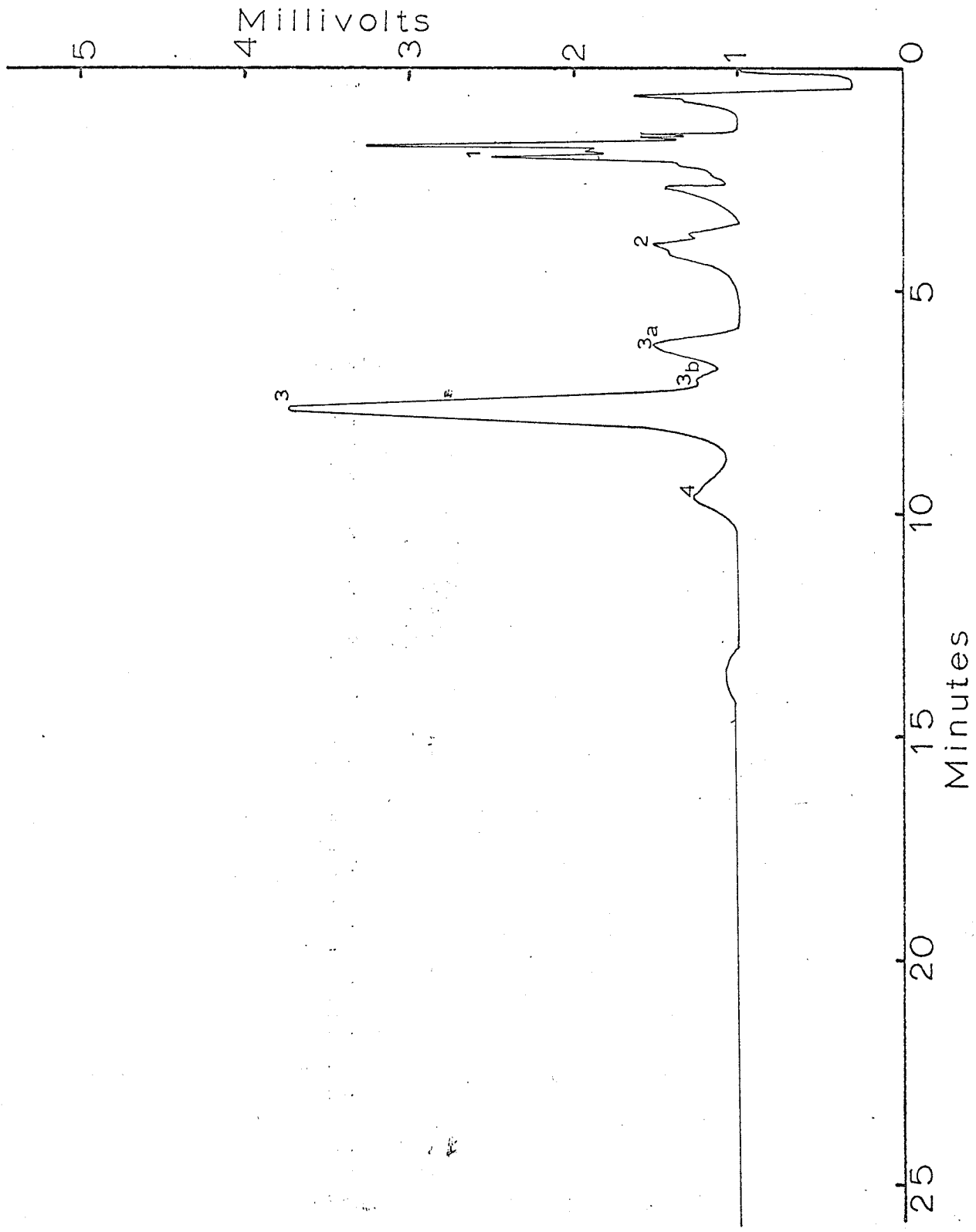

Millivolts
5
4
3
2
1
0
1
2
5
10
15
20
25
Minutes

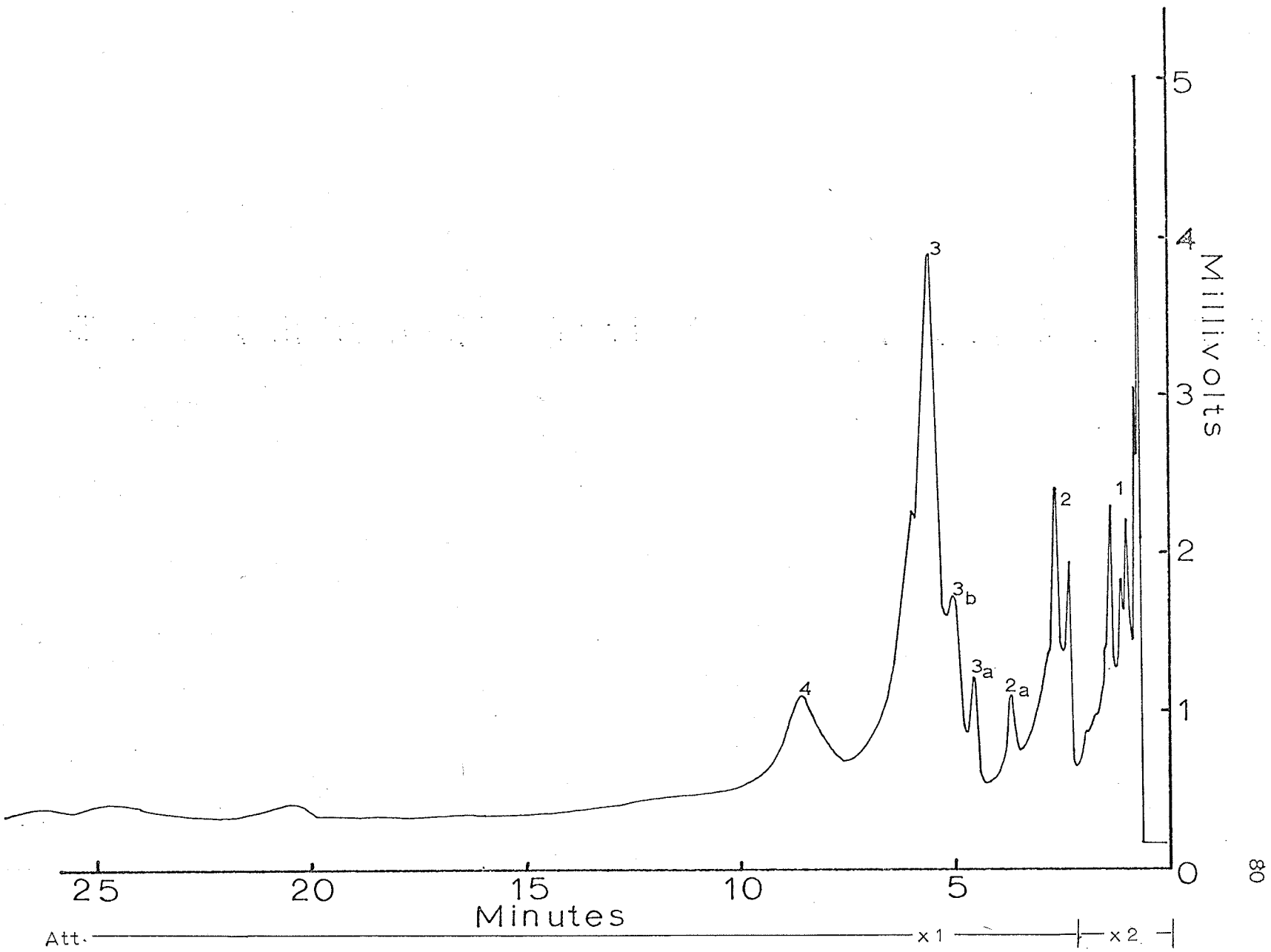
FIGURE 32. Pyrogram of *Aerobacter Arogenes* DNA.

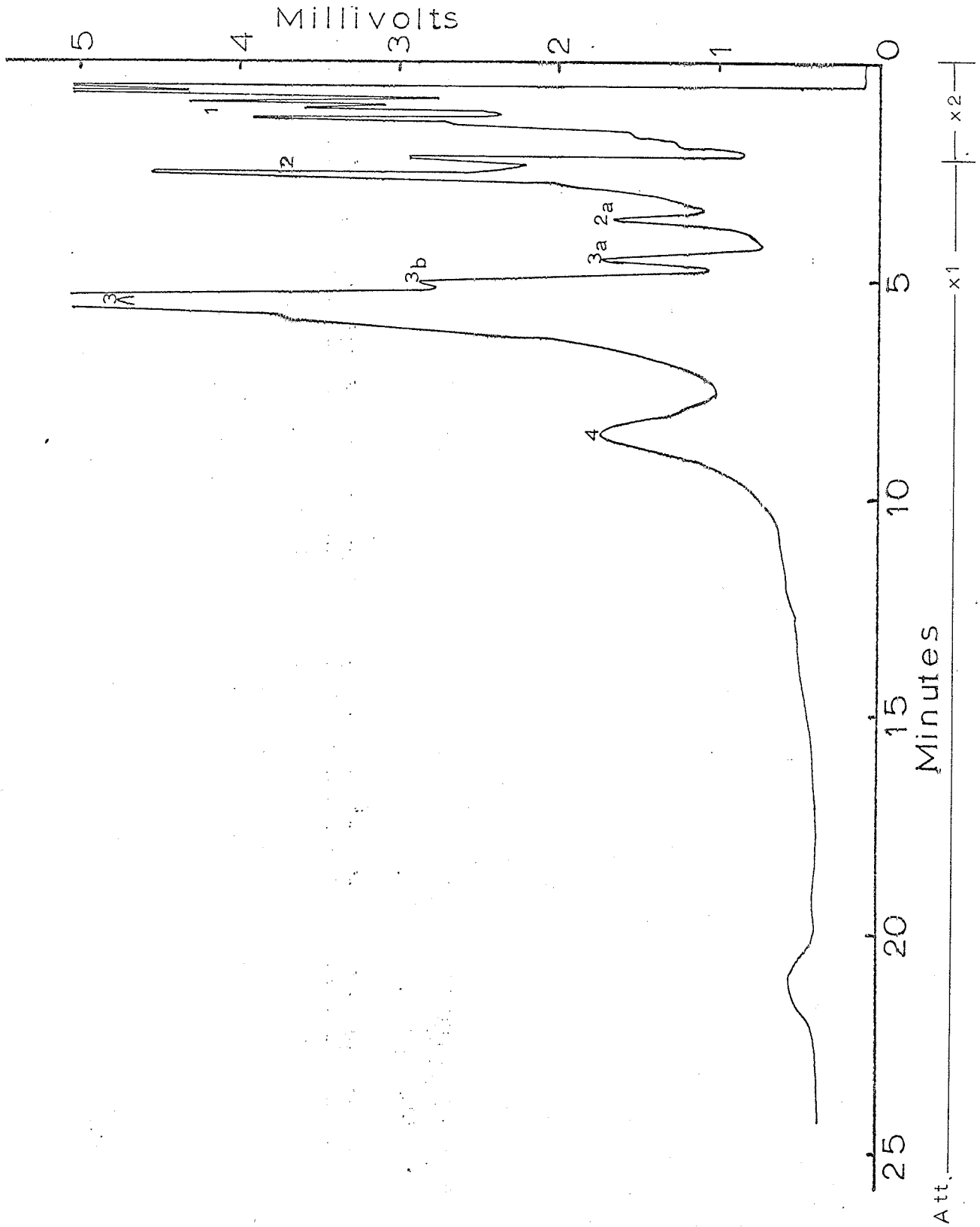
Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2, x1 (as shown)
Sample Size: 50 µg.
Column : #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min.at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.


Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
6
5
10
15
20
35
Minutes
x2
x4
x1
Att


Millivolts
5
4
3
2
1
0
1
2
3
3a
3b
4
5
10
15
20
25
Minutes


Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
10
15
20
25
Minutes


Millivolts
5
4
3
2
1
0
25
20
15
10
5
Minutes
Att.
x 1
x 2
1
2
2a
3
3a
3b
4


Millivolts
5
4
3
2
1
0
1
2
2a
3a
3b
3
4
x2
x1
5
10
15
20
25
Minutes
Att.

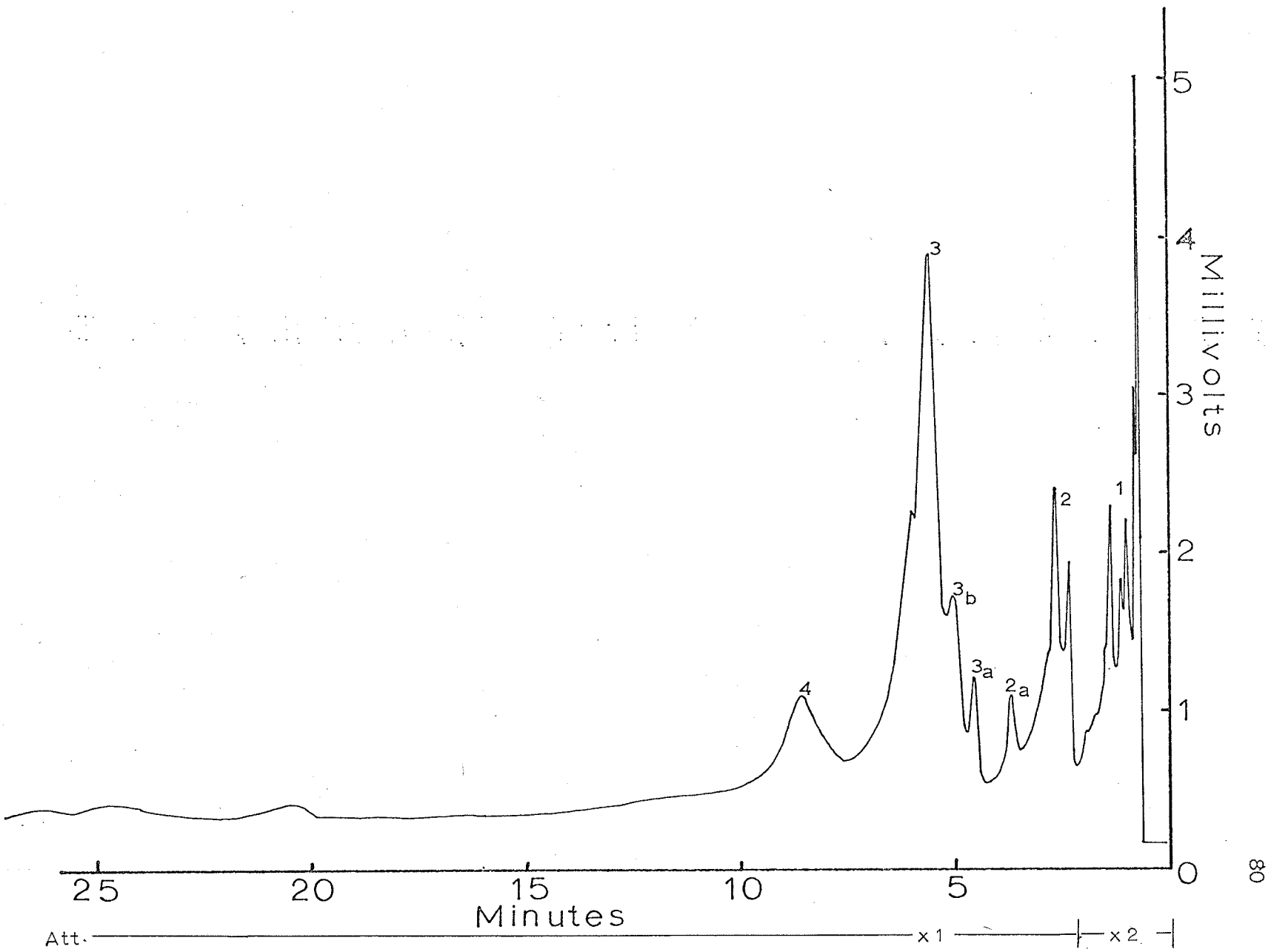
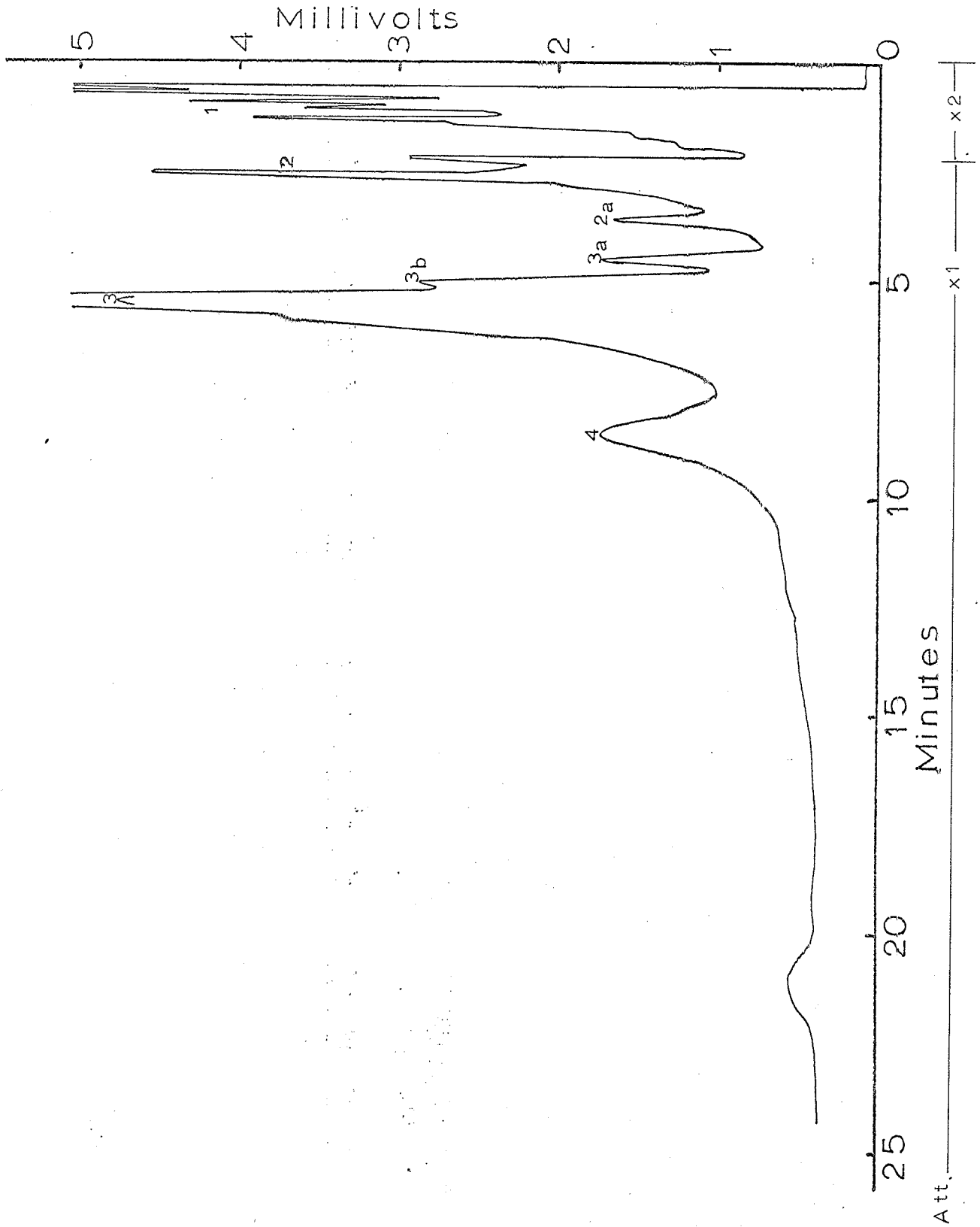

Millivolts
5
4
3
2
1
0
25
20
15
10
5
Minutes
Att.
x 1
x 2
1
2
2a
3
3a
3b
4

FIGURE 32-A. Repeat Pyrogram of Aerobacter Arogenes DNA.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2,x1 (as shown)
Sample Size: 60 µg.
Column: #3.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 53°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml./min.
Powerstat Setting: #80.
Pyrolysis Time: 7 seconds.


Millivolts
5
4
3
2
1
0
1
2
2a
3a
3b
3
4
x2
x1
5
10
15
20
25
Minutes
Att.

comparison of the pyrograms of 50 micrograms of guanosine or adenosine with those of 50 micrograms of thymidine or cytidine indicated that the yield of products from the pyrimidine nucleosides was much greater than the yield (on a mole-for-mole basis) from the purine nucleosides. Hence, for a mixture of nucleosides containing equi-molar amounts of purine and pyrimidine nucleosides (e.g., DNA), the pyrolysis peaks would result essentially from the pyrimidines. Contributions to the peak areas from the purine nucleosides would be negligible for the pyrolysis conditions used. The final chromatogram in each series represents the pyrolysis of a deoxyribose nucleic acid. This pyrolysis pattern can be seen as a composite of the patterns found for the various nucleosides. In some cases two pyrograms are given to show the reproducibility of the pyrolyses.

For any one compound, a linear relationship could be obtained between the area of any given peak in the pyrogram and the amount of sample applied to the coil. Figures 33 to 35 show several of these relationships.

An interesting result appeared when the plots for two successive calibration curves were compared (Figure 35). Although the slope of the two curves is the same, the curve for one calibration shows a larger peak area per microgram of sample pyrolysed than the curve for a subsequent calibration. The conditions of pyrolysis, according to the powerstat setting and pyrolysis chamber temperature, were identical. The only difference between the two calibrations is to be found in the number of times the coil was fired. It is suggested that the decrease

FIGURE 33. Calibration Curve for Cytosine: Plot of Peak Area[1] Versus Weight of Sample Applied to Pyrolysis Coil.

[1]Peak #3, Figure 13.

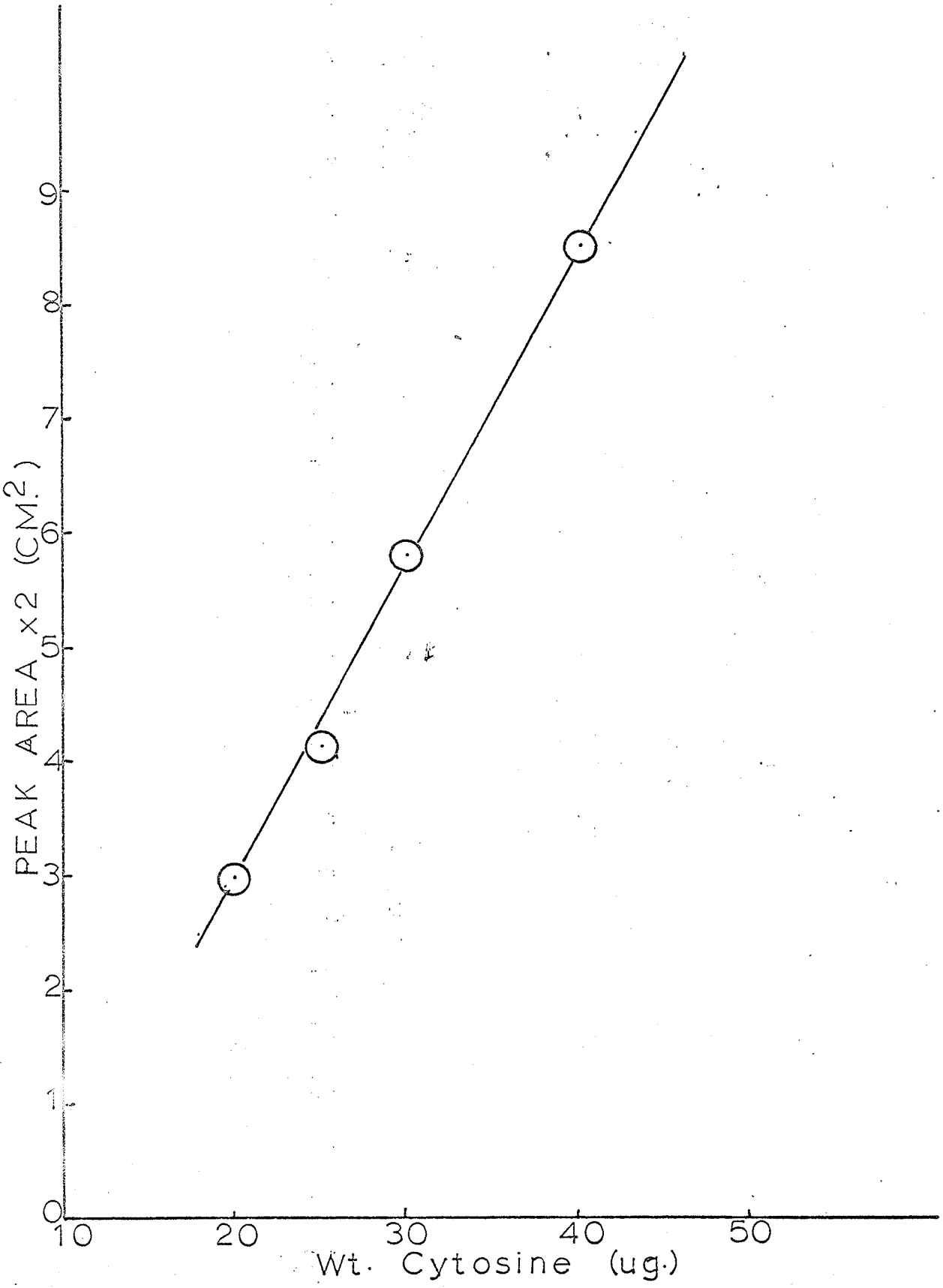

PEAK AREA x2 (CM.2)
0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
Wt. Cytosine (ug.)

FIGURE 34. Calibration Curve for Thymine: Plot of Peak Area[1] Versus Weight of Sample Applied to Pyrolysis Coil.

[1]Peak #5, Figure 17.

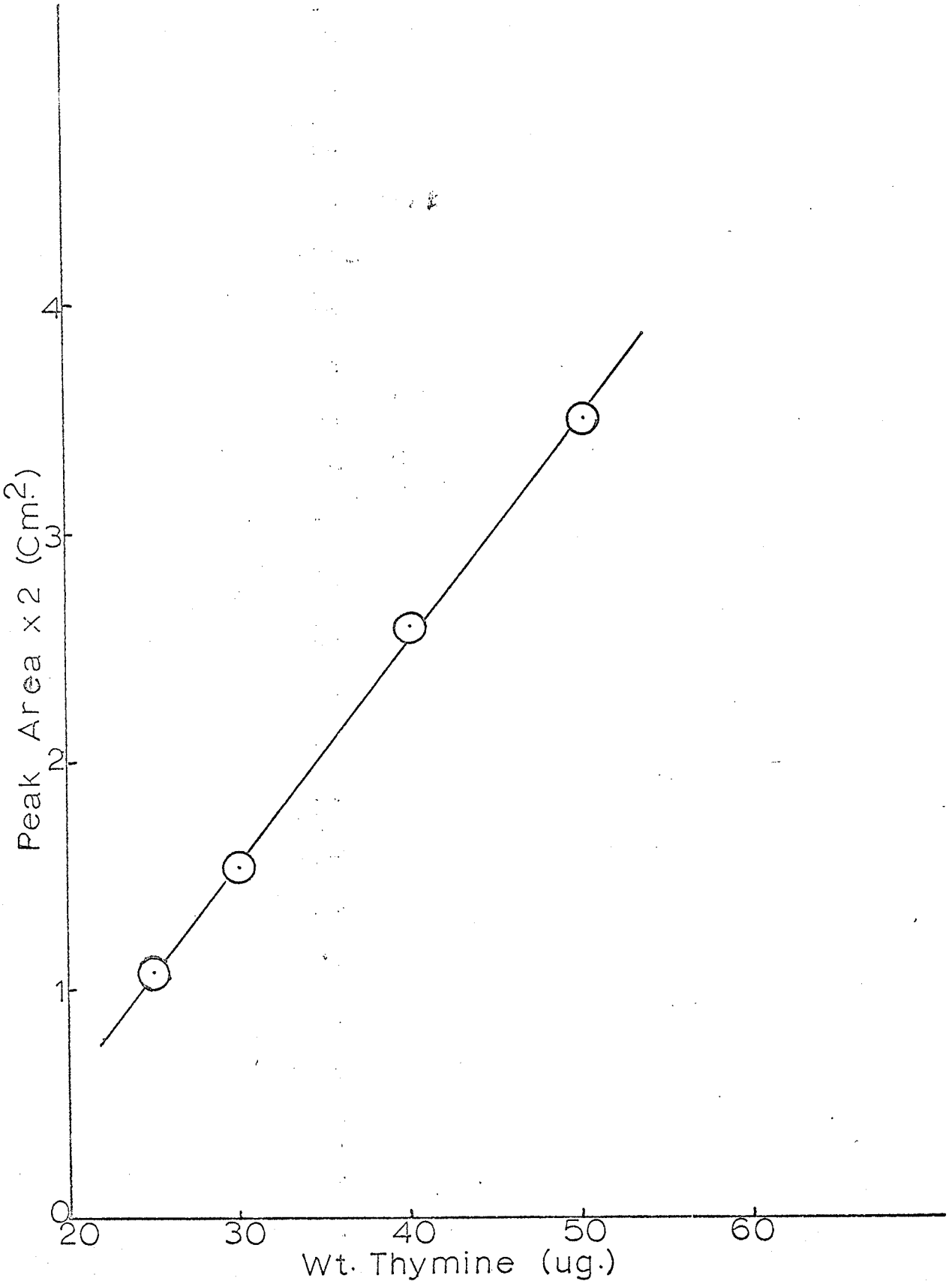
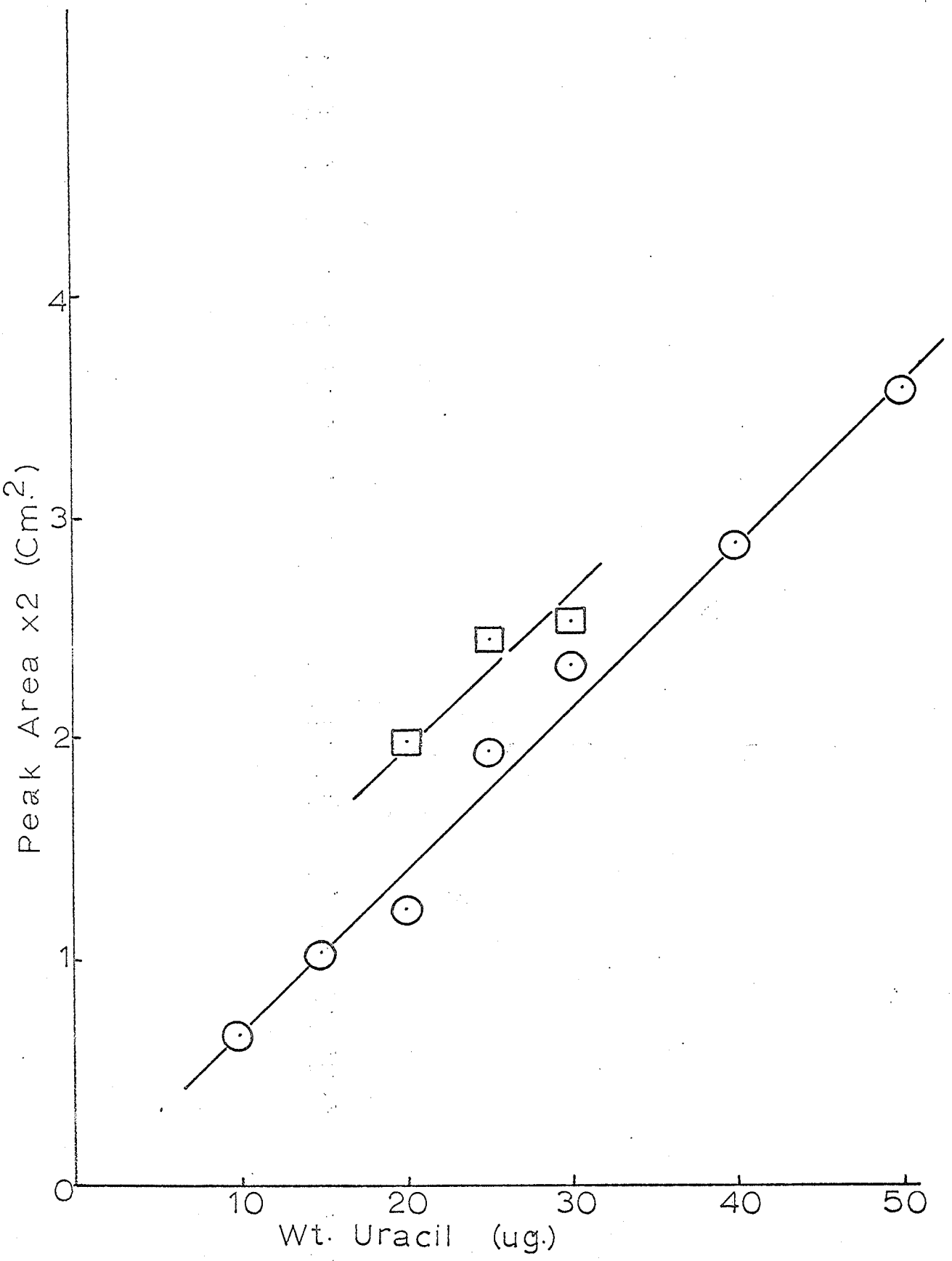

Peak Area x 2 (Cm.2)
0
1
2
3
4
20
30
40
50
60
Wt. Thymine (ug.)

FIGURE 35. Calibration Curve for Uracil: Plot of Peak Area[1] Versus Weight of Sample Applied to Pyrolysis Coil.

[1]Peak #3, Figure 15.


Peak Area x2 (Cm.2)
0
1
2
3
4
10
20
30
40
50
Wt. Uracil (ug.)

in coil temperature for the same powerstat setting, reported by Jennings and Dimick, is sufficient, even after fifteen trials, to change the yield of pyrolysis products significantly. The observed lower yield for the second set[1] of pyrolyses is consistent with this hypothesis.

Calculations based on the relative peak areas are shown for cytosine in Table 7. The ratio of one peak area to another can be seen to have a constant value within the limits of error encountered in measuring the peak areas. However, in several pyrolysis trials, pyrograms were observed which yielded anomalous values for these ratios. In most cases these trials were repeated to give consistent results. The fact remains, however, that these unexpected results can appear and that, for the purposes of quantitative studies, results such as these must be anticipated. Any quantitative calibration based on relative peak areas would be meaningless in these anomalous cases. The results of one such series for thymine are shown in Tables 8-A and 8-B.

Another difficulty which arose consistently in the course of these studies was the appearance of unexpected and irreproducible peaks in several pyrograms. These peaks appeared despite the precaution of cleaning the coil in a stream of oxygen between each pyrolysis. In another instance, the firing of the cleaned coil as a blank gave rise to a series of unexplained peaks. The repeated firing of the blank coil eventually eliminated these peaks. These peaks were presumably

[1] Lower curve in Figure 35.

TABLE 7. Ratio of Peak Areas For Cytosine[1]

Peak #[3]	Peak Area x 2(Cm^2)	Sample Size (in micrograms)	Ratio 1/2
3	3.00	20	1.83 ± 4%[2]
4	1.64		
3	4.14	25	2.04
4	2.03		
3	5.82	30	1.60
4	3.63		
3	5.39	40	2.00
4	2.70		
3	8.54	40	2.11
4	4.05		
		Average:	1.91

[1]Pyrolysis products chromatographed on column #2.

[2]The percentage error is based on an estimated accuracy of ±0.01 cm in measuring the dimensions of the triangulated peak areas.

[3]The numbers refer to the corresponding peaks in Figure 13.

TABLE 8-A. Peak Areas For Thymine[1]

Peak #[2]	Sample Size (in micrograms)	Area x 2 (Cm^2)
2[3]	20	2.08
3		0.40
4		0.71
5		0.96
2	25	2.76
3		0.77
4		3.70
5		1.08
2	30	4.06
3		1.05
4		2.52
5		1.55
2	30	6.88
3		1.10
4		2.49
5		1.44
2	40	5.41
3		0.85
4		4.93
5		2.60
2	50	10.30
3		1.17
4		5.63
5		3.50

[1]Pyrolysis products chromatographed on column #2.

[2]The numbers refer to the corresponding peaks in Figure 17.

[3]Composite of second group of peaks in Figure 16, and similar group in other Figures designated also as #2.

TABLE 8-B. Ratio of Peak Areas For Thymine

Ratio Taken [1]	Sample Size (in micrograms)	Ratio
2/4	20	2.93 ± 4%
"	25	0.75
"	30	1.61
"	30	1.76
"	40	1.10
"	50	1.82
2/5	20	2.17
"	25	2.56
"	30	2.62
"	30	4.78
"	40	2.08
"	50	2.94
4/3	20	4.28
"	25	4.81
"	30	2.40
"	30	2.26
"	40	5.80
"	50	4.80
4/5	20	1.78
"	25	3.42
"	30	1.63
"	30	1.73
"	40	1.90
"	50	1.61

[1] From Table 8-A.

caused by the collection of non-volatile pyrolysis products on the coil, in the pyrolysis chamber, or in the column head. The pyrolysis apparatus could not be cleaned conveniently in the middle of a series of pyrolyses. The peaks did not seem to bear any relation to the pyrolysis which had preceded their appearance.

The pyrograms of the quaternary salt are shown in Figures 36 to 38. The negative peaks on the chromatograms obtained using the Pye chromatograph are attributed to the presence of water in the pyrolysis products. The chromatograms obtained on the Hy-Fi apparatus using a different column and different pyrolysis conditions would show no peak for water because of the detection system used.

Tables 9-A and 9-B and 10-A and 10-B and Figures 39 to 40 give the results of the pyrolysis of mixtures of two pyrimidines and of mixtures of two of the corresponding nucleosides. Plots of the various peak areas and ratios of peak areas versus the composition of the mixtures are shown in Figures 41 to 51.

Work by Levy on the pyrolysis of mixtures of porphyrins (88-A), has shown how the relative peak areas on the pyrograms can be used to determine the composition of the mixture:

(a) Let the ratio of the areas of two peaks on the pyrogram of one compound be A.

(b) Let the ratio of the areas of two identical peaks on the pyrogram of the second compound be B.

(c) Let the ratio of the two peaks on the pyrogram of the mixture of compounds be M.

FIGURE 36. Pyrogram of Triethyl Methyl Ammonium Iodide.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x8,x4 (as shown)
Sample Size: Approx. 20 μg.
Column: #2.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 33°C.
Nitrogen Flow: 24 ml/min. at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Powerstat Setting: #65.
Pyrolysis Time: 7 seconds.

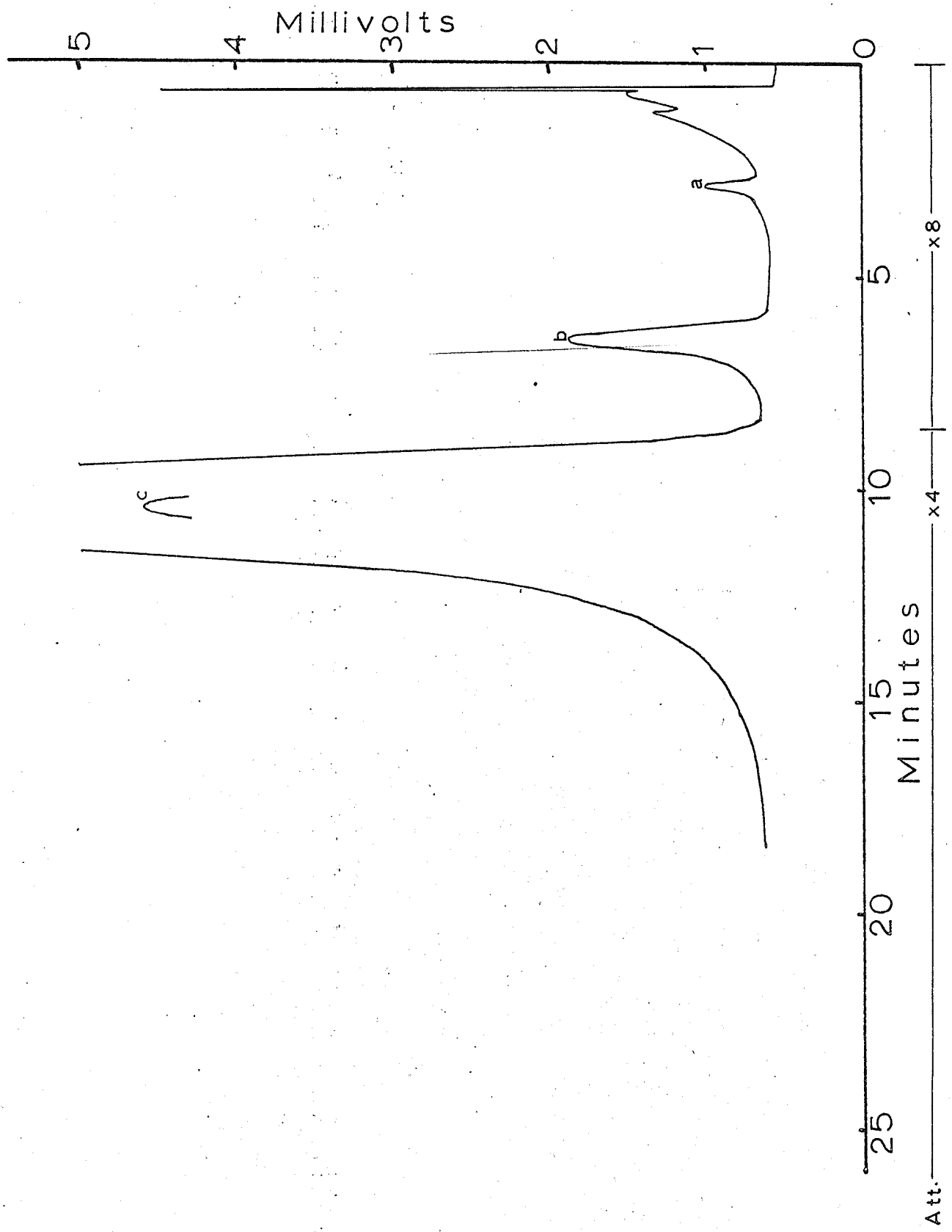

Millivolts
5
4
3
2
1
0
a
b
c
5
10
15
20
25
Minutes
×8
×4
Att.

FIGURE 37. Repeat Pyrogram of Triethyl Methyl Ammonium Iodide.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x4,x8 (as shown).
Sample Size: Approx. 25 µg.
Column: #2
Conditions: Preheater Temperature: 100°C.
Column Temperature: 34°C.
Nitrogen Flow: 24 ml/min. at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #65.
Pyrolysis Time: 7 seconds.

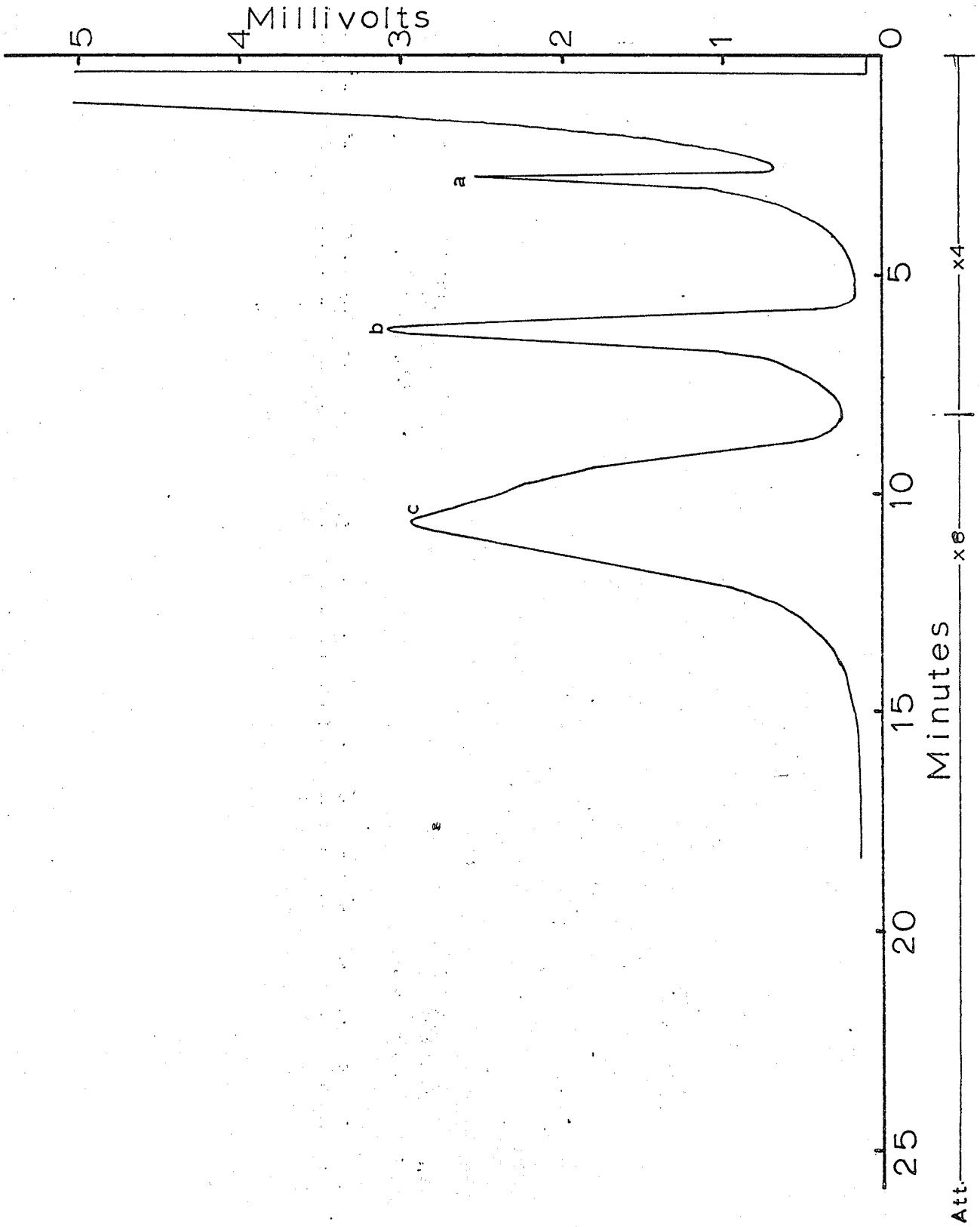

Millivolts
5
4
3
2
1
0
a
b
c
5
10
15
20
25
Minutes
x4
x8
Att.

FIGURE 38.[1] Pyrolysis of Triethyl Methyl Ammonium Iodide Using the Pye Argon Chromatograph.

Detector Sensitivity: Voltage 1250;
Attenuation x3
Sample Size: Approx. 50 µg.
Column: Squalane, 20% on 80/90 mesh Anachrom A.
Conditions: Column Temperature: 100°C.
Argon Flow: 75 ml/min.
Variac Setting: #40 (to give an estimated coil temperature of 1000°C).
Pyrolysis Time: 4 seconds.

[1]Peaks a and b are situated in the negative "water peak" found for experiments done using this chromatograph.

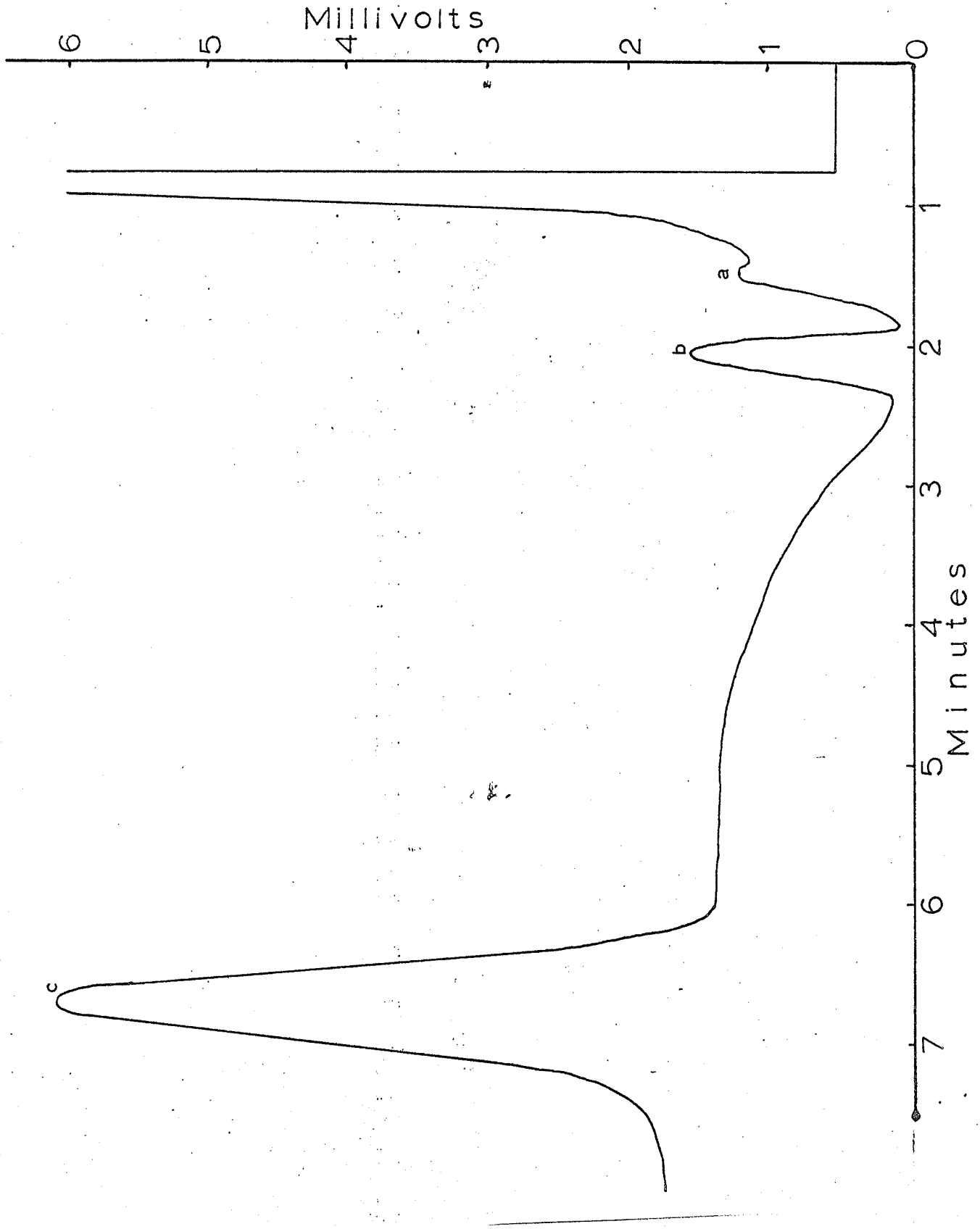

Millivolts
6
5
4
3
2
1
0
a
b
c
1
2
3
4
5
6
7
Minutes

TABLE 9-A. Peak Areas For Mixtures of Thymine and Cytosine[1]

Peak #[3]	Peak[2] Area x 2 (Cm^2)		% Thymine	Total Sample Size
	a	b		
3	13.05	-	30	50 µg
4	12.00	-		
5	2.21	-		
3	12.15	8.83	40	50 µg
4	13.23	7.10		
5	3.40	-		
3	9.19	6.78	50	50 µg
4	8.29	6.78		
5	2.20	2.40		
3	6.94	7.30	60	50 µg
4	8.50	7.43		
5	-	3.21		
3	4.98	4.85	70	50 µg
4	7.10	6.66		
5	2.38	3.20		

[1]Pyrolysis products chromatographed on column #2.

[2]The two results represent calibration experiments performed on two separate occasions.

[3]The numbers refer to the corresponding peaks in Figure 39.

TABLE 9-B. Ratios of Peak Areas for Thymine-Cytosine Mixtures.

Ratio Taken [2]	% Thymine	Ratio[1]	
		a	b
3/4	30	1.09	-
	40	0.92	1.24
	50	1.11	1.00
	60	0.95	0.98
	70	0.70	0.73
3/5	30	5.9	-
	40	3.58	-
	50	4.17	2.82
	60	-	2.27
	70	2.09	1.51
4/5	30	5.44	-
	40	3.90	-
	50	3.76	2.83
	60	-	2.32
	70	2.98	2.08
4/3	30	0.92	-
	40	1.09	0.86
	50	0.90	1.00
	60	1.23	1.02
	70	1.43	1.37

[1]The two results represent calibration experiments performed on two separate occasions.

[2]From Table 9-A.

TABLE 10-A. Peak Areas for Mixtures of Thymidine and Cytidine[1]

Peak #[2]	Peak Area x 2 (Cm^2)	% Thymidine by weight	Total sample size (micrograms)
3a	1.70	20	50 µg
3b	8.69	"	"
3	15.40	"	"
4	5.42	"	"
5	4.82	"	"
3a	1.71	30	50 µg
3b	8.17	"	"
3	15.70	"	"
4	4.69	"	"
5	4.76	"	"
3a	1.15	40	50 µg
3b	4.50	"	"
3	10.10	"	"
4	2.56	"	"
5	2.92	"	"
3a	2.35	50	50 µg
3b	6.92	"	"
3	12.50	"	"
4	5.96	"	"
5	1.80	"	"
3a	2.53	60	50 µg
3b	6.39	"	"
3	12.80	"	"
4	4.76	"	"
5	0.56	"	"
3a	1.04	70	50 µg
3b	3.71	"	"
3	11.10	"	"
4	3.96	"	"
5	1.01	"	"
3a	3.52	80	50 µg
3b	3.90	"	"
3	12.10	"	"
4	3.38	"	"
5	0.54	"	"

[1]Pyrolysis products chromatographed on column #3.

[2]The numbers refer to the corresponding peaks in Figure 40.

TABLE 10-B. Ratios of Peak Areas for Thymidine-Cytidine Mixtures.

Ratio Taken[1]	% Thymidine	Ratio
3b/3a	20	5.10
	30	4.78
	40	3.91
	50	2.94
	60	2.52
	70	3.56
	80	1.11
3/3a	20	9.06
	30	9.19
	40	8.79
	50	5.32
	60	5.06
	70	10.70
	80	3.44
3/3b	20	1.77
	30	1.92
	40	2.24
	50	1.81
	60	2.01
	70	2.99
	80	3.09
3/4	20	2.84
	30	3.36
	40	3.95
	50	2.10
	60	2.69
	70	2.80
	80	3.58
4/3a	20	3.18
	30	2.74
	40	2.22
	50	2.52
	60	1.88
	70	3.80
	80	0.96
4/5	20	1.12
	30	0.99
	40	0.88
	50	3.32
	60	8.50
	70	3.92
	80	6.26

[1]From Table 10-A.

FIGURE 39. Typical Pyrogram of Mixture of Pyrimidines.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1
Sample Size: 50 µg.
Composition: 30% Thymine, 70% Cytosine.
Column: #2.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 32°C.
Nitrogen Flow: 24 ml/min. at 15 P.S.I.G.
Hydrogen Flow: 25 ml/min. at 10 P.S.I.G.
Powerstat Setting: #80
Air Flow: 300 ml/min.
Pyrolysis Time: 7 seconds.

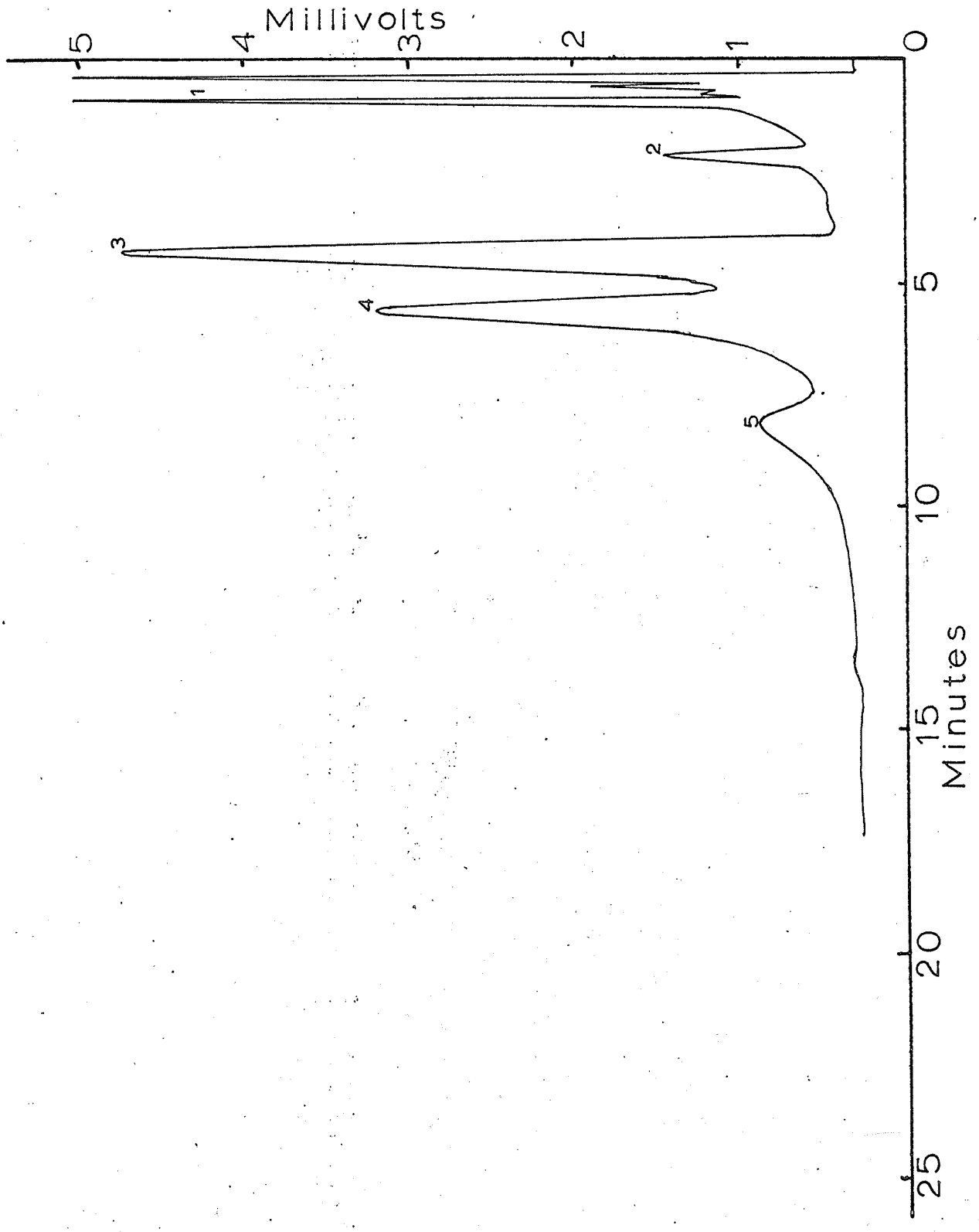

Millivolts
5
4
3
2
1
0
1
2
3
4
5
5
10
15
20
25
Minutes

FIGURE 40. Typical Pyrogram of Mixture of Nucleosides.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x4,x2,x1 (as shown).
Sample Size: 50 µg.
Composition: 30% Thymidine, 70% Cytidine.
Column: #3.
Conditions: Preheater Temperature:110°C.
Column Temperature:52°C.
Nitrogen Flow: 30.8 ml/min. at 13.5 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10.5 P.S.I.G.
Air Flow: 300 ml/min.
Powerstat Setting: #80
Pyrolysis Time: 7 seconds.

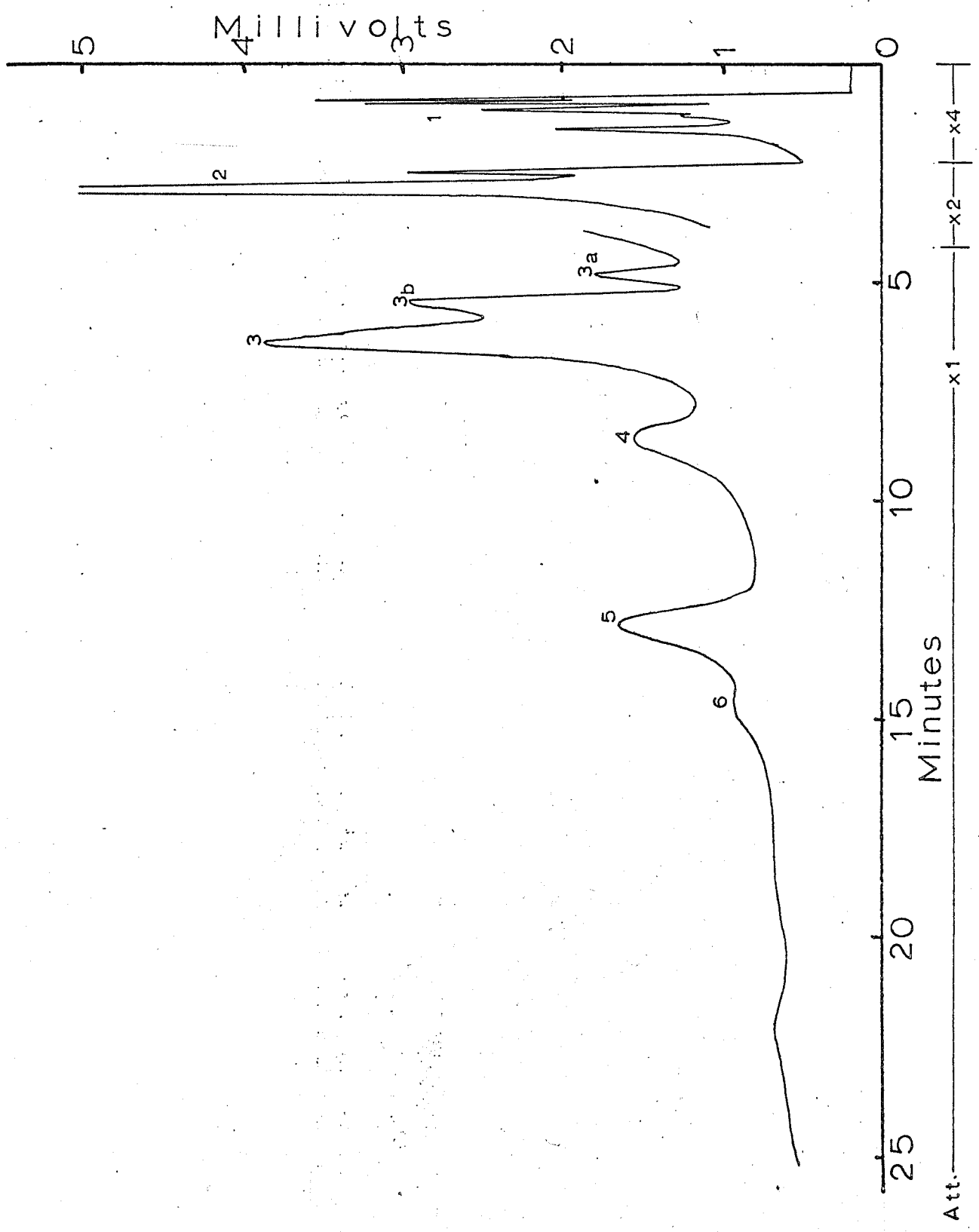

Millivolts
5
4
3
2
1
0
1
2
3a
3b
3
4
5
6
x4
x2
x1
5
10
15
20
25
Minutes
Att.

FIGURE 41. Calibration Curve for Pyrimidine Mixture. Plot of Peak Area[1] Versus Percent Thymine for Thymine-Cytosine Mixture.

[1]Peak #3, Figure 39.

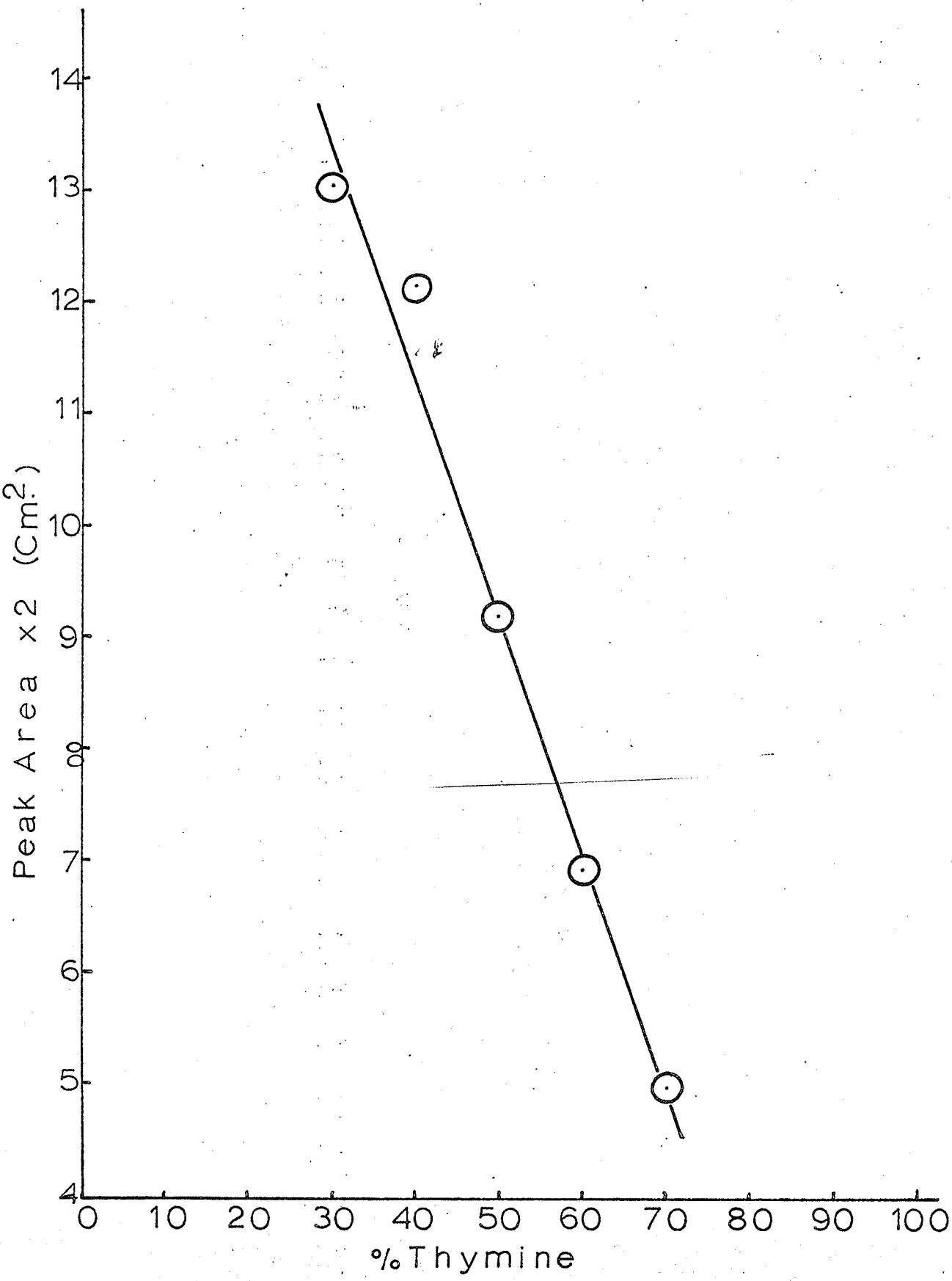

Peak Area x2 (Cm.²)
14
13
12
11
10
9
8
7
6
5
4
0
10
20
30
40
50
60
70
80
90
100
% Thymine

FIGURE 42. Calibration Curve for Pyrimidine Mixture. Plot of Peak Area[1] Versus Percent Thymine for Thymine-Cytosine Mixture.

[1]Peak #4, Figure 39.

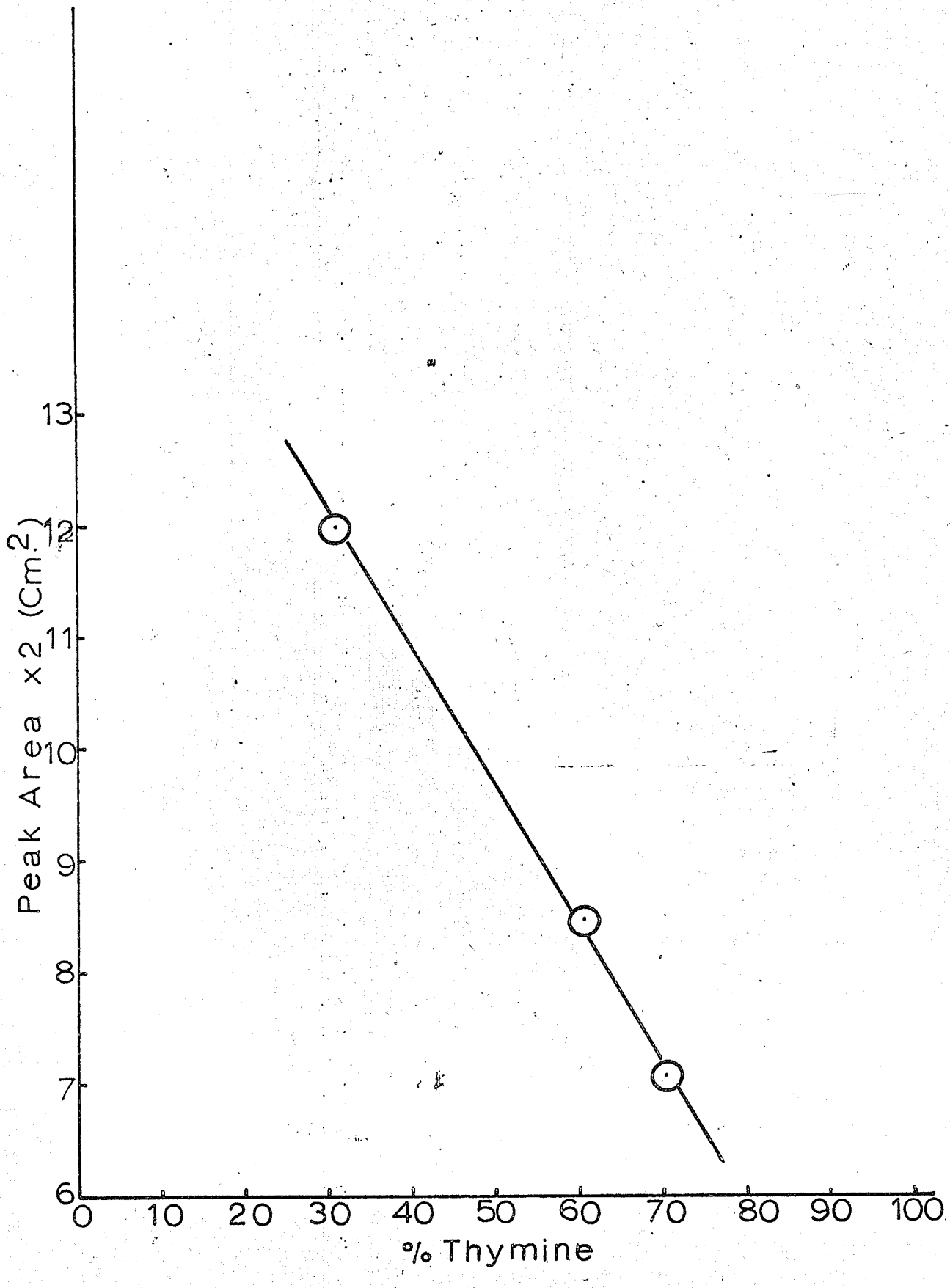

Peak Area x2 (Cm.2)
13
12
11
10
9
8
7
6
0
10
20
30
40
50
60
70
80
90
100
% Thymine

FIGURE 43. Calibration Curve for Pyrimidine Mixture. Plot of Ratio of Peak Areas[1] Versus Percent Thymine for Thymine-Cytosine Mixture.

[1]Ratio of Peak Areas: 4/3, Figure 39 (see Table 9-B)

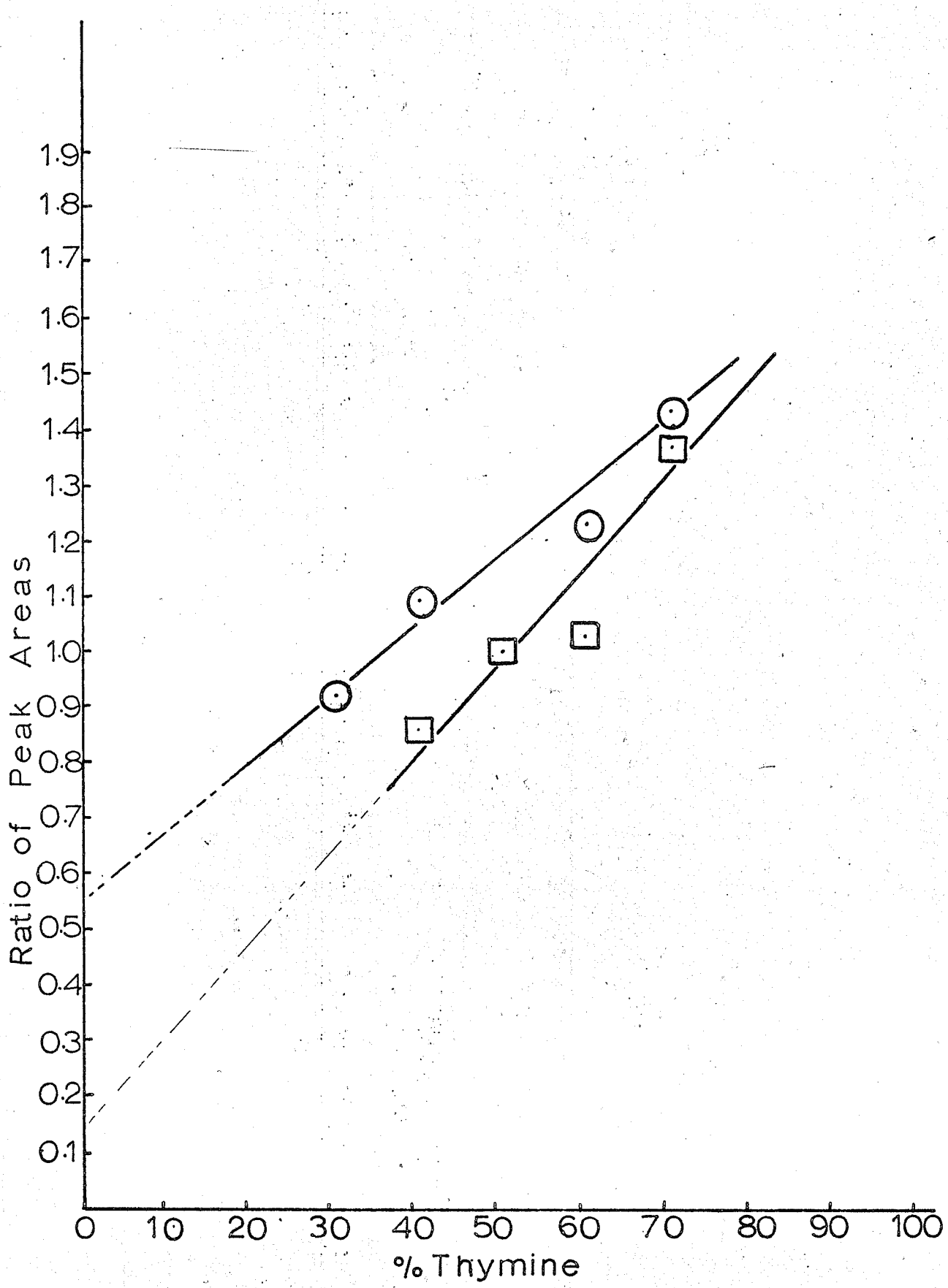

Ratio of Peak Areas
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
70
80
90
100
% Thymine

FIGURE 44. Calibration Curve for Pyrimidine Mixture. Plot of Ratio of Peak Areas[1] Versus Percent Thymine for Thymine-Cytosine Mixture.

[1]Ratio of Peak Areas:3/5, Figure 39 (see Table 9-B).

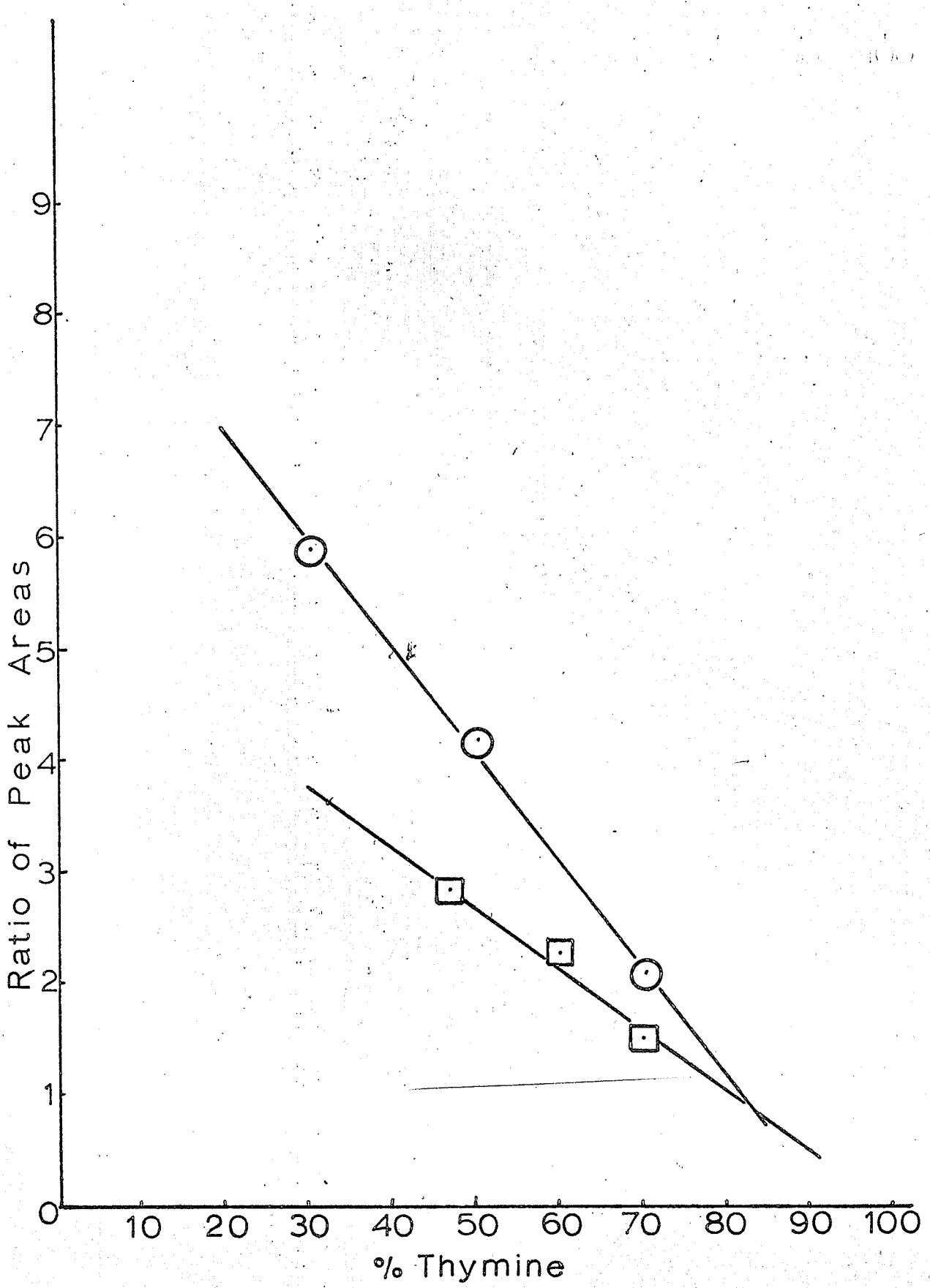

Ratio of Peak Areas
0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
100
% Thymine

FIGURE 45. Calibration Curve for Nucleoside Mixture. Plot of Peak Area[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Peak #3a, Figure 40.

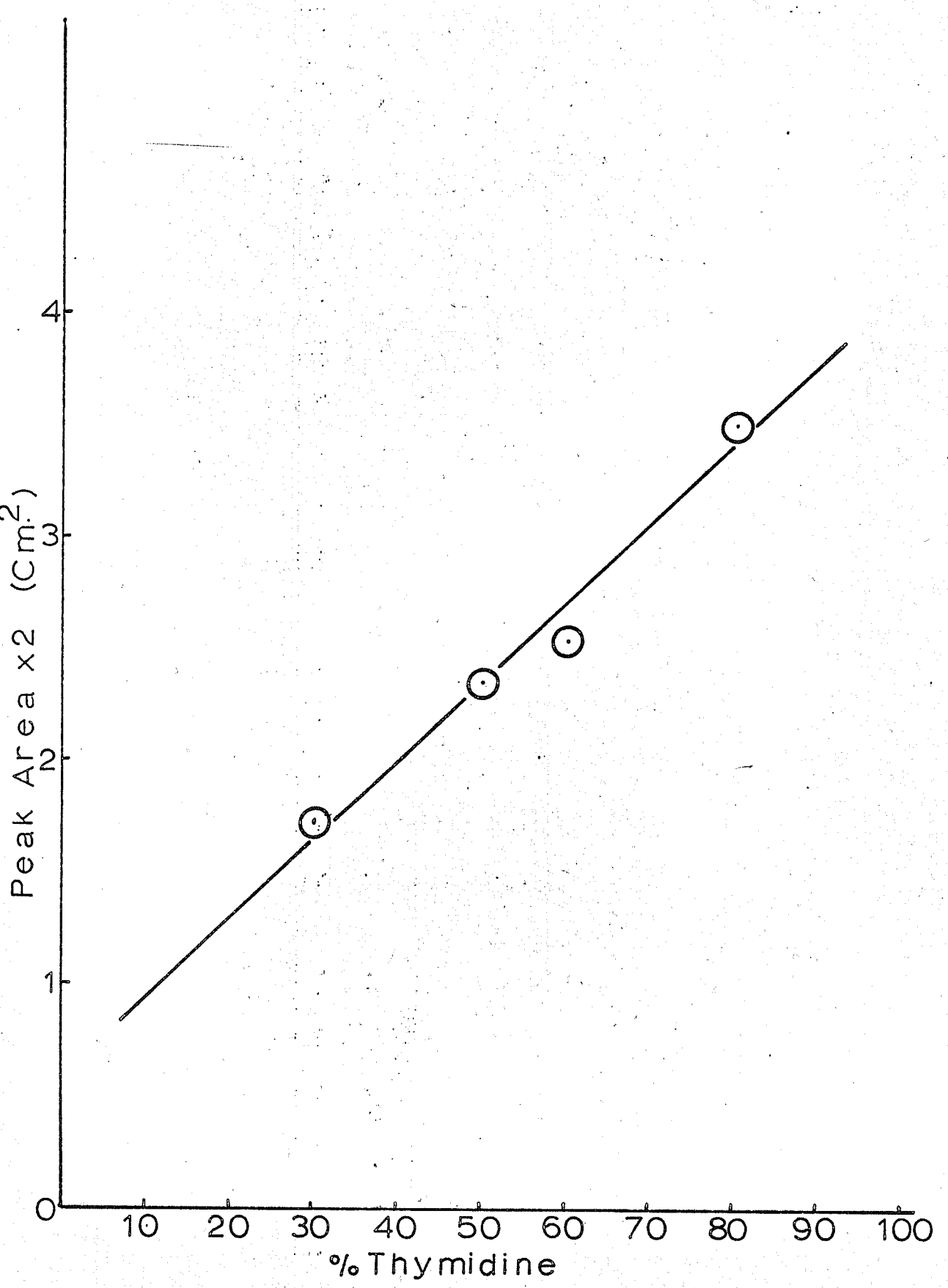

Peak Area x2 (Cm.2)
0
1
2
3
4
10
20
30
40
50
60
70
80
90
100
% Thymidine

FIGURE 46. Calibration Curve for Nucleoside Mixture. Plot of Peak Area[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Peak #3b, Figure 40.

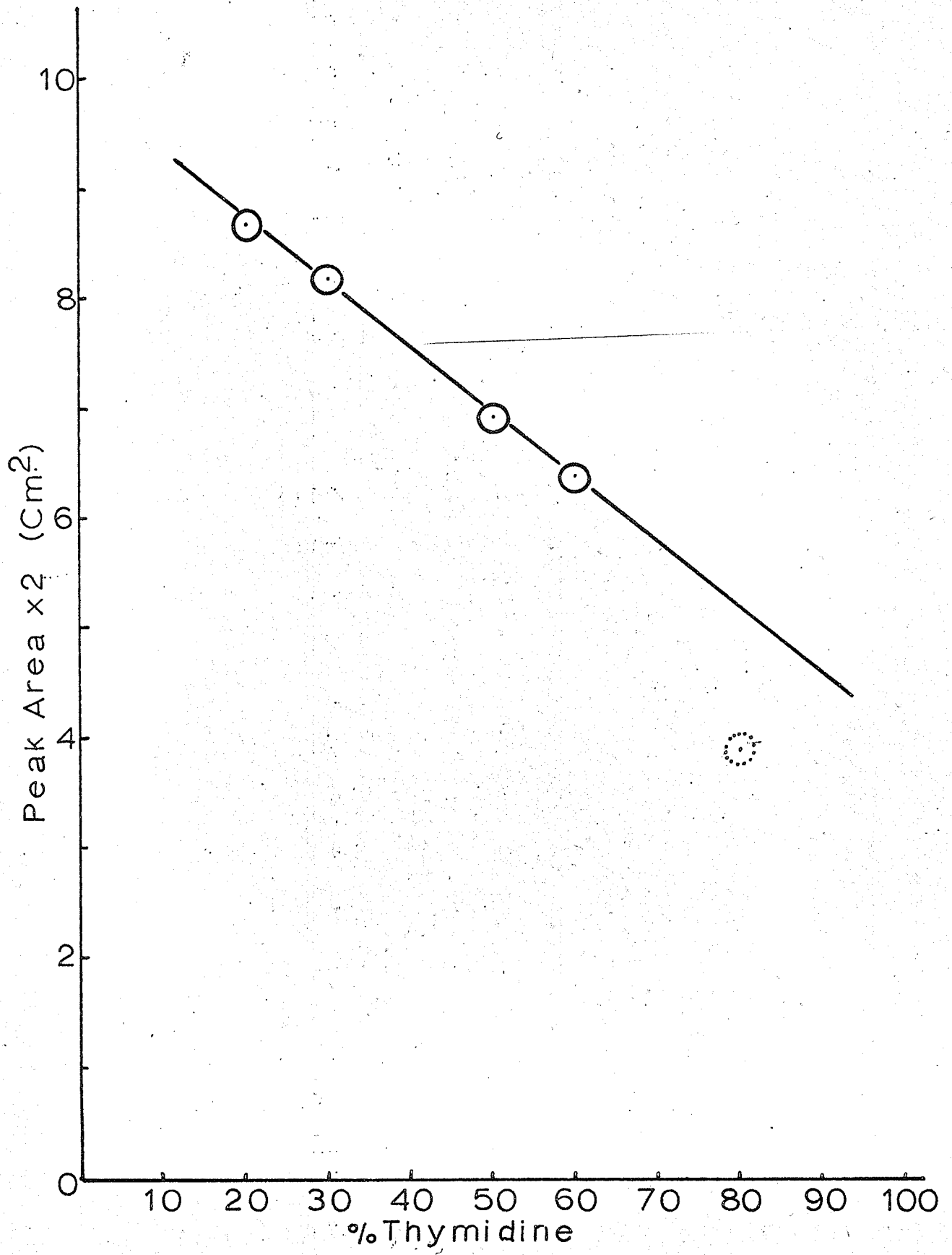

Peak Area x2 (Cm.2)
0
2
4
6
8
10
10 20 30 40 50 60 70 80 90 100
% Thymidine

FIGURE 47. Calibration Curve for Nucleoside Mixture. Plot of Peak Area[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Peak #5, Figure 40.

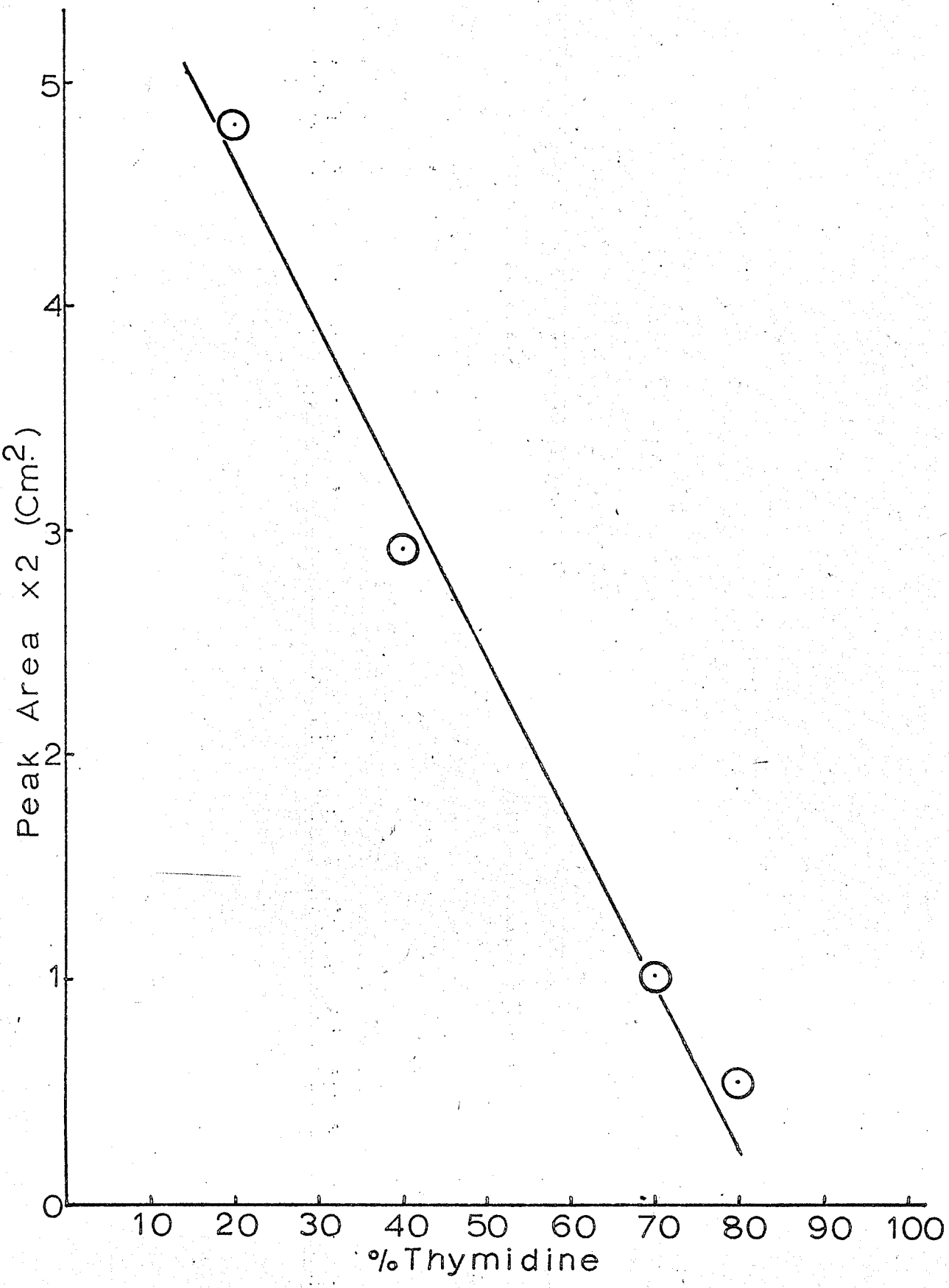

Peak Area x2 (Cm.2)
0
1
2
3
4
5
10
20
30
40
50
60
70
80
90
100
%Thymidine

FIGURE 48. Calibration Curve for[1] Nucleoside Mixture. Plot of Ratio of Peak Area[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Ratio of Peak Areas: 3b/3a, Figure 40 (see Table 10-B)

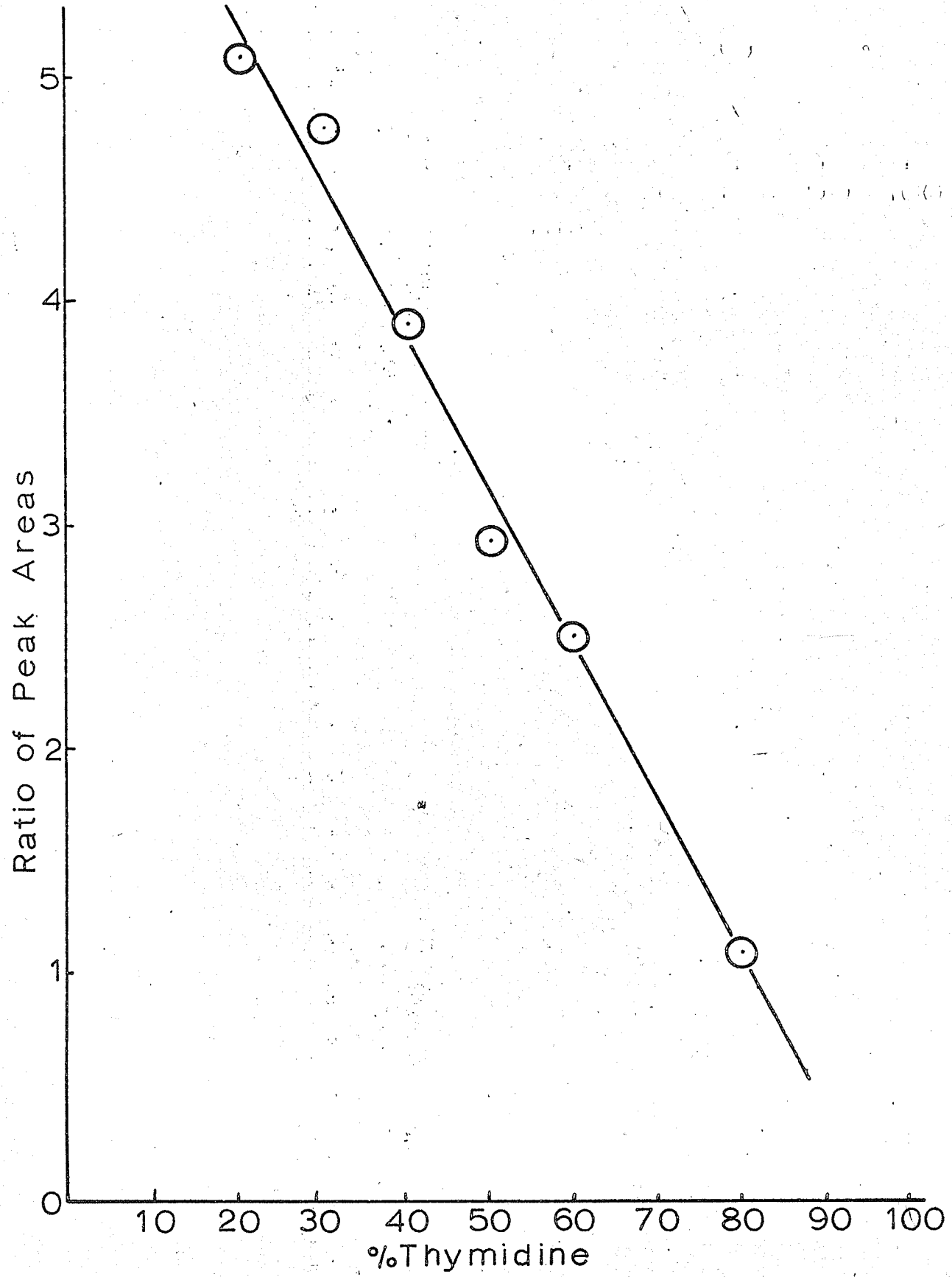

Ratio of Peak Areas
0
1
2
3
4
5
10
20
30
40
50
60
70
80
90
100
%Thymidine

FIGURE 49. Calibration Curve for Nucleoside Mixture. Plot of Ratio of Peak Areas[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Ratio of Peak Areas: 3/3b, Figure 40 (see Table 10-B)

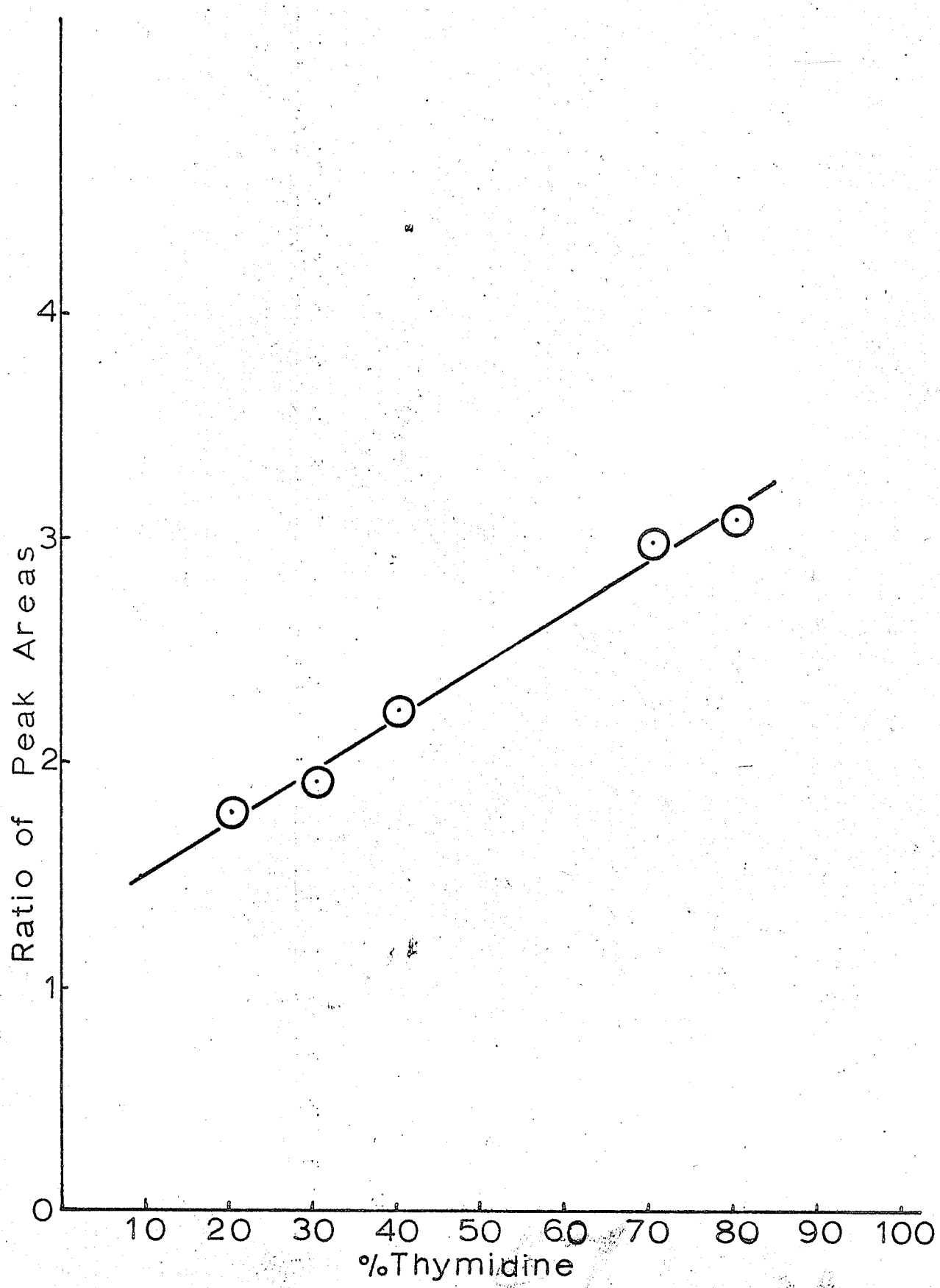

Ratio of Peak Areas
0
1
2
3
4
10
20
30
40
50
60
70
80
90
100
%Thymidine

FIGURE 50. Calibration Curve for Nucleoside Mixture. Plot of Ratio of Peak Areas[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1]Ratio of Peak Areas: 3/3a, Figure 40 (see Table 10-B)

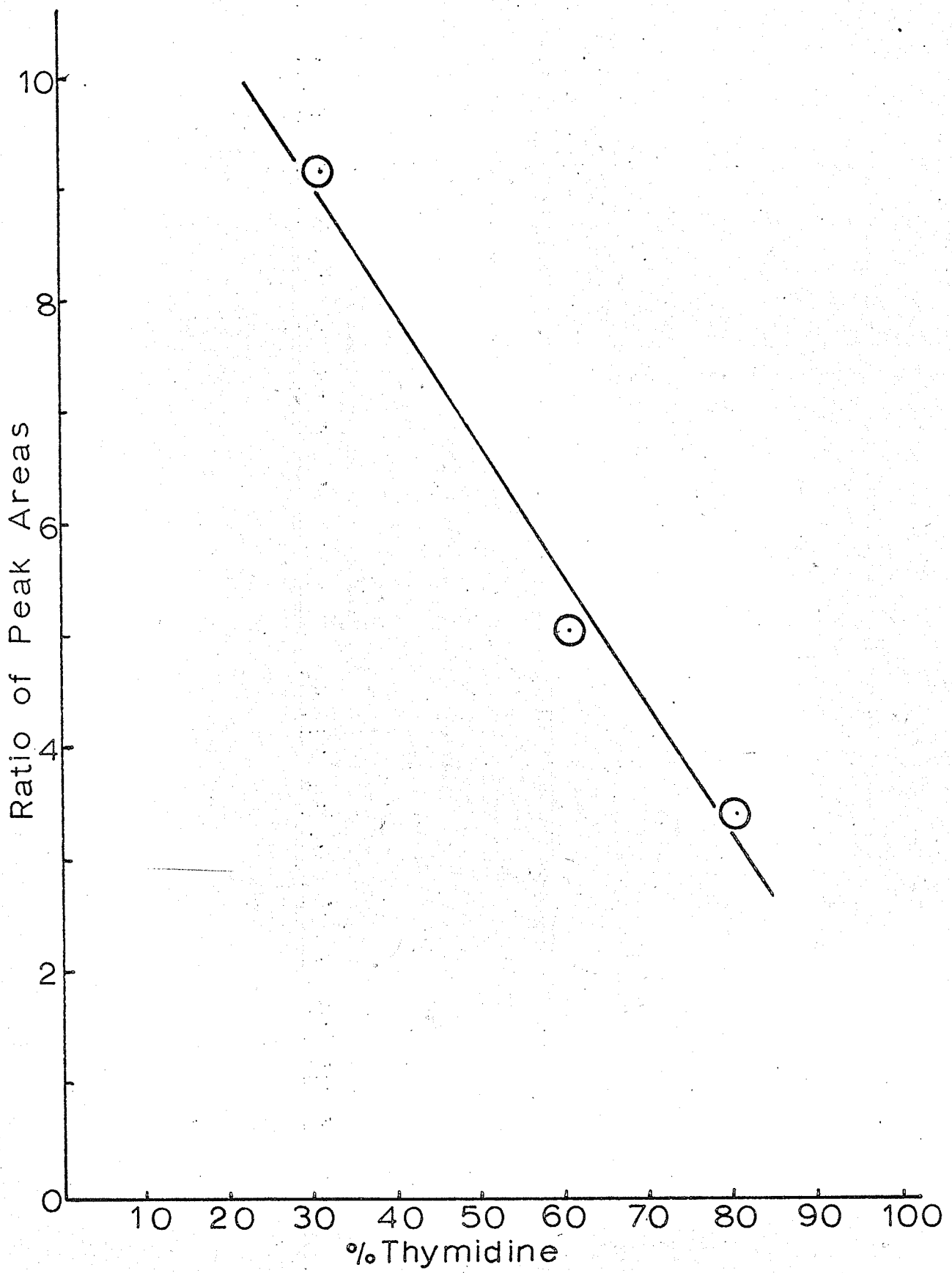
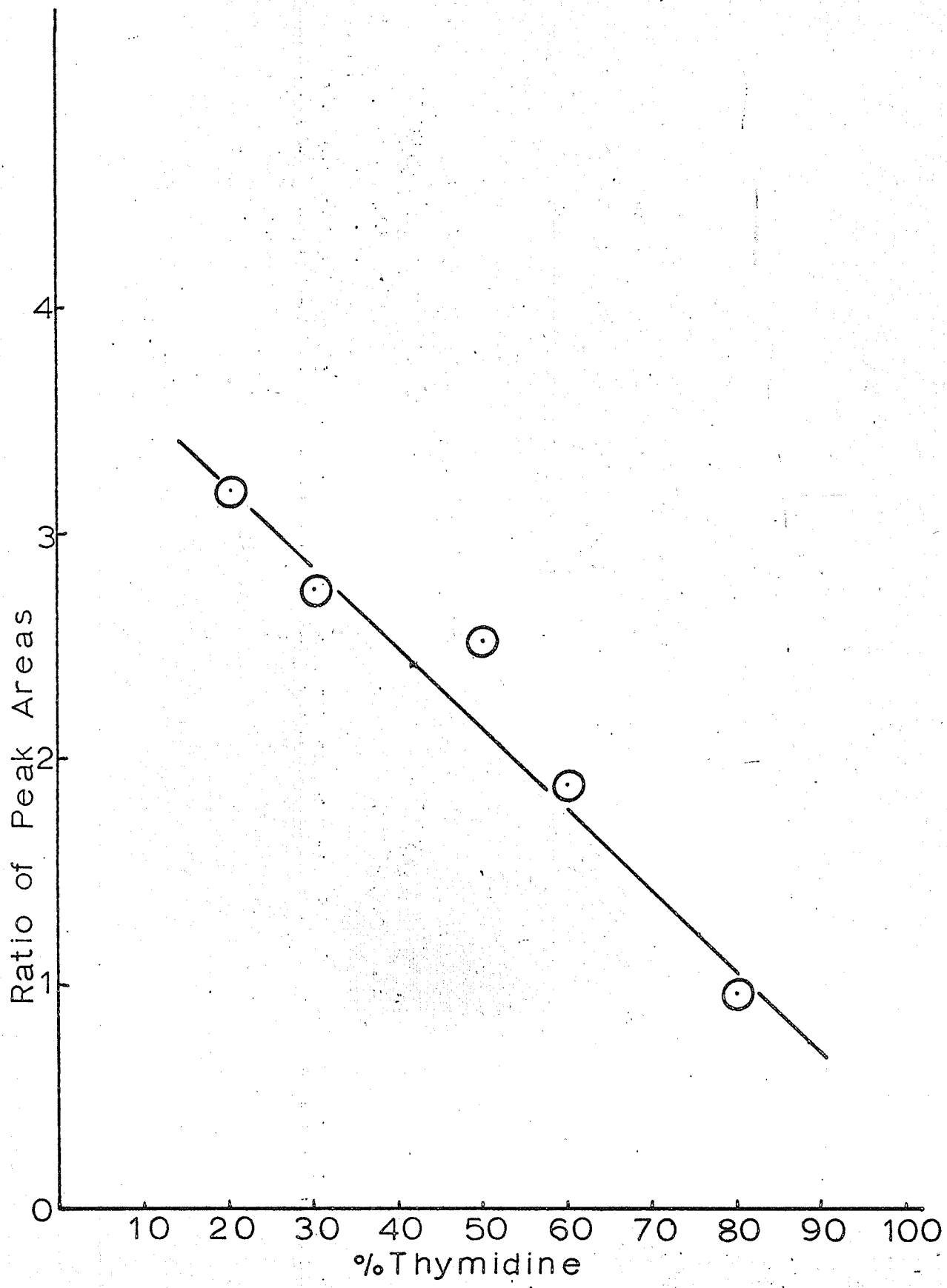

Ratio of Peak Areas
0
2
4
6
8
10
10 20 30 40 50 60 70 80 90 100
% Thymidine

FIGURE 51. Calibration Curve for Nucleoside Mixture. Plot of Ratio of Peak Areas[1] Versus Percent Thymidine for Thymidine-Cytidine Mixture.

[1] Ratio of Peak Areas: 4/3a, Figure 40 (see Table 10-B)


Ratio of Peak Areas
0
1
2
3
4
10
20
30
40
50
60
70
80
90
100
% Thymidine

If the fraction of the total weight of mixture comprised by the first compound is f_a and that of the second compound is f_b then the ratio of areas for the mixture can be expressed:

$$M = (f_a)(A) + (f_b)(B) \quad \text{---------- (1)}$$

Since $f_a + f_b = 1$

$$M = f_a(A) + (1-f_a)(B) \quad \text{---------- (2)}$$

or

$$f_a = \frac{M-B}{A-B} \quad \text{---------- (3)}$$

Where M, A and B represent the ratio of peak areas for the mixture and pure compounds respectively. Equation (3) is the one Levy used to calculate the composition of the porphyrin mixtures.

If equation (2) is rearranged:

$$M = f_a(A-B) + B \quad \text{---------- (4)}$$

A plot of the ratio of peak areas for mixtures versus the composition of the mixture should thus yield a straight line of slope (A-B) and intercept B. This plot could be used as a calibration curve to determine the composition of any mixture of the two compounds. It was hoped that the calibration curve prepared for the mixtures of nucleosides could also be used to estimate the relative concentration of pyrimidine in a DNA molecule. The absolute value of the peak areas could then be related to the total amount of pyrimidine present in the DNA molecule (a plot of total weight of pyrimidine versus peak area would be used).

The relative areas of the peaks on the DNA pyrograms, which correspond to similar peaks on the pyrograms of the mixtures, should thus

bear a relation to the pyrimidine content of the DNA molecule. As was already mentioned, the contribution of the purines to these peak areas should not (on the basis of the pyrolyses of the separate nucleosides) alter these ratios significantly. From the calibration curves it should be possible to calculate the percent thymine in the pyrimidine content of the DNA molecule. The percentages found in this manner (from the data in Tables 11-A, 11-B, 12-A and 12-B) for the two nucleic acids are given in Table 13 along with the reported values of these percentages (89). No estimate of the purine to pyrimidine ratio can be made from these pyrolysis results.

TABLE 11-A. Peak Areas for Calf Thymus DNA[1]

Trial	Peak #[2]	Area	Sample Size.
1	3	4.05	30 µg
	4	2.16	
2	3	3.00	30 µg
	4	3.60	
3	3	2.38	30 µg
	4	1.65	
4	3	6.42	30 µg
	4	5.20	
	5	1.01	
5	3	4.52	30 µg
	4	2.52	
	5	0.73	

[1]Pyrolysis products were chromatographed on column #2

[2]The numbers refer to the corresponding peaks in Figures 20 and 21.

TABLE 11-B. Ratios of Peak Areas for Calf Thymus DNA.

Ratio Taken[1]	Trial	Ratio
4/3	1	0.53
	2	1.20
	3	0.69
	4	0.81
	5	0.56
	Average (omitting Trial 2)	0.65
3/5	4	6.35
	5	6.20
	Average:	6.27

[1] Table 11-A

TABLE 12-A. Peak Areas for Aerobacter Arogenes DNA[1]

Trial	Peak #[2]	2 x Area (cm^2)	Sample Size
1	3a	1.85	50 µg
	3b	4.23	
	3	15.49	
	4	4.24	
2	3a	0.81	50 µg
	3b	3.91	
	3	11.50	
	4	3.42	
3	3a	1.57	60 µg
	3b	9.44	
	3	22.20	
	4	6.84	

[1] Pyrolysis products chromatographed on column #3.

[2] The numbers refer to the peaks in Figures 32 and 32-A.

TABLE 12-B. Ratios of Peak Areas for Aerobacter Arogenes DNA.

Ratio Taken	Trial	Ratio
3/3a	1	8.35
	2	14.20
	3	14.10
3/3b	1	3.66
	2	2.94
	3	2.36
	Average (excluding Trial #1)	2.65
3/4	1	3.65
	2	3.36
	3	3.26
3b/3a	1	2.28
	2	4.80
	3	6.00
	Average:	4.36
4/3a	1	2.29
	2	4.22
	3	4.35

TABLE 13. Percent Thymine in Pyrimidine Content of DNA.

DNA	Trial	Ratio Used[1]	Calibration Curve	Percent Experimental	Percent Actual[2]
Calf Thymus	Average of four trials	4/3=0.65	Figure 43[3]	10	55
			Figure 43[4]	30	"
Calf Thymus	Average of two trials	3/5=6.27	Figure 44[3]	25	55
Aerobacter Arogenes	Average of three trials	3b/3a = 4.36	Figure 48	32	45
	Average of two trials	3/3b =2.65	Figure 49	56	45
	1	3/3a= 8.35	Figure 50	34	45
	1	4/3a= 2.29	Figure 51	45	45

[1] Table 12-B.

[2] On the basis of data in "The Chemistry of Nucleic Acids" (89).

[3] Upper calibration curve.

[4] Lower calibration curve.

For the purposes of this research it was not necessary to identify the peaks resulting from the pyrolyses. Nevertheless, the retention behaviour of the products of purine and pyrimidine pyrolyses, reported by Jennings and Dimick, was observed for the Hallcomid-KOH and Hallcomid M-18/Carbowax 600 columns (Columns #2 and #3). The retention time studies (Tables 14-A to 15-B) suggest that not all the products found in these studies correspond to the compounds identified by Jennings and Dimick.

TABLE 14-A. Results of Relative Retention Time Studies[1] on Column #2.

Compound	R.R.T.$_a$[2]	R.R.T.$_b$[3]
Acetone	1	0.26
Acetonitrile	1.83	0.47
Benzene	3.89	1
Methanol	2.68	0.69

[1] Conditions of chromatograms similar to those for pyrolysis experiments (Column temperature: 32°C, nitrogen flow rate: 24 ml/min.)

[2] Relative retention time with respect to acetone (2.37 minutes.)

[3] Relative retention time with respect to benzene (9.20 minutes)

TABLE 14-B. Relative Retention Times of Peaks on Pyrograms.

Figures	Peak #	R.R.T.$_a$[1]	R.R.T.$_b$[2]	Suspected Composition
9 - 21	2	1	0.26	Acetone
9 - 21	3	2.00	0.48	Acetonitrile
13 - 21	4	2.60	0.64	Methanol
17,18	5	3.39	0.87	Propionitrile[3]

[1] Relative retention time with respect to acetone peak on pyrogram.

[2] Relative retention time with respect to benzene (9.20 minutes).

[3] On basis of relative retention time data reported by Jennings and Dimick (39).

TABLE 15-A. Results of Relative Retention Time Studies[1] on Column #3.

Compound	R.R.T.$_a$[2]	R.R.T.$_b$[3]
Acetone	1	0.33
Acetonitrile	1.77	0.58
Benzene	3.06	1
Hexane	1.05	0.35
Isopropyl alcohol	3.08	1.01
Methanol	1.75	0.57
Propionitrile	2.89	0.95

[1] Conditions of chromatograms similar to those for pyrolysis experiments (Temperature: 53°C; Nitrogen flow rate:30.8 ml/min.)

[2] Relative retention time with respect to acetone (2.40 minutes).

[3] Relative retention time with respect to benzene (7.25 minutes).

TABLE 15-B. Relative Retention Times of Peaks on Pyrograms

Figures	Peak #	R.R.T.$^{1}_{a}$	R.R.T.$^{2}_{b}$	Suspected Composition
22,23,25,26,28, 29,30,31,32, 32-A	2	1	0.37	Acetone
22-32-A	3b	2.00	0.73	-
24-32-A	3	2.23	0.81	-
23-32-A	4	3.15	1.15	-
26,27,28	5	4.70	1.71	-

[1] Relative retention time with respect to acetone peak on pyrogram.

[2] Relative retention time with respect to benzene (7.25 minutes).

The effect of freezing the head of the chromatographic column is seen in the chromatograms of Figures 16-B, 22-A, 29-A and 30-A. As would be expected of any programmed temperature technique, the peaks of the "frozen" pyrolyses are sharper and better resolved than those on the isothermal chromatogram.

RESULTS OF SWEAT ANALYSIS

A preliminary chromatogram of body odour is shown in Figure 52. Chromatograms of the vapours above the gauze sponges collected from the first group of patients are typified by Figure 53. Another chromatogram obtained by injecting several cm^3 of distilled water into a bottle contain-

FIGURE 52. Chromatograms of Under-Arm Vapours.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 2 ml of vapour.
Column: # SE-30, 10% on 60/80 Neutraport S.
Conditions: Preheater Temperature: 100°C.
Column Temperature: 150°C to approx. 200°C.
Nitrogen Flow: 24 ml/min.
Hydrogen Flow: 25 ml/min.
Air Flow: 300 ml/min.

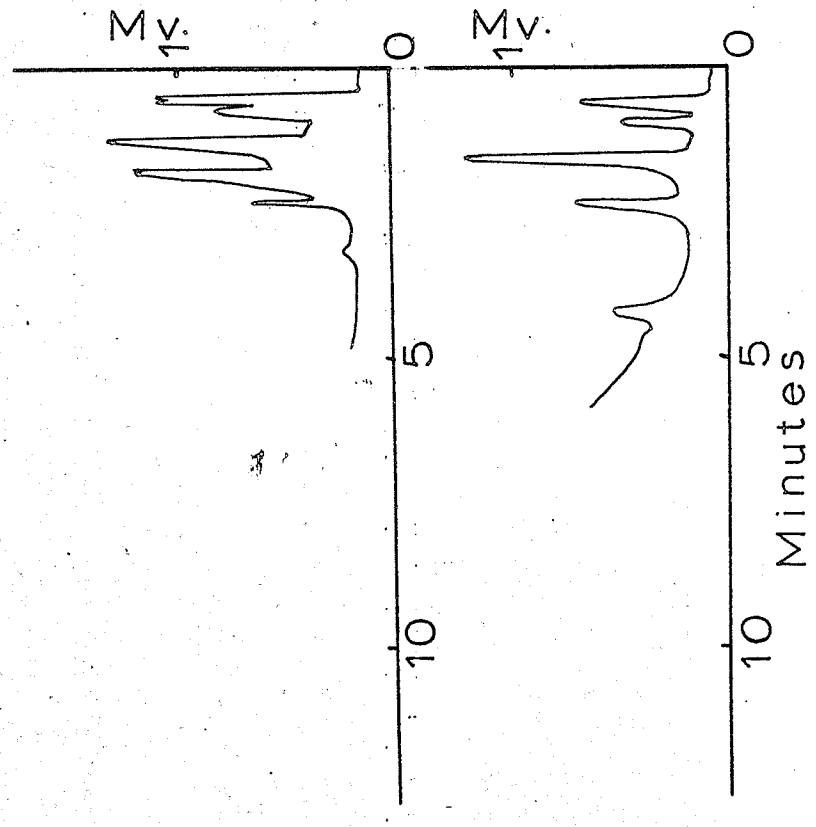
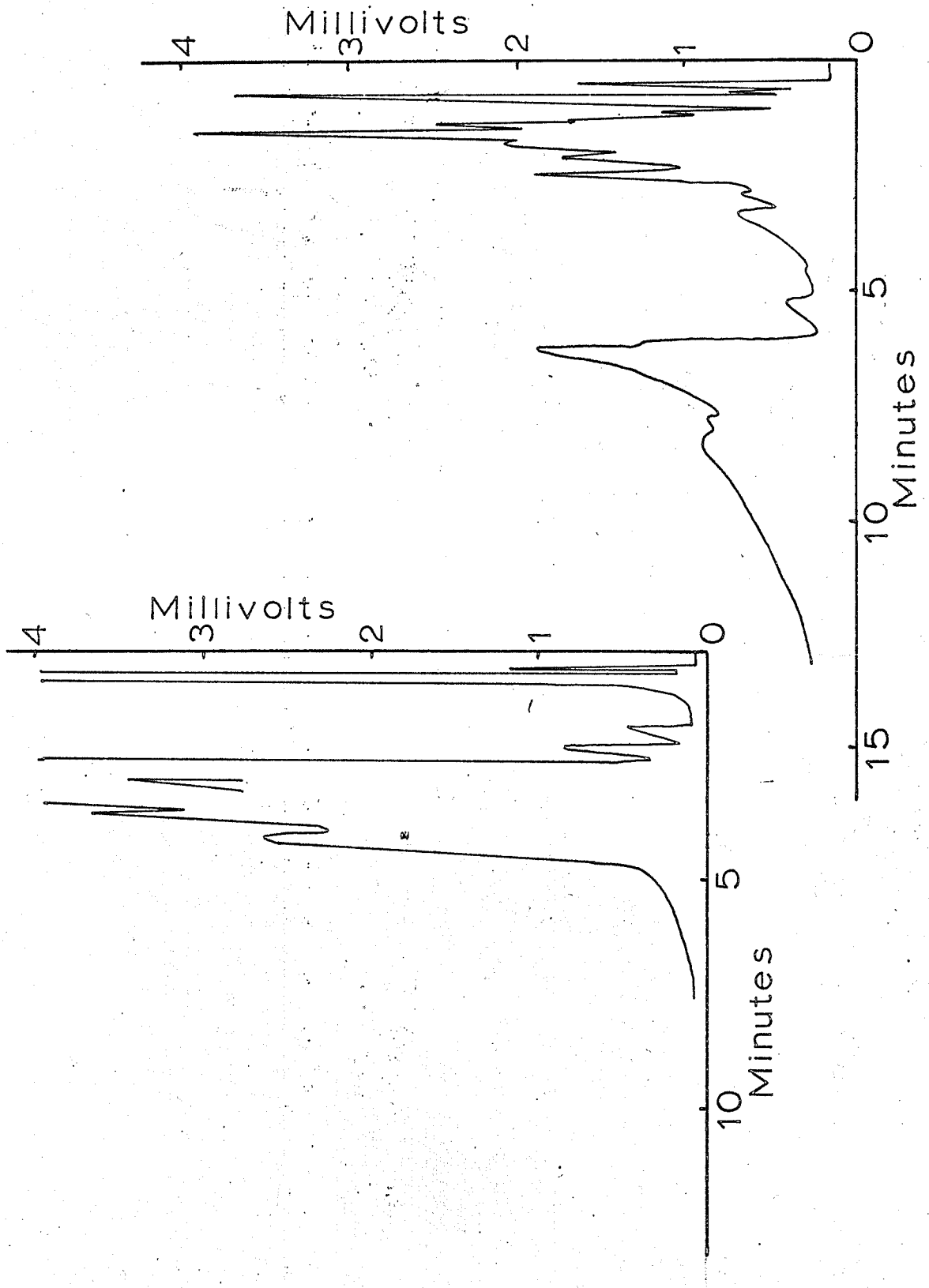

Mv.
0
Mv.
0
5
5
10
10
Minutes

FIGURE 53. Typical Chromatogram of Sweat Vapours Collected on Pads Extracted With Ether and Acetone. A Similar Chromatogram of the Vapours Displaced by Distilled Water from the Treated Pads is Shown Above the Chromatogram of Sweat Vapours (left insert).

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2
Sample Size: 1 ml. vapours.
Column: SE-30, 10% on 60/80 Neutraport S.
Conditions: Preheater Temperature: 119°C.
Column Temperature: 110°C.
Nitrogen Flow: 25 ml/min.
Hydrogen Flow: 23 ml/min.
Oxygen Flow: 300 ml/min.


Millivolts
4
3
2
1
0
5
10
15
Minutes
Millivolts
4
3
2
1
0
5
10
Minutes

ing an unused sponge and analysing the vapour above the sponge is also shown. Although the dry sponges gave no response when checked with the chromatograph, the water from the sweat appeared to displace some impurities from the pad. Either the extraction technique used in the first instance did not work, or the ether-acetone mixture used for this purpose introduced these impurities to the sponges.

The vapours of the sweat collected on the second set of sponges gave no response on the chromatograph at all (Figure 54).[1] When the sweat was centrifuged from the sponges and injected directly onto the SE-30 column, no compounds could be detected even when the maximum sensitivity (impedance 10^9 ohms) was used. The method used to collect the sweat was thus quite effective in eliminatingmany contaminants from the sweat.

The ability of the chromatographic system to handle aqueous samples containing small amounts of acidic compounds is demonstrated by the chromatograms in Figures 55 and 56.

Depending on a subject's sweating rate, the method of sweat collection yielded from three to four cm^3 of sweat per sponge in a thirty minute interval. The fractionation of the sweat with the vacuum apparatus constructed was quite easily performed. No chromatographic results were obtained for any of the fractions collected.

Pyrolysis of acidic and basic residues showed no distinguishing peaks when normal and schizophrenic sweat was examined (Figure 57).

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[1] The two peaks observed can be attributed to contaminants rather than to the sweat vapours.

FIGURE 54. Typical Chromatogram of Sweat Vapours Collected on Pads Extracted with Water.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x1.
Sample Size: 300 µl vapour.
Column: SE-30, 10% on 60/80 Neutraport S.
Conditions: Preheater Temperature: 110°C.
Column Temperature: 100°C.
Nitrogen Flow: 24 ml/min.
Hydrogen Flow: 23 ml/min.
Oxygen Flow: 300 ml/min.

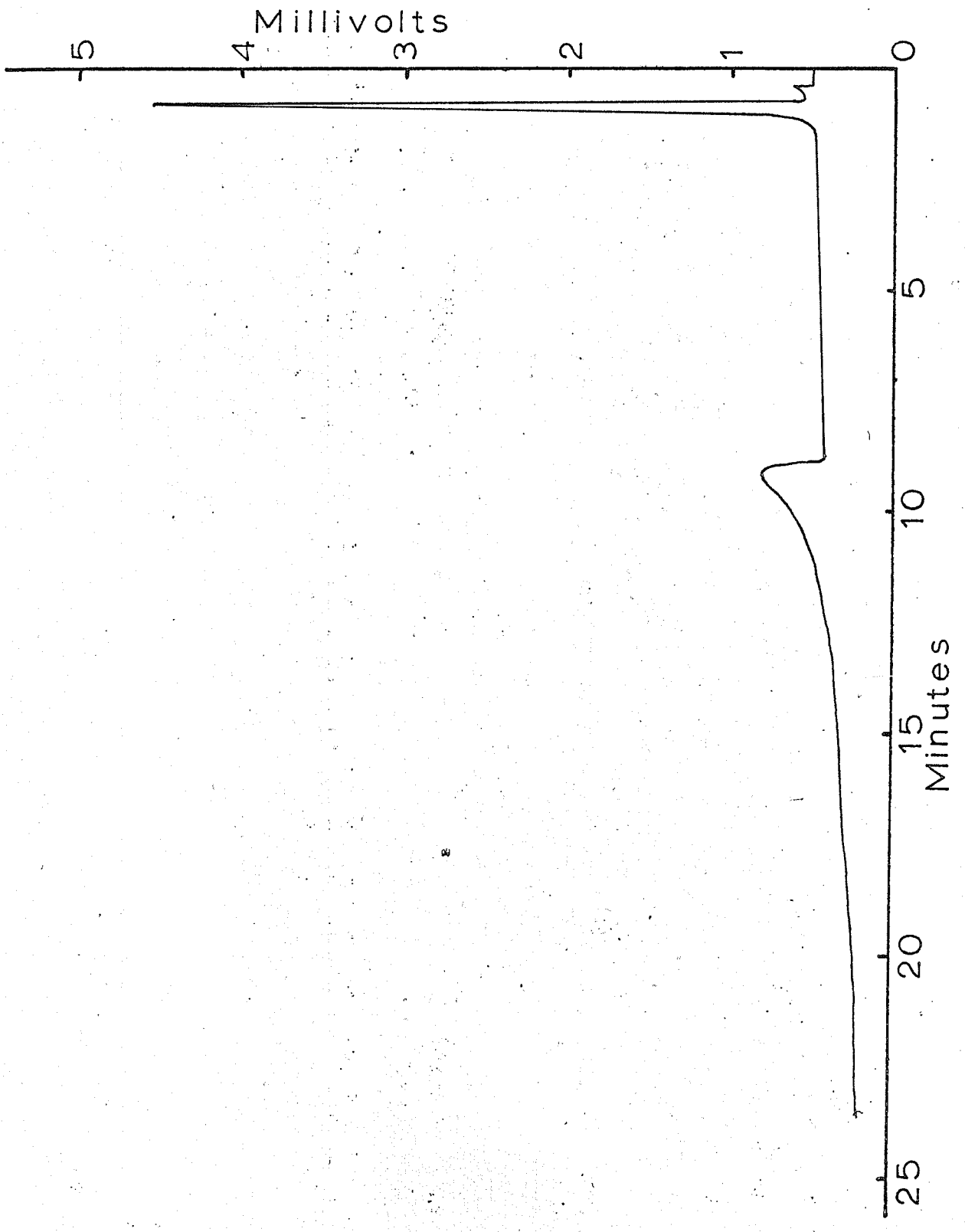

Millivolts
5
4
3
2
1
0
5
10
15
20
25
Minutes

FIGURE 55. Chromatogram of Vapours Above a Solution of Fatty Acids Containing Isobutyric, Isovaleric, Isocaproic and Heptanoic Acids[1]

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x2
Sample Size: 100 µl of vapour.
Column: PEGS 1%-H_3PO_4 0.4% on 60/70 Teflon 6.
Conditions: Preheater Temperature: 120°C.
Column Temperature: 104°C.
Nitrogen Flow: 23 ml/min. at 12. P.S.I.G.
Hydrogen Flow: 24 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.

[1]Peaks #1,2,3 and 4 respectively.

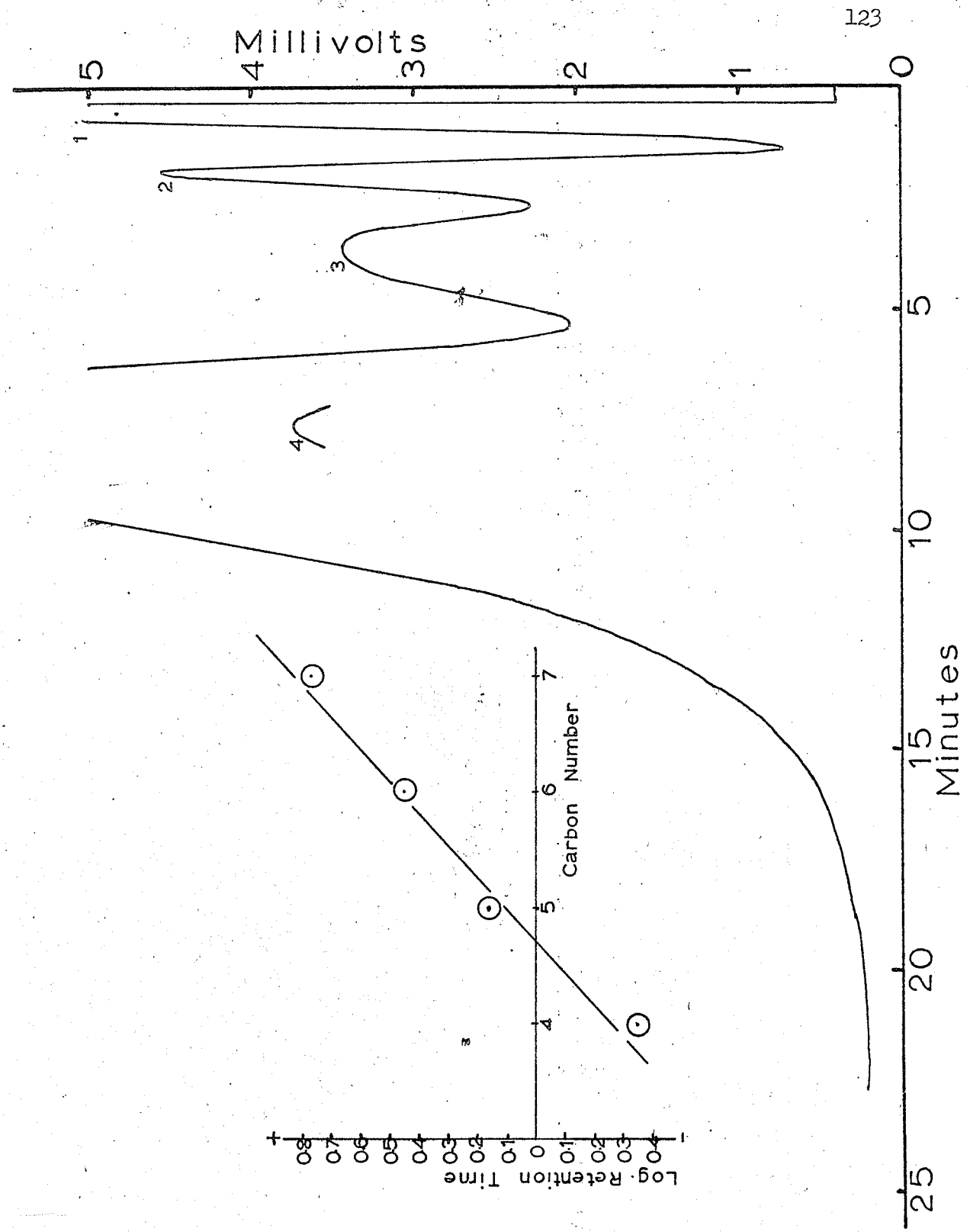

Millivolts
5
4
3
2
1
0
1
2
3
4
5
10
15
20
25
Minutes
Carbon Number
4
5
6
7
Log. Retention Time
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4

FIGURE 56. Chromatogram of a Dilute Aqueous Solution of Acetic[1], Pyruvic[2], and Lactic[3] Acids.

Detector Sensitivity: Impedance 10^9;
Output 10x,
Attenuator x4.
Sample Size: 50 nanolitres of dilute (approx.1%) solution.
Column: #PEGS 1% -H_3PO_4 0.4% on 60/70 Teflon 6.
Conditions: Preheater Temperature:170°C.
Column Temperature:177°C.
Nitrogen Flow: 23 ml/min.at 12 P.S.I.G.
Hydrogen Flow: 24 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.

[1]Peak #1.

[2]Peak #2

[3]Double Peak: #3 and #4.

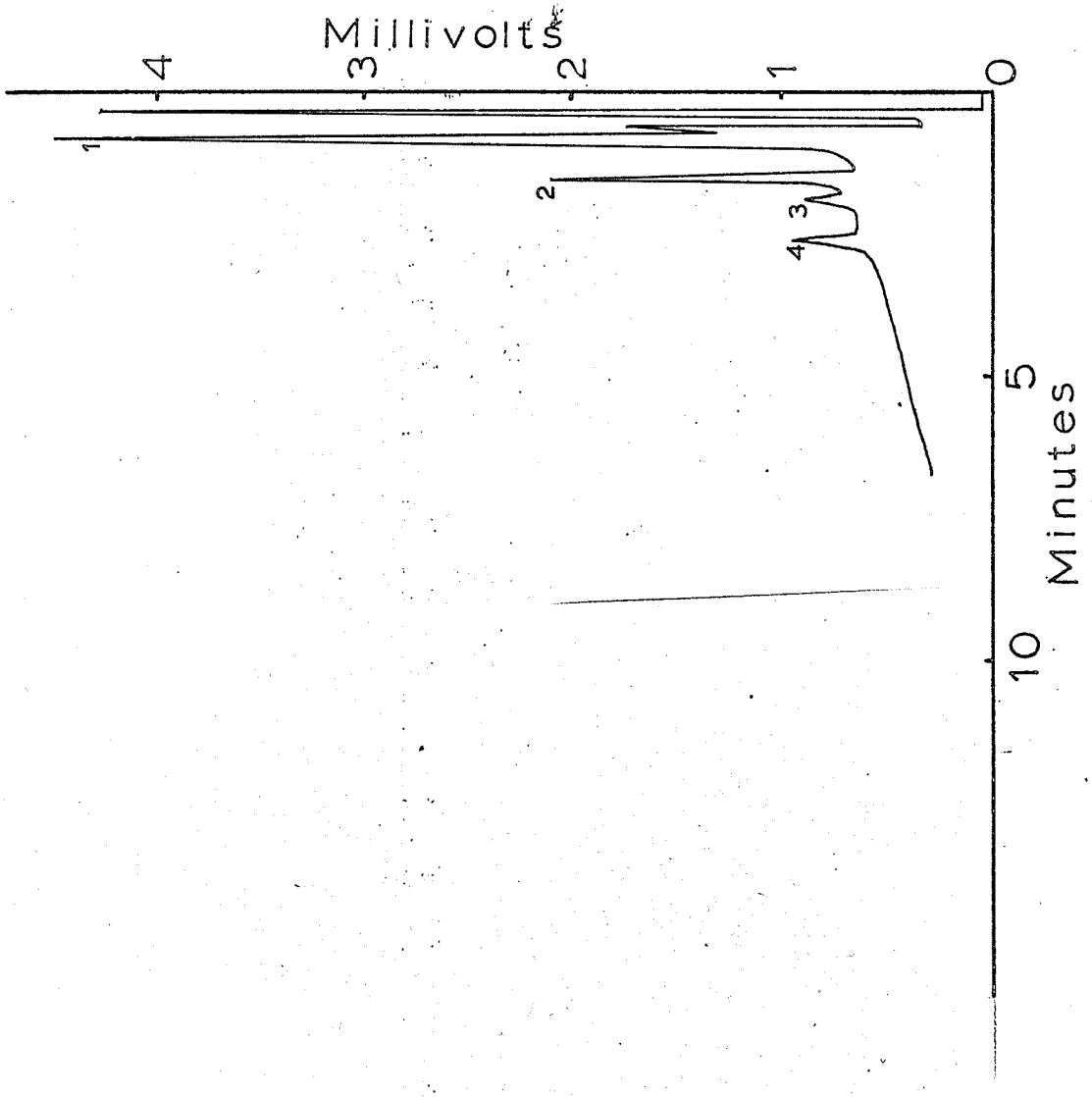
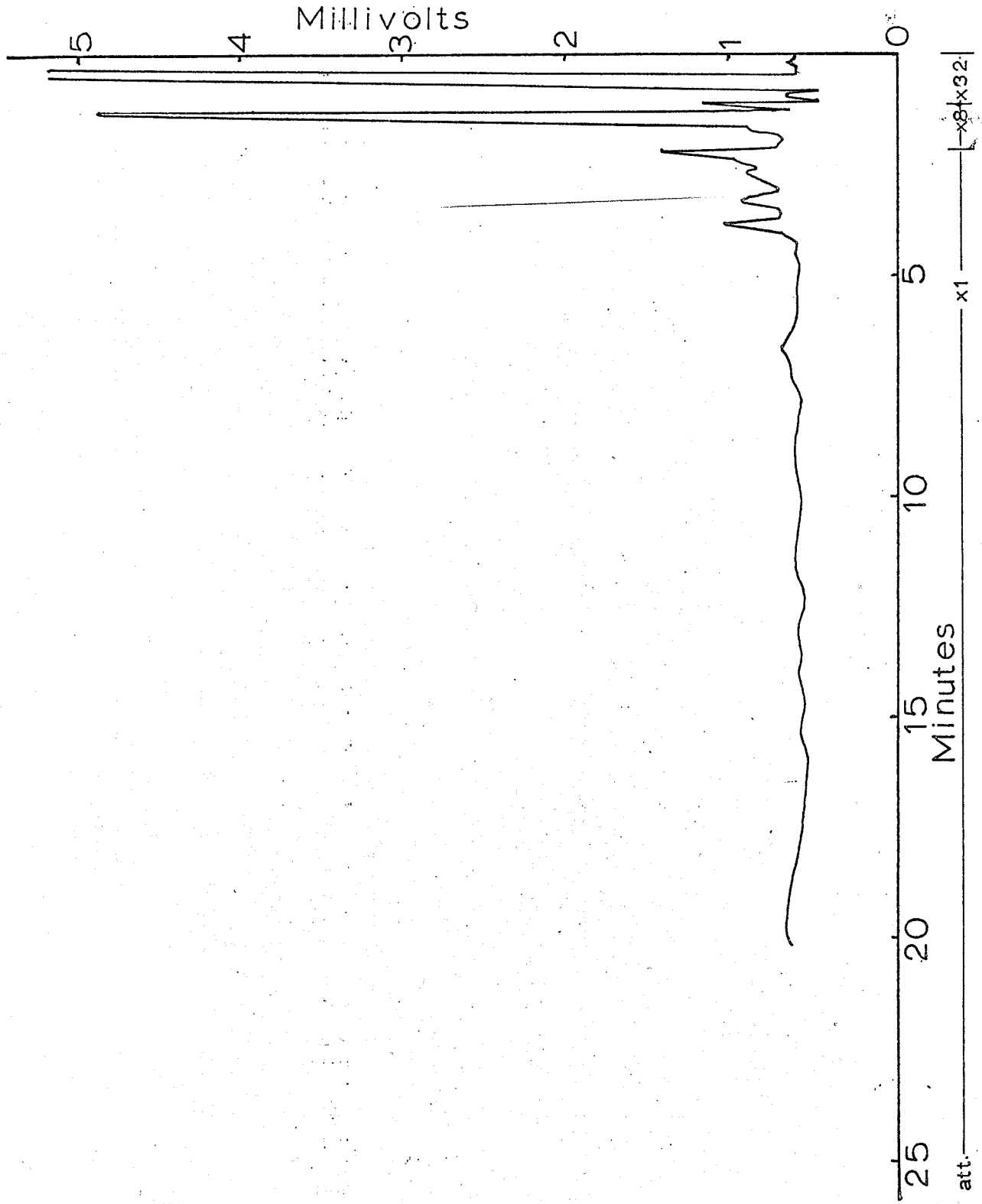

Millivolts
4
3
2
1
0
1
2
3
4
5
10
Minutes

FIGURE 57. Typical Pyrogram of Basic Residue from Sweat Fractionation.

Detector Sensitivity: Impedance 10^9;
Output 10x;
Attenuator x32,x8,x1 (as shown)
Sample Size: unknown.
Column: Carbowax 1540; 2% on Neutraport-S (#1, Table 6)
Conditions: Preheater Temperature:100°C.
Column Temperature: 57°C.
Nitrogen Flow: 21 ml/min. at 20 P.S.I.G.
Hydrogen Flow: 27 ml/min. at 10 P.S.I.G.
Air Flow: 300 ml/min.
Pyrolysis Setting: #80.
Pyrolysis Time: 7 seconds.


Millivolts
5
4
3
2
1
0
5
10
15
20
25
Minutes
att.
x1
x8
x32

DISCUSSION

DISCUSSION

These studies point out once again the value of the pyrolysis gas chromatographic technique in identifying compounds, in differentiating between closely related molecular species and in yielding a quantitative measure of these compounds. If the nucleosides from the hydrolysis of some nucleic acid were separated, for example by thin layer chromatography, the pyrolysis technique could be easily used both to confirm the identity of any species and to give a quantitative estimate of the amount of substance in the hydrolysate. The results of the pyrolysis of mixtures of the nucleosides show that the technique could also serve as a check on the purity of any one fraction separated from a hydrolysate. However, the experiments with mixtures of nucleosides did not yield a method for determining the composition of DNA. If, as hoped, it were possible to find at least one distinguishing peak for each component in the mixture, the quantitative estimate of each compound could be easily made. Unfortunately, no such distinguishing peaks could be found for the purine and pyrimidine nucleosides pyrolysed. In these cases, where the peaks overlapped one another, or where the peaks resulted from the same decomposition products, the quantitative correlation between the peak areas and the composition of the mixture (Figures 41 to 51) could not be used to estimate the DNA composition with any degree of accuracy.

The quantitative calibrations demonstrated that care must be taken in interpreting the results obtained by this method. Although the relationship between the peak area and sample size was linear, the shift of the curves between calibrations (Figure 35) suggests that

systematic errors could be introduced in to any quantitative results. The shift of these curves is attributed to an ageing of the pyrolysis coil which results in a lower pyrolysis temperature for the same powerstat setting. If some alteration of the apparatus could ensure that the coil temperature for all the pyrolyses would be identical, it is expected that this shift would not occur. It is felt that the recommended use of phosphoric acid to aid in the pyrolysis might have caused the unexpected peaks found in the pyrolyses. Although some residue did collect in the pyrolysis chamber over a period of time, it is felt that this residue (which was removed between experiments) could not have given rise to all of the extra peaks. Furthermore, when the same column that was used for the pyrolysis studies was used to examine aqueous solutions, similar peaks also appeared (presumably due to a desorption process). It is suggested that the decomposition products of the phosphoric acid (which yielded no peaks when the one percent solution was pyrolysed as a blank) might collect in the head of the column, acting as a trap for some pyrolysis products. Compounds from a subsequent pyrolysis would desorb these materials, allowing them to travel down the column to appear in the next chromatogram.

The attempt to correlate the pyrimidine content of the nucleic acids with the peak areas on the pyrogram did not meet with much success (Table 13). The results obtained by the "method of mixtures" did not agree with the values reported for the nucleic acids analysed. No estimate of the purine to pyrimidine content could be made. Nevertheless, the technique can definitely be used to characterise the nucleic acids

and to measure them quantitatively in a solution. Several peaks (2a and 4a) in the pyrograms of the aerobacter arogenes DNA (Figures 32, 32-A) which could not be attributed to the decomposition products of the nucleosides studied may result from the 5-methyl cytosine present in this nucleic acid.

The failure in the attempt to estimate the composition of the DNA could easily be due to a "shift" in the calibration curve between the time the mixtures of nucleosides were pyrolysed and the time the DNA's were pyrolysed. In addition, it is possible that the purine deoxyribosides[1] in the DNA may have contributed to the peak areas used for calibration. If this were the case (even though on the basis of the studies of the purine ribosides, this type of contribution was expected to be minimal), the calibration curves obtained from the thymine-cytosine or thymidine-cytidine mixtures would be of no use for estimating the composition of the DNA. Mixtures of deoxyribosides would then be required for the calibration.

The results obtained from the pyrolysis of the quaternary salt indicate that the pyrolysis gas chromatographic technique might prove useful for the analysis of quaternary-type compounds (such as acetyl choline) to be found in biological fluids.

For the purposes of identification at least, the practice of freezing the head of the column during the pyrolysis seems advantageous. The pyrolysis of the compounds on the coil does take a finite amount of time. It is suggested that, when the freezing technique is used, the pyrolysis products which issue in a stream from the coil (this

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[1]These compounds were not available for the pyrolysis studies.

effect was seen through the pyrex columns) are frozen in a plug at the head of the column. When this portion warms up, the entire plug is introduced to the column at once (Figures 16-B, 22-A, 29-A and 30-A). As a result, the resolution of the peaks is improved. This improved resolution may also be partly the result of the programmed temperature effects which operate simultaneously.

The Pye chromatograph did not prove to be very useful for pyrolysis gas chromatographic studies. Both the instrument's low sensitivity, and the impaired response of the detector caused by common pyrolysis products such as CO_2 and H_2O detracted from any advantages this detection system may have offered.

The check on the identity of the pyrolysis products of the pyrimidines as anticipated from the work by Jennings and Dimick (39) raises doubt as to the composition of all the peaks except the acetone peak. Although the relative retention times for the pyrolysis peaks on the Hallcomid column (Column #2) corresponded closely to those for the compounds reported (39) by these workers, the retention data on the Hallcomid/Carbowax 600 column (Column #3) do not confirm the identities (see Tables 14A to 15B).

The identification of all the peaks is not an easy task. Unfortunately, it was necessary to take the pyrolysis apparatus apart before the retention time studies could be performed for the column used. It was thus difficult to reproduce column conditions identical to those which prevailed at the time the pyrolysis was performed. The arrangement used for the studies on the Pye chromatograph is more suitable for this identification procedure. Immediately after a pyrolysis, a mixture

of the suspected products can be injected directly into the column and the chromatogram of the synthetic mixture can be compared with the pyrogram.

The sweat study did not meet with much success. The method of sweat collection yielded uncontaminated sweat samples in quantities that were thought to be adequate for the purpose of gas chromatographic analysis. The failure to obtain peaks from the sweat is thought to be due to several factors. In the first instance, the concentration of compounds appearing in sweat is very low. Secondly, it is felt that many of the organic compounds collected in sweat must first build up on the skin surface before dissolving in the sweat as it spreads out over the skin. If this were the case, the thorough washing procedure followed would remove these constituents and thus lower their concentration in whatever sweat was collected. Furthermore, work by Lester (90A) on blood serum and aqueous solutions has shown that the analysis of very small amounts of acetic, lactic, and pyruvic acids is hampered by column adsorption effects. For aqueous samples containing less than 1 mg/liter of acid, Lester emphasises the necessity of using a formic acid-saturated carrier gas. This procedure was not used for the sweat analyses. The concentration of a larger volume of sweat would thus be required to yield a detectable amount of material. There is no reason why a treatment of this larger sample could not yield analyses for such components as lactic and pyruvic acids, or for steroids. The method of sweat collection developed is much better suited to the controlled study of sweat than that employed by Smith and co-workers.

If the odour reported by Smith is (as Posner et al maintain) the

result of the action of an organism on the skin surface, the method of collection used in this study would not isolate the responsible compound. The compound would be washed away even before the sweat collection began. As the chromatograph had no trouble in sensing body odours, perhaps the best approach would have been to collect under-arm vapour samples from the subjects by using a vacuum line and a liquid nitrogen trap. The abnormal degradation product would then appear among the other degradation products of the skin surface fat.[1] Smith's observation that the patients she chooses to sample all seem to have a "waxy complexion" suggests that the odour may be traced to a difference in skin composition of the schizophrenic patients and not to a difference in the composition of the sweat.

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[1]Skin surface fat consists of hydrocarbons, sterol esters, waxes, glycerides, and free fatty acids, (90).

SUMMARY

SUMMARY

A gas chromatographic study of the pyrolysis of the components of nucleic acids (purines, pyrimidines and their nucleosides) showed that, on the basis of the chromatograms obtained, all of these compounds yield similar degredation products. The pyrograms could be used for a qualitative and quantitative analysis of any single compound, but could not be used to give information about the base composition of the DNA's pyrolysed.

In an attempt to isolate the compound or compounds responsible for an odour associated with schizophrenic patients, sweat was collected from both normal and schizophrenic individuals and injected onto several gas chromatographic columns. No detectable levels of organic compounds were found in the eccrine sweat collected by the methods used.

APPENDIX

APPENDIX

DISCUSSION OF VARIOUS ASPECTS OF GAS CHROMATOGRAPHY

THE COMPONENTS OF GAS-LIQUID CHROMATOGRAPHY

The mechanics of conventional or packed column gas-liquid chromatography were outlined schematically in Figure 1. The column consists of a tube (various geometric forms are used) which is filled with a non-reactive, coated, sandy material. The solid supposedly acts only as a support for the stationary liquid phase which covers it and which exhibits a low vapour pressure at the temperature of the surrounding thermostated compartment. Provision is made for a sample and carrier gas inlet at one end of the column and for a differential (or integral) detector at the other. The small sample of volatile mixture to be separated is introduced into the front end of the column by some injection device. The column is maintained at a certain temperature and a constant current of gas is passed through it. This gas is the eluent; it transports the components of the mixture in the form of a vapour through the column where they are retained by the stationary liquid to different extents in the course of the chromatographic process. The individual "bands" which emerge, separated by zones of carrier gas, are sensed by a delicate detecting device capable of indicating the presence of components in a qualitative and quantitative manner.

The efforts in the development of this technique were aimed primarily at the perfection of adequate and sensitive detecting devices and at the discovery of the most favourable operating conditions, stationary liquids, and solid supports. The performance of the equipment depends

on how these various factors influence the chromatographic process. For example, among other things, the separating power of the column depends on the nature and amount of stationary liquid (91,92), the particle size and nature of the support (93), the uniformity of packing, the length and diameter of the column, the temperature, the composition, velocity and pressure distribution of the carrier gas, the properties of the components in the solution to be separated, and on the size of sample introduced to the column. For the purposes of any work using gas chromatography, a brief consideration of each of these parameters is in order.

THE INJECTION SYSTEM

The first consideration might well begin with the introduction of the sample. The necessity of using small samples for this operation (94) poses problems of accuracy and reproducibility. For liquid samples, most needs are met by the commercially available micro-syringes which are used to inject the sample through a silicone rubber plug at the head of the column. Another procedure (95) employs a sealed ampoule which is crushed in the path of the carrier gas. For gaseous samples,more accurate work is done by using the carrier gas to displace a known volume of sample from a calibrated chamber. However, the ease and utility of the syringe technique has led to the development and use of teflon-tipped, gas-tight syringes. As with all the techniques used in gas chromatography, the injection procedure is subject to many variations. Methods other than the ones already mentioned are generally described in the literature along with the particular problem they were designed to meet.

To increase the efficiency of any injection system, it has been demonstrated that one need only pre-heat the injection port. Pollard and Hardy (96) showed that the temperature of the injection port profoundly influenced the peak shape (and hence the column efficiency) and that best results could be expected when the port temperature was maintained at, or above, the boiling point of the least volatile component of any mixture. This technique is of great assistance in pyrolysis studies as well as in the study of a fluid such as sweat, where in both cases high-molecular weight substances of low volatility may be anticipated.

One particular method of sample introduction, developed by the Scientific Kit Company, is most useful for reproducibly injecting small samples for retention time studies. The sample is sucked into a calibrated metal capillary which has been previously evacuated. When the capillary is introduced into the preheated injection port, the sample vaporises onto the column. The use of these "Nano-Jector" syringes was described in the experimental part of this thesis.

CHROMATOGRAPHIC COLUMN MATERIALS

Given a tube (copper, glass or otherwise), a bucket of sand, and a can of grease, any technician can put together a working column for vapour phase chromatography. However, a careful consideration of the more subtle characteristics of the materials used, and of the technology of putting these materials together, must be made before any serious work can be attempted.

THE STATIONARY PHASE

The partitioning solvent used is perhaps the most crucial factor in determining the success or failure of any particular separation. Although it was first believed that the wide range of stationary phases available to the analyst would render no separation improbable, it is found that the rather stringent specifications that the stationary phase must meet do limit the choice. The substance to be used must not react irreversibly with the solutes, it must be stable and non-volatile under the prevailing column conditions, and it must possess some solvent power for the substances to be separated by the column. In addition, the stationary phase should be reasonably fluid over the range of operating temperatures.

The choice of liquid substrate is usually made in a relatively simple manner. Firstly, the characteristics of the components of any mixture are roughly matched with those of the available stationary phases. The use of a "like dissolves like" criterion will usually indicate a satisfactory liquid phase. However, a knowledge of the basis of the simple criterion is necessary for dealing successfully with any perplexing separation that may arise.

Three types of cohesion, or interaction forces, combine collectively to determine the relative volatility of the solutes. The Keesom (97), or orientation forces, result from the interaction between the permanent dipoles of the solvent and solute molecules. One important type of this orientation force is the hydrogen bond which often plays a large role in chromatographic separations. The potential of interaction falls

off as the sixth power of the distance between the dipoles and is inversely proportional to the absolute temperature. One thus finds that orientation forces decrease with increasing temperature, and that the interactions are more pronounced for solvents with large dipoles concentrated in small molecules. A second type of force, the Debye or Induction force, results from an interaction between a permanent dipole in either the solute or solvent and an induced dipole in the other. This force is relatively small in comparison with the orientation forces, but examples of separations depending on this type of interaction (e.g. the separation of benzene from cyclohexane) can be cited. Debye forces are independent of the temperature. The last type of interaction found in solute-solvent systems is the London or Dispersion force. This force arises from the action of the electric field generated by the oscillating dipoles generated between nuclei and electrons. Dipoles, in phase with the instantaneous dipoles producing them, are induced by some species in other polarisable molecules. These temperature-independent dispersion forces are the only source of attraction between two non-polar substances. The knowledge of the nature of these forces which determine the solvent-solute interaction provides a more sophisticated basis for the choice of a stationary phase. In this light, the simple criterion of "like dissolves like" can be well understood and used to advantage.

Martire (98) has developed an expression relating the activity coefficient (and hence the retention volume) of a solute in a dilute solution to three parameters characteristic of these solution forces.

When sufficient physical data are available for both the stationary phase and the solutes to be separated, the relative solute volatilities can be predicted. Furthermore, when all the column operating conditions are known, the actual retention times can be calculated. Martire's calculations have been successful in several cases, but the need for more experimental verification, and for an investigation of the limits and degree of accuracy of the approach, is only too apparent.

Theory can usually indicate the best variety of liquid phases to consider. From that point on, the analyst must rely on a wealth of empirical data which has been well catalogued (99). With a sixth sense and a search of the literature, an analyst invariably can find a liquid phase that will be adequate.

In this regard, the suppliers of stationary phases are most helpful with their frequent compilations of the applications and useful temperature ranges of the available liquid phases. However, these distributors may be just a bit too helpful in their provision of literally hundreds of available partitioning liquids. Without a doubt, one of the six stationary phases recommended in 1956 by the London Symposium Committee (100) should first be considered for use (Table 16).

The instinct for selecting a stationary phase may be described as a subconscious feeling for the many aspects to consider. The selectivity of the solvent, the amount of solvent, the solvent polarity and the working temperature range of the stationary phase must all be considered. In addition techniques such as the use of multiple, or mixed bed columns (101, 102), or the use of chemically active columns (103) can be called upon

for difficult analyses. The empirical results, together with the theoretical aspects of solution theory, both play a part in solving any difficult analysis problem.

TABLE 16. Liquid Phases Recommended by The London Conference (1956).

Stationary Phase.	Applications	Recommended Working Temperature Range
Benzyldiphenyl	aromatic compounds and halogen compounds in general	60 - 120°C
Diglycerol	phenols, alcohols aliphatic amines	20 - 150°C
Dimethylformamide	low boiling paraffins and olefins	-20 - 20°C
Dinonylphthalate	general purpose, especially suitable for esters and ketones	20 - 130°C
n-Hexadecane	lower hydrocarbons	20 - 75°C
Squalane	hydrocarbons, especially branched chain and cyclic alkanes.	20 - 160°C

THE SOLID SUPPORT

The support must receive almost as much attention as the liquid phase used. Much effort has gone into the search for an ideal support to use for packed column gas-liquid chromatography. Aside from the diatomites and household detergent, investigators have tried some dozen or so materials (104) in the hope of finding a rugged support having a large, uniform, chemically inert surface area. The support must also be able

to absorb large quantities of viscous liquids and yet still run freely and pack into a column to offer the maximum permeability to gas flow.

Of the many supports available, few meet all the requirements of an ideal support. The diatomataceous earth supports, such as Celite, Decalite, Fosalsil, Chromosorb, and the firebricks, Sil-o-Cel and Sterchamol, are used in most cases for packed columns. Materials such as the teflons, glass beads (105), or porous glass (106,107), may be employed where special support characteristics are desired.

The main point of interest in these supports (aside from the packing characteristics) lies in their adsorptive properties. The specific retention volume should be independent of the solvent to support ratio. Studies have shown however (108) that this is not the case. It seems that even with solvent concentrations as high as 10 percent by weight, the support is still involved in the overall process (in addition to any surface energy effects that may be present). Of particular concern here is the use of lightly loaded supports (109, 110,111) which can be employed to analysecompounds at temperatures as much as 250 degrees below their boiling points. The adsorptive effect must be accounted for in cases such as these. This effect may be manifested not only in the tailing of chromatographic peaks, but also in the chemical alteration of the sample as it passes through the column (112).

The adsorption of samples on diatomite materials is attributed to the van der Waals sites and Hydrogen-bonding sites inherent in the support.

To deal with the effects of these sites, several approaches have been tried. Firstly, workers have used trace quantities of surface active compounds (for example, corrosion inhibitors (113))having hydroxyl, primary amine, or some other polar groups which will deactivate the adsorption sites. In addition this thin coating, approaching monomolecular film thickness, acts as a glue to span the interface between the liquid phase and the surface of the support. Another variation of this "tail reducer" technique involves the coating of the support with several percent of a non-volatile acid (114) or base (115). Alternatively, the sites can be treated chemically to alter the adsorption characteristics. The earliest support treatment involved the acid and/or base washing of the support. It is not quite clear what is accomplished by this operation, except that the treatment alters the appearance of the support (116), removes small amounts of metal impurities, and apparently improves the column efficiency. On the assumption that the adsorptive character of the silaceous support is attributed to the presence of the silanol groups, it was suggested (117) that modification of this group would beneficially alter the nature of the surface. Reagents such as hexamethyldisilazane (HMDS) (118) and dimethyldichlorosilane (DMCS) have been used (119) to form silyl ethers from the surface hydroxyls. Even with the HMDS treatment some residual adsorption is observed, but much of this can be further eliminated by employing a "tail reducer". The combined surface treatment-tail reducer technique appears to be the most effective way of eliminating adsorption effects.

Another technique used to eliminate these effects is to cover the diatomite surface with an appropriate coat. In this manner the support used has the surface characteristics of the coating material and the structure of the particular diatomite used. Coatings such as gold, silver (120) and teflon(121) have been used with varying degrees of success. Still another treatment of the diatomite recommended by Keulemans (122) requires the baking of the size-graded support at a temperature of 1350°C for a six to twelve hour period. Keulemans claims that this treatment closes the smaller pores of the support, tending to make the overall adsorbing surface more uniform. The more uniform liquid film that results when this material is coated is thought to improve the efficiency of the separation.

Aside from the various diatomaceous supports, materials such as metal helices, sodium chloride, sand, unglazed tile, tide, teflon, and glass beads have all been used (123). Most of these supports find rather limited use and offer little advantage, if any, over the diatomite support for general applications.

The analyst's choice of support will generally depend on the mixture he is working on. For the most part, the decision will be based on rhyme rather than reason as a result of the varied and controversial claims of both commercial firms and investigators in the field. Specific problems (for example, amine analysis) often give birth to a specific support treatment which "works" although one is not exactly sure why.

Perhaps as important as the choice of non-interacting support is

the selection of particle size and distribution. It is now apparent (124) that the best column performance is yielded by supports having the smallest particle size and the narrowest possible range of sizes.

Studies on sweat or pyrolysis products require a knowledge of the complications a "bad" (in terms of the separation required) support might introduce. The advantage to be gained by combining the always-present selectivity of the support with that of the solvent to effect a difficult separation has also been reported (125).

THE MOBILE PHASE

To complete the discussion of column materials, the choice of the mobile phase must be considered. Many high-purity gases are now commercially available for gas chromatographic purposes. The gas chosen depends to a great extent on the detection system being used, for the sensitivity of several detectors (for example the Mass Density Balance and the Thermal Conductivity Detector) is dependent on the mobile phase. The theoretical approach to the problem of solute distribution between two phases and to the problem of diffusion in the mobile phase favours the use of the higher molecular weight gases. However, considerations such as the mass transfer properties of the system, the availability of the gas, the expense, and chemical reactivity of the gas usually limit the choice. Aside from the occasional use of such carriers as nitric oxide, steam and sulphur hexafluoride, nitrogen, hydrogen, and the noble gases, helium and argon are most often employed. For trace analysis studies, the purity of the carrier gas used is also an important factor.

THE DETECTOR

With the knowledge of the foregoing discussion, an analyst can carefully select a column capable of dealing with any complex mixture. However, unless he is equally judicious in the choice of a detector or detectors to couple with this column, he may undo the good work the column may be performing (i.e., the column may be separating the components, but the detector may not detect the separation). Furthermore, with the use of multi-column systems (126), the analyst can, by selecting the proper combination of detectors, facilitate compound identification.

Over the past ten years, workers have developed more than twenty types of detectors, each operating on a different principle (see Table 17).

TABLE 17. List of Detectors for Gas Chromatography

Detector	Reference (s)
Thermal conductivity	Purnell (127)
Surface potential change	Phillips (128)
Dielectric change	Turner (129)
Infrared spectrophotometer	Martin and Smart (130)
Far ultraviolet absorption	Kay (131)
Polarographic detection	Nedorost (132)
Heat of adsorption detector	Dudenbostel and Priestley (133)
Interferometer	Zderic, Bonner and Greenlee (134)
Radioactive detection	(135)
Gas density meter	Martin and James (136)
Hydrogen flame temperature detector	Scott (137,138)

continued

TABLE 17 CONTINUED

Detector	Reference (s)
Flame ionisation detector	Sternberg et al (139) Perkins et al (140)
Thermionic diode	Hudson and King (141)
Discharge detector	Hardy and Pretorius (142) Karmen and Bowman (143)
Radiofrequency discharge	Bowman and Karman (144)
Cross section β-ray capture	Boer, H.(145) Lovelock et al (146)
Argon β-ray ionisation	Lovelock (147) (147-A) Purnell (148)
Electron capture	Lovelock and Gregory (149)
Ultrasonic whistle	Testerman and McLeod (150)
Mass spectrometric detection	Varadi (151)

Faced with this multiplicity of choice, the analyst must be aware of the advantages and disadvantages of each device. The major requirements of a detector are high sensitivity and stability, combined with a rapid response to any change in the composition of the chromatographic effluent. The detection is based on some physical property which undergoes a change in magnitude because of the presence of sample in the carrier gas. The change in the physical property is converted via some transducer into a change in an electrical property which is further modified to yield a readout signal. For each detector, the mechanism of response and the property of the sample to which the detector is responding must be considered. The sensitivity and response time of the system to the compounds issuing from the column, and the limit of detection which may be expected of the system, must also be noted. The potential sources of noise in the detector

response and the fraction of the normal signal level which can be sensed should be known. Finally, the manner in which the response of the system is related to the sample size and the way the system's response may alter the nature of the sample as it passes through the detector must be considered. These different considerations allow a comparison of the various detectors for use in any analytical problem.

The instantaneous signal yielded by a detector may correspond to the concentration of some component in the carrier gas, to the rate of introduction of that component into the detector, to the total amount of sample material which has passed through or into the detector, or the signal may correspond to the rate of change of concentration of sample component within the detector. All these modes of response are found among the various detectors used. The signal may be further altered electronically to read out in any of a number of ways. Most frequently, one encounters systems which yield either measurements of concentration, or of the rate of introduction of sample into the detector.

Detectors such as the thermal conductivity cell, the gas density balance, the electron mobility device, the electron capture detector and the far-ultraviolet absorption cell, all yield a direct measure of the concentration of sample in the carrier gas. In several of these systems (thermal conductivity and gas density balance), the important factor is the concentration of sample relative to the carrier gas (e.g., parts sample per unit volume of carrier gas) whereas in other detectors (electron capture, electron mobility and u.v. absorption) the volume con-

centration (in moles substance per unit volume of effluent) is the important concentration factor. In the interpretation of the response of such detectors, the distinction of the two "classes" of concentration detectors must be recognised. The concentration-type detector will be relatively insensitive (as compared with the rate of introduction sensing devices) to flow fluctuations occurring during the passage of a peak. However, these detectors may be affected by changes in the total pressure in the detector chamber, and they may yield a diminished response if additional carrier is introduced into the column effluent between the column and the detector.

The response of devices such as the hydrogen flame and β-ray argon ionisation detectors is a direct measure of the rate of introduction of sample into the detector. In contrast to the concentration measuring devices, these systems will be sensitive to flow fluctuations, but relatively insensitive to changes in total pressure.

A convenient method of classifying detectors, either as "bulk property detectors" or "specific property detectors", at once distinguishes between the so-called "normal sensitivity devices" and the "ultra-high sensitivity devices". The bulk property detectors measure some unique bulk property of the carrier gas and sample together. The thermal conductivity cell and gas density balance represent devices of this type. In these detectors, the property measured is not, in general, more than ten-fold discriminatory between pure carrier and pure sample. Thus a sensitivity of the detector to one part of sample in 10^6 parts of carrier gas (1 ppm) requires an extremely fine measurement of the property in question.

Detectors of the "specific property" type include the hydrogen flame ionisation detector, the beta-ray ionisation detector, the far-ultraviolet absorption detector, the electron capture detector and the photo-ionisation detector. In these systems, the property measured is 10^3 to 10^7 fold greater for the sample than for the carrier, thus permitting a high sensitivity of detection without as fine a measurement of the property. Unfortunately these specific property detectors work at low current levels in high impedance circuits, so that electrical measurements cannot be made with the same degree of precision as is possible in the case of the bulk property detectors.

Of the many detectors available, a selection can be made from about six common units which more than adequately provide the versatility needed for general analytical purposes. A laboratory furnished with a thermal conductivity cell, a flame ionisation detector, a beta-ray ionisation detector, a cross section ionisation detector, an electron capture unit, and a gas density balance, is well supplied. Other detectors used are usually more limited in their applications than those listed above.

For the purposes of pyrolysis-gas-chromatography and the analysis of trace components in aqueous media, the hydrogen flame ionisation detector is undoubtedly best suited to the analyst's needs. Not only is this detector one of the most sensitive available, but it is also one of the few detectors whose response is not impaired by the presence of carbon dioxide and water. This latter property is perhaps the most valuable for the type of work attempted in the pyrolysis and sweat studies. Unfortunately,

this detector destroys the sample. Thus if sample identification by retention data alone proves too difficult, a change to other detectors which allow the effluent to be collected for further study must be considered.

Enough is now known about the most commonly used detectors to permit a rather brief outline of their most important characteristics (152). More extensive descriptions of the detection systems are to be found in the references cited in Table 17.

THE THERMAL CONDUCTIVITY DETECTOR

In the thermal conductivity detector, a change in the thermal conductivity of the effluent from the column leads to a change in the rate of heat removed from a filament or thermistor sensing element. The resultant change in the temperature of the sensing element leads to a change in the resistance. This resistance change unbalances a wheatstone bridge circuit of which the sensing element forms one arm. The theoretical treatment of the thermal conductivity of mixtures is rather complex. In general, the thermal conductivity of a mixture of two gases is not a linear function of composition. It is thus difficult to predict the way in which a sample will alter the thermal conductivity of the column effluent. However, the response of the detector to a given compound can be calculated to within 3 percent of the observed value (153, 154). For samples of the size encountered in gas chromatography, the response of the detector is linear with respect to the amount of substance present in the carrier gas.

The main sources of noise for the detector come from temperature

and flow fluctuations. Instability in the detection system may also be introduced by thermocouple effects at various contacts, and by bridge power-supply fluctuations. With special precautions, this detection system can be made sensitive to 20 to 50 parts of sample per 10^9 parts of carrier gas at the detector. Normally a sensitivity in the 1 ppm (part per million) range at the detector is observed.

THE GAS DENSITY BALANCE

This detector, developed by Martin and James (136), operates on the principle that vapours of different density distribute in a unique manner between two vertically displaced segments of a reference gas stream. Lighter vapours diminish the flow in the upper reference stream, whereas heavier gases diminish the flow in the lower reference stream according to the construction of the detector (see for example, Keulemans (8) p.78 or Fig.5) The flow decreases are sensed as temperature increases in the thermistor or filament sensing elements in the reference stream segments of the apparatus. The change in temperature effects a resistance change in the sensing element which (as in the case of the thermal conductivity detector) unbalances one arm of a wheatstone bridge circuit. An output signal is thus produced.

The response of this detector is directly proportional to the molecular weight of the sample. It can thus be used (155) to aid in the identification of the separated components of a mixture. The response is also linear with respect to the sample size, and can be observed for sample concentrations in the 1 to 10 ppm range at the detector. The main source

of noise for this detector is found in detector-temperature fluctuations.

THE HYDROGEN FLAME IONISATION DETECTOR

This detector employs a high temperature flame to provide the necessary energy and reactive intermediates to strip and crack organic sample molecules. The subsequent reaction of the sample fragments with flame intermediates (139) produces ion fragments which are collected by the application of an electrical field. The resulting current is modified electronically via a high-impedance circuit, to yield a recorder signal.

The response of this system is roughly proportional to the number of carbon atoms in the sample. In addition, the configuration of the molecule, and the presence of fluorine, nitrogen, oxygen and metal atoms in any sample can play an important role in the detector response. The very low background signal produced when no sample is present in the detector (10^{-11} to 10^{-12} ampere) permits a high sensitivity. The linear response of the system can be demonstrated for sample sizes ranging from the detection limit of 8×10^{-14} gram atoms of carbon per second to the point where the sample level is 3 percent of the hydrogen concentration at the detector. Of particular interest is the absence of response of this detection system to carbon dioxide, water and carbon disulphide.

The chief sources of noise for this system originate with contaminants in the gas streams going to the detector and with the amplifier-recorder system used.

THE ARGON BETA-RAY DETECTOR

This form of ionisation detector operates on the basis of two reactions: the excitation of argon to its metastable state by electron bombardment; and the ionisation of vapour molecules by the transfer of the energy stored in the metastable atoms. The high ionisation efficiency of this system permits measurements at extreme sensitivity with relatively simple amplifiers. The detector can be connected directly to a high input-impedance recorder.

The system yields a positive response to all vapours whose ionisation potential is less than, or equal to, the energy of the metastable argon atoms (ca. 11.7 electron volts). The response to different substances is primarily determined by the frequency of collision between the test molecules and the metastables. This response is fairly closely related to the mass of substance introduced to the detector per unit time, and is independent of the molecular species. With the smaller molecules (molecular weights less than 100) collisions with the metastables are more frequent, and a greater signal for a given mass results.

The attractive characteristics of the argon detector are its high ionisation efficiency and its ability to respond in a non-destructive manner to most volatile organic and inorganic compounds. The disadvantages encountered are the system's inability to handle very large vapour samples and its tendency to lose sensitivity in the presence of water vapour or air (these may be present in the carrier gas). The limit of detection of this device has been estimated at 10^{-14} moles per second at the detector (156) for propane .

THE ELECTRON CAPTURE DETECTOR

This detector uses recombination effects to yield a signal. A radioactive source ionises the carrier gas to provide a free electron gas in the detection chamber. A potential just sufficient to collect all the free electrons is maintained across the ion chamber. When an electron-capturing substance enters the cavity, a decrease in current related to the concentration of test substance is observed. Basic to the operation of the detector is the fact that the rate of recombination of positive and negative molecular ions is some 10^5 to 10^8 times greater than between free electrons and positive ions, and the fact that the drift velocity of the electrons is much greater than the mobility of the negative ions. The introduction of sample into the detector thus markedly affects the standing electron current.

The response of the system depends on the electron affinity of the sample. This characteristic varies with the energy of the free electrons in much the same way as light absorption varies with the energy of the incident photons (156). By varying the mean free electron energy in the detector cavity, the sensitivity of the device to a variety of compounds can be altered. Depending on the carrier gas used and the cavity potential, mean electron energies ranging from 10 electron volts in argon to 0.1 electron volt in carbon dioxide can be obtained. The use of the Pulse Sampling Technique, as described by Lovelock (157), permits the observation of the variation in electron absorption cross-section with the mean electron energy. The type of variation observed will be characteristic of a given compound and thus can serve for identification

purposes. The selective response of this detector permits sensitivities to as little as 10^{-15} moles of sample per second. The quantitative interpretation of the detector response to such small samples is often a more difficult operation than is first apparent (158).

THE CROSS-SECTION DETECTOR

This detector, which was one of the first to be developed, employs ionising radiation from some radioactive source to effect a primary ion current. The current is proportional, among other things, to the effective cross-section of ionisation of the vapour in the detector chamber. A gas with a low cross-section (hydrogen or helium) is chosen for the mobile phase of the chromatographic system. A current increase proportional to the concentration of any other vapour in the detector will be observed.

The response of this detector for any substance can be calculated. A knowledge of the atomic cross-sections of the constituent elements for the particular bond arrangement of any compound is required for this purpose. The response is linear with respect to sample size from the detection limit (ca. 5×10^{-9} moles per second of propane) to 100 percent sample within the detector. The main disadvantage of this detector is its relatively low (when compared with other available detectors) sensitivity. The main advantage is its linearity of response to most compounds over a very wide concentration range. By scaling the detector down, it is possible to obtain a detector sensitivity of about 10^{-9} grams per second of propane (159). This version of the detector is adequate for

all applications of gas chromatography except high-dilution trace analysis.

COMPOUND IDENTIFICATION

There are three general methods available for the identification of peaks in gas chromatography (160). The most commonly used approach is based on the retention data alone (161). The simplest form of this method is to compare the retention time of a certain peak in a chromatogram with that of a known substance in another chromatogram. If the behaviour of the known and unknown substances is found to be identical on at least two columns of differing specificity, one can be fairly certain of their identity. Confirmation can be achieved by the "marker" technique whereby the suspected component is added to the mixture and the change in peak height observed for the particular component. A second variation of this first method employs special plots of the retention data. For example, in a study of a homologous series of compounds, a plot of the logarithm of the retention time versus the carbon number yields a straight line.[1] An unknown homologue can be identified using this plot once its retention volume is determined. There are also other graphical representations of this type that can be used to characterise a substance or family of substances (162). In addition to these graphical methods, Kovats (163) and Miwa (160) have both independently devised a system for compound identification on the basis of the retention volume data. The method seems to be based on the idea (rightly or wrongly) that the thermodynamic properties of any molecule can be thought

[1] An example of the type of plot is seen in Figure 55.

of as a sum of the individual contributions of the component parts of the molecule. Kovats uses polar and non-polar columns to compare the behaviour of all organic compounds with the behaviour of the normal paraffins. The extent of deviation in behaviour of a certain compound from that of the paraffins is attributed to a specific grouping in the compound. An extensive tabulation of these deviations can, according to the method, ascertain whether an unknown belongs to a family of 1-nitriles, aromatics, 1-alcohols and so on. Most of the functional groups have been characterised by these deviations, thus facilitating their identification as unknown peaks in a chromatogram. The details of this method of identification have been well reviewed (164).

Aside from the use of retention data, two other general approaches can be employed to identify chromatographic peaks. The first of these involves the use of a dual, selective-response detector system (165, 166). As has been mentioned, the various detectors now in use each depend on a different property of a compound to evoke a response. The combination of response given to two detectors is usually unique for a given type of compound. There is no detector yet which is completely selective in its response to all types of compounds, although the electron capture unit and the mass spectrometer detector approach this criterion. A combination of detectors such as these yields a powerful tool for the purposes of compound identification.

The second identification procedure which does not use the retention data requires the isolation of the effluent as it comes from the detector. When a substantial amount of the desired material has been obtained, any

conventional physical or chemical means may be used to identify the peak. Recently the methods of qualitative organic analysis have been scaled down for this type of approach. Both catalytic combustion (167,168) and derivative preparation techniques (169) are now used for purposes of identification.

In most cases, identification based on the retention data alone will suffice. However, when a complex problem is encountered, the use of a combination of techniques is desirable.

THE VAN DEEMTER EQUATION

The efficiency of a gas chromatographic column is expressed by a quantity called the "height equivalent to a theoretical plate" (H.E.T.P). This quantity refers to the length of column required for the complete equilibration of the sample vapour between the gas and liquid phases as the plug passes down the column. This piece of the column may be thought of as one stage in a series of hypothetical Craig extraction vessels which are joined to form the whole column.

In his investigations of the gas chromatographic process, Van Deemeter (170) was able to derive an expression relating the column efficiency (in terms of the H.E.T.P.) to the linear velocity of the mobile phase and to the various constants associated with the construction of the packed column. The expression derived for a packed column is of the form

$$H = A + \frac{B}{u} + Cu \qquad (1)$$

where u is the linear gas velocity along the column. From the plot of H as a function of u (Figure 58), it can be seen that there exists some optimum gas velocity (corresponding to the minimum on the curve) for which the column operates at peak efficiency (lowest H.E.T.P.). This plot underlines the disadvantage of operating at too low gas velocities where the curve rises steeply.

In its more explicit form, the equation appears:

$$H = 2\lambda dp + 2\gamma \frac{D_{gas}}{u} + \frac{8}{\pi^2} \frac{k^1}{(1+k^1)^2} \cdot \frac{df^2}{D_{liq}} u \quad \text{---------- (2)}$$

where the constants are defined:

λ : the packing coefficient

dp : the average particle diameter

γ : the void space correction

D_{gas} : the diffusion coefficient[1] in the gas phase

k^1 : a constant related to the distribution coefficient k, by

$$k^1 = k \frac{\text{(fraction of column occupied by liquid phase)}}{\text{(fraction of column occupied by gas phase)}}$$

df : the liquid film thickness

D_{liq} : the diffusion coefficient in the liquid phase.

Equation (2) expresses in one statement the effect a number of factors may have on the overall column efficiency. For instance, decreasing the average particle size and making the packing uniform will reduce the first term of equation (2) and thus increase the column efficiency. The use of a carrier gas with a small diffusion coefficient (a high molecular weight) will reduce the second term of equation (2). An elimination of column "void space"

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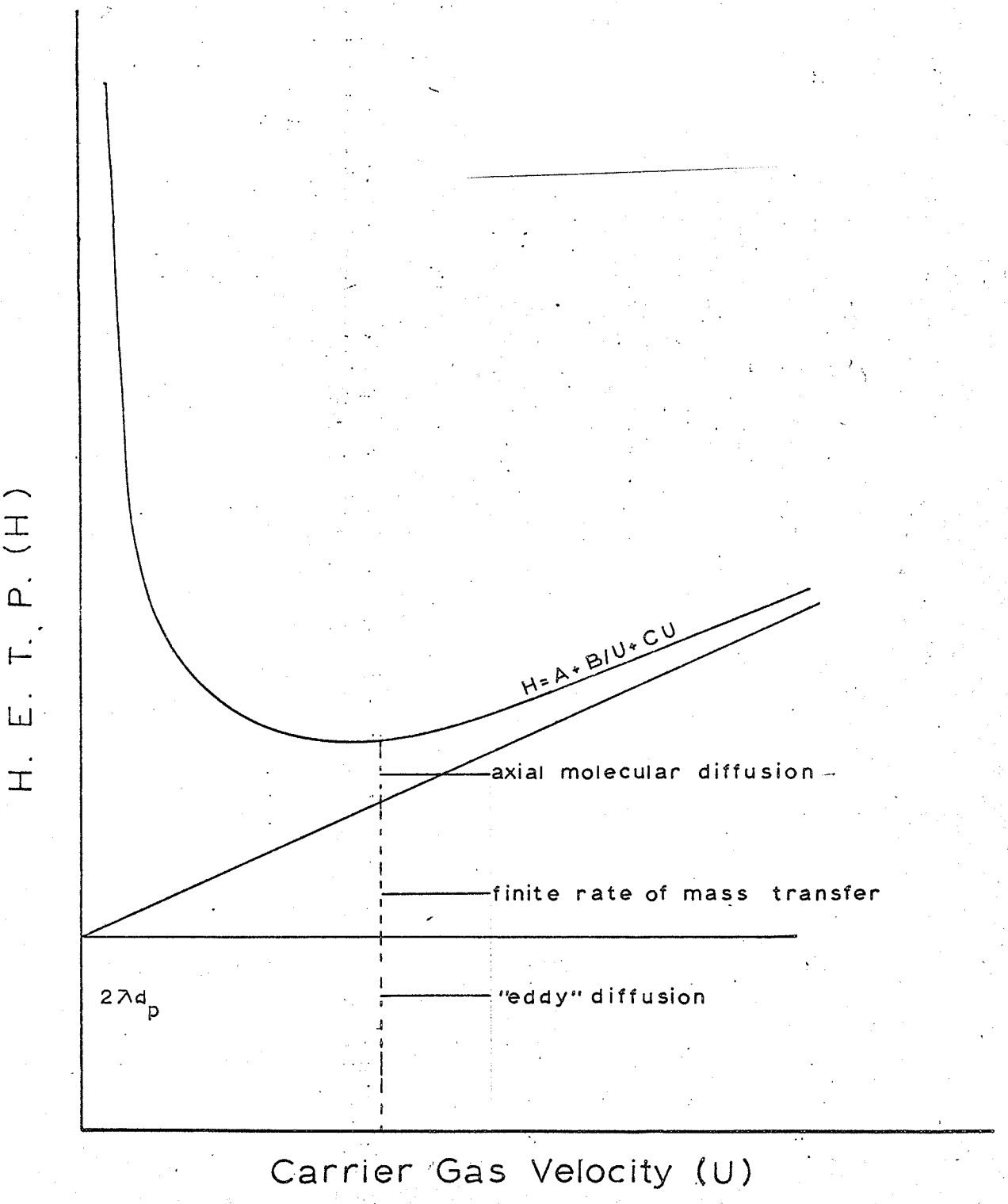
[1] The diffusivity, D_{gas}, is proportional to $1/P$ and $1/(M_s M_g)^{1/2}$, where P is the pressure in the system, and where M_s and M_g are the molecular weights of samples and carrier gas respectively.

FIGURE 58. Van Deemter Plot Showing Variation of Column Efficiency (HETP) With Carrier Gas Velocity.


H. E. T. P. (H)
H=A+B/U+CU
axial molecular diffusion
finite rate of mass transfer
2λd_p
"eddy" diffusion
Carrier Gas Velocity (U)

will also decrease this second term and increase the column efficiency. The adverse effect of large film thicknesses on column efficiency is seen in the third term of the equation. The effect of the diffusion coefficient in the liquid phase (which is related to the liquid phase viscosity) and of the partition coefficient are also expressed in the third term of equation (2). This equation relating the many column factors to the column efficiency gives a good guide to the construction of any chromatographic column.

In the course of any analysis, a van Deemter-type plot for any particular column (a plot of the carrier gas velocity versus the HETP) will be useful in determining the optimum carrier gas velocity.

THE LITERATURE

The literature concerned with gas chromatography is admittedly difficult for one person to tabulate. Nevertheless, many excellent texts, several cumulative literature compilations, and relatively few periodicals which cover most of the developments in the field, make the job of following the literature a manageable one. One convenient list of these texts, cumulative reviews and current publications which aid in the understanding of this field is given in Table 18.

TABLE 18. Guide to The Gas Chromatographic Literature

Texts:	Burchfield, H.P. and E.E. Storrs. Biochemical Applications of Gas Chromatography, Academic Press, New York and London (1962)

continued

TABLE 18 CONTINUED

Texts: continued.	Keulemans, A.I.M. Gas Chromatography, Second Edition, ed. C.G. Verver, Reinhold Publishing Corp., New York (1960)
	Nogare, S. Dal, and R.S. Juvet, Jr. Gas-Liquid Chromatography, Theory and Practice, Interscience Publishers, New York-London (1962)
	Purnell, Howard. Gas Chromatography, John Wiley & Sons, Inc., New York-London (1962)
	Szymanski, H.A. (editor), Lectures on Gas Chromatography 1962, Plenum Press, New York (1963)
Cumulative Reviews:	Analytical Chemistry Annual Reviews
	Gas Chromatography Abstracts ed. C.E.H. Knapman, Butterworths, London.
	in Gas Chromatography, Academic Press, New York and London (1958,1961, 1962) Symposia held under the auspices of the Analysis Instrumentation Division of the Instrument Society of America.
	Journal of Gas Chromatography.
	Preston Abstracts (Preston Technical Abstracts Co., Evanston, Illinois)
Periodicals:	Analytical Chemistry
	Journal of Chromatography
	Journal of Gas Chromatography

NOMENCLATURE RECOMMENDATIONS AND DEFINITIONS

Although the terminology which was developed for the gas chromatographic technique is not too extensive, it may be confusing at times. To assure some kind of uniformity, several nomenclature recommendations have been made at various conferences on gas chromatography (171,172,173, 174). Table 19 lists a few common terms encountered in the literature.

TABLE 19. Some Terms Used in Gas Chromatography

Term	Definition
Capillary columns	This term is used to refer to open tubular columns of the Golay type. The stationary liquid is coated on the wall of the column rather than on a conventional solid support.
Chromatogram	This refers to the record of the analysis as depicted in Figure 59.
Dead Space	Any part of the chromatographic apparatus not being used for the separation process.
Liquid Load	i.e., the amount of liquid phase used to coat the support.
Peak	The detector response to the column effluent (Figure 59).
Programmed Temperature Chromatography	The temperature of the column is altered during the course of the chromatographic process.
Pyrolysis Gas Chromatography	A technique used to study non-volatile compounds. The thermal degredation products are analysed with a gas chromatograph.

continued

FIGURE 59. A Typical Chromatogram for Defining Terms.

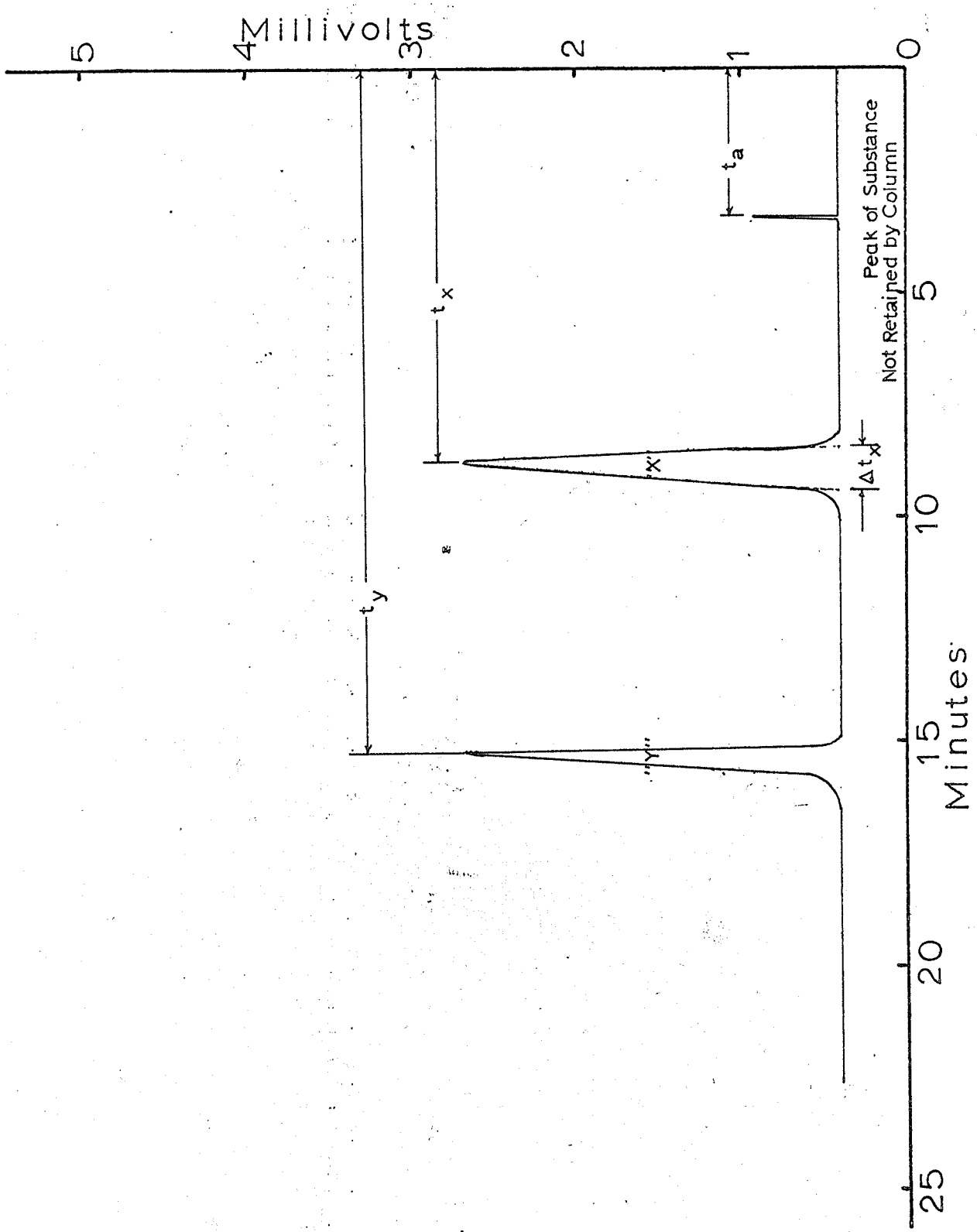

Millivolts
5
4
3
2
1
0
t_a
t_x
t_y
Peak of Substance
Not Retained by Column
Δt_x
'X'
'Y''
5
10
15
20
25
Minutes

TABLE 19 CONTINUED

Term	Definition
Pyrogram	A chromatogram obtained from the products of pyrolysis of a compound.
Resolution	An expression of the ability of a column to separate any two compounds. Expressed: $B = ty-tx/tx$ (Figure 59).
Retention Time	The time taken for a substance to pass down a chromatographic column. This is more accurately tabulated as the Retention Volume, the volume of carrier gas used to elute the compound.
Relative Retention Time	The time of elution for one compound relative to the time of elution of some standard (174) substance.
Theoretical Plate	A term derived from the Plate Concept of chromatography, referring to a hypothetical Craig extraction vessel. The number of plates for a given column is expressed: $N = 16(tx/\Delta tx)^2$
Height Equivalent to a Theoretical Plate	The total number of plates divided by the column length. This value expresses the height of individual column portions, which if lumped into as many continuous absorption vessels, would cause the column so transferred to reproduce in theory the observable times on the elution curve (Figure 59).

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