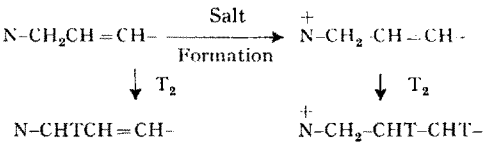
determined in a β-ray spectrometer. The Figure presents the results of thin layer chromatography of each sample of tritiated VLB before purification and again after six recrystallizations.

Discussion. When either VLB or VLB sulfate was tritiated by the Wilzbach method the bulk of the radioactivity in the product is not associated with VLB. Two major tritiated by-products, one fast moving, the other slow moving, are formed (Figure). Both are readily removed by recrystallization of VLB as the sulfate salt.

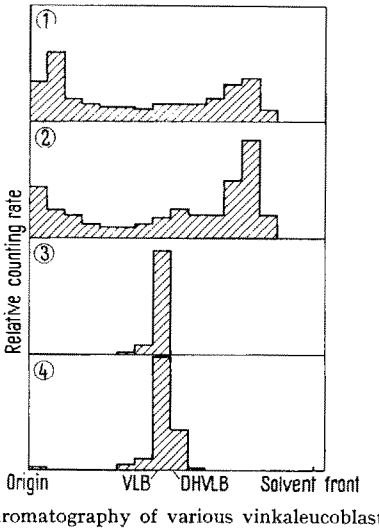
There are several interesting differences between the results obtained by tritiation of the salt compared to the free base. For example, six recrystallizations of VLB sulfate obtained from exposure of the free base produces material of nearly constant specific activity, while in case of the material exposed as the salt constant specific activity is not reached. The specific activity of the product is also considerably higher in the former case. The most notable difference, however, is that the product from exposure of the salt contains substantial amounts of dihydro VLB while the material exposed as free base is essentially free of this reduced by-product (Figure). Since reduction of double bonds is known[5] to be an important side reaction during Wilzbach tritiation, dihydro VLB had been expected to be present as an impurity. The structure of VLB is now well enough known[6] so that it can be said that the double bond involved in dihydro-VLB formation is in the vindoline portion of the molecule. This double bond is in a position allylic to a basic nitrogen:



It is not possible at present to explain why the double bond in the salt form is susceptible to reduction while that in the free base is not. It is interesting to note, however, that the catalytic reduction of the similarity placed double bond of vindoline goes readily in acid solution but not in the presence of base[7]. Furthermore, in recent experiments in these laboratories by NEUSS, it has been found that the catalytic reduction of VLB to dihydro-VLB proceeds only when VLB is present in the salt form.

The reason for the higher specific activity obtained during tritiation of the free base is not apparent, but it is possible that in this case the methylene carbons adjacent to nitrogen are more readily exchangeable than is true when the nitrogen is ionized. Another possibility is that the base in the amorphous form offers more surface for exchange than does the crystalline salt form.

Thus it has been possible to prepare tritiated VLB of constant specific activity which contains no dihydro-VLB. It is of course possible that other impurities which co-crystallize with VLB: SO_4 and co-chromatograph with VLB may be present[8].



Thin-layer chromatography of various vinkaleucoblastine samples. (1) VLB: SO_4 after tritiation; (2) VLB base after tritiation; (3) same as (2) but after six recrystallizations as sulfate salt; (4) same as (1) after six recrystallizations.

Zusammenfassung. Das Alkaloid Vincaleukoblastine (VLB) wurde als Base bei Anwendung der Wilzbach-Technik titriert. Die Verbindung, bis zur konstanten Aktivität gereinigt, enthielt kein Dihydro-VLB mehr. Das Alkaloid, als Sulfatsalz dem Tritium unterworfen, inkorporiert die Substanz nur geringfügig, wobei das Tritium enthaltende Dihydro-VLB ensteht.

R. E. MCMAHON

Lilly Research Laboratories, Indianapolis (Indiana, U.S.A.), March 18, 1963.

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Determination of Adenine in Hydrolyzates of Ribonucleic Acids with the Aid of Comparative Titration

One of the principal methods of research into nucleic acids is the determination of the molecular proportions of the bases of these acids. At present, several methods are used to determine the proportions of bases. The most widely used method is that of paper chromatography which, upon separation of the individual bases, permits their measurement—spectrophotometry in UV. Another method used in practice consists in fractionation of a mixture of bases with the aid of an ion exchanger and, upon elution, in repeated measurements in UV. These methods yield sufficiently accurate results; however, their performance is rather time consuming.

For this reason, it is advisable to look for new methods which would essentially speed up the process of analysis. Thus, the method of direct spectrophotometry of RNA hydrolyzate has been worked out; however, it is little used, for the treatment of the results is rather intricate, and moreover the determination in the presence of substances absorbing in UV is inaccurate.

Polarographic methods for the determination of the components of nucleic acids were used for the first time by

HEATH[1], who found that it was only adenine that was polarographically active. Up to the present time, however, we have not succeeded in determining adenine polarographically in a mixture of other bases of nucleic acid, e.g. in the hydrolyzate of RNA—GOWELSKI[2]. PALEČEK[3] demonstrated the possibility of determining adenine with the aid of oscillographic polarography and found that also an excess of cytidine or cytidylic acid in the mixture does not interfere with the determination process.

For the quantiative determination of adenine in a mixture of all bases of RNA (ribonucleid acid), we employed the method of comparative titration according to KALVODA[4], originally meant for determining pure substances; we modified this method and used it for determining adenine right in the acid hydrolyzate of RNA.

Methods. Principle of the method of comparative titration: on the screen of the oscillopolarograph are obtained two curves of the function $(dE/dt) = f_1(E)$, one for the solution investigated, the other for the solution of basic electrolyte which is titrated with the measuring solution of the reference depolarizer so long, until both curves are identical. In this original form, the method can be used—as the author puts it—only for the determination of pure substances. In the presence of other substances in the mixture, the shape of the curve changes and thus the curve indentation, referring only to one substance, cannot well be compared with the indentation on the other curve caused by the same substance, though in the presence of other admixtures.

For this reason, we modified this method so that we established the difference in the concentrations of adenine between the solution of RNA hydrolyzate and between the same solution, to which, for instance, 1 ml of basic electrolyte had been added. Thus, a difference in A (adenine) concentrations arises between the diluted and the undiluted solution of hydrolyzate, and this difference manifests itself in a diminution of the depth of indentation in the diluted solution. After compensating the concentration difference—indicated by the same indentation depth of adenine in both solutions—it is possible to calculate the A amount in the hydrolyzate from the consumption of adenine titration solution of known concentration.

Since, by adding adenine titration solution, the total volume, and thus also the concentration, changes, we can calculate the A amount in the added volume of basic electrolyte according to the formula:

$$\text{Adenine mg} = \frac{B \cdot V_1}{V_1 + V_2} \qquad (1)$$

where B = amount of adenine consumed during titration
V_2 = volume of titration solution (consumption)
V_1 = volume of basic electrolyte added to hydrolyzate.

If we determine the phosphorus content in the RNA hydrolyzate, we can calculate the adenine content in molar %.

The apparatus used for measuring was a Polaroscope P 576. After work with a vibration electrode the instrument was adapted by building in an additional resistance, which made it possible to work with an alternating current component of 0.03 mA.

For titration with two capillaries, capillaries of the same internal diameter were used. In order to ensure synchronous separation of the mercury drops, connection of the capillaries by means of a small glass rod, in the place where the tapper was active, proved to be of good advantage.

In a similar way we can also work with one capillary, at the best with one of the vibration type according to FIBY[5], which furnishes a stable picture on the screen of the picture tube, using the light trace according to KALVODA[6]. The light trace serves as indicator of the depth of indentation. Under these conditions the process of determination is shortened and stirring during titration done away with, for the vibrating electrode itself is a good stirrer.

The vibrator for the capillary was constructed in the Institute workshop according to the design by FIBY[5].

Prior to determination the hydrolyzate was diluted with 1 N H_2SO_4 to a concentration of 40–100 γ of pure RNA per 1 ml solution. RNA concentration was determined by measurement of extinction at 260 mμ using E-extinction-1% RNA solution after hydrolysis $E\,\frac{1\text{ cm}}{260} = 310$. The established amount of RNA is then converted into phosphorus.

Process of Determination. (a) Titration with two capillaries: Into each vessel 3 ml RNA hydrolyzate solution were pipetted. Upon compensating on the instrument both curves to the same shape, 1 ml 1 N H_2SO_4 was added into one of the vessels. Thus, a difference in the depth of indentation between the curves arose and consequently it was necessary to compensate that difference by adding adenine titration solution, until complete identification of the indentations on the curves was reached. From the consumption of titration solution the adenine content in 1 ml is calculated with the aid of formula (1).

(b) Titration using light trace: In this process vibration electrodes were used. The working method is the same as in item (a)—as control of the depth of indentation serves the light trace.

Errors in Determination. Probable error in either procedure was calculated from three determinations and amounted to ±7%.

Paper Chromatography. All RNA preparations given in the paper were, upon hydrolysis, analysed for the com-

	Adenine in mol% Chromatography	Oscillopolarography
(a) RNA from yeast	25.0	24.5
(b) RNA from yeast	26.3	25.4
(c) RNA from mouse liver	18.2	19.3
(d) RNA from rat liver	18.1	19.0
(e) RNA from ex pancreas	14.2	15.0

Preparations used: (a) RNA from yeast—product of Messrs. Light. (b) RNA from yeast, isolated according to CRESTFIELD[7]. (c) RNA from mouse liver, isolated according to KIRBY[8]. (d) RNA from rat liver, isolated according to KIRBY[8]. (e) RNA from ox pancreas—preparates made available by Dr. S. ZADRAŽIL, Czechoslovak Academy of Sciences, Prague. Adenine-'Lachema', its purity was verified by paperchromatography.—As titration solution was used A in a concentration of 20 γ/ml in 1 N H_2SO_4.—RNA hydrolysis: 1 mg RNA hydrolyzed with 0.1 ml 1 N H_2SO_4 in sealed glass test tube at 100°C for 1 h. (Note: HCl cannot be used for hydrolysis, as chlorides in 1 N H_2SO_4 environment as basic electrolytes interfere with oscillopolarographic determination.)

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position of their bases according to the method developed by KIRBY[8,9].

In the Table, the results of chromatographic and oscillopolarographic analyses of the A content in RNA are given for the sake of comparison.

Zusammenfassung. Auf dem Prinzip der Komparationstitration wird eine schnelle oszillographische Methode für quantitative Adeninbestimmung in saurem Hydrolysat der RNS entwickelt.

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Institute of Biophysics, Czechoslovak Academy of Sciences, Brno (Czechoslovakia), January 28, 1963.

[9] *Acknowledgment.* My thanks are due to Dr. E. PALEČEK for the communication of the themes and the interest with which he followed this work.

STUDIORUM PROGRESSUS

Local Metabolic Response to Physio-Pathological Demands: The Pentose Phosphate Pathway

In the effort to understand and combat disease, the study of the pathways and intermediate products of metabolism plays an ever-increasing role. It is obvious that any qualitative or quantitative changes occurring in the metabolic pattern will produce consequent functional alterations in the cells affected.

In vitro studies have shown that, under normal conditions, glucose breakdown takes place *via* both the Embden-Meyerhoff (glycolytic) and pentose phosphate (direct oxidative) pathways in the arterial wall, liver, kidney, pancreas, adrenals and the spleen; whereas, in the veins, cardiac and striated muscles, central nervous system and gastro-intestinal tract, glucose is metabolized *via* the glycolytic route only[1–7].

This diversity in the pattern of glucose metabolism in different tissues implies that their requirements and utilization of the various intermediate metabolites provided by these pathways vary according to their structure and function. Metabolic needs alter under different physiological and pathological conditions. It has been shown, for instance, that the pentose shunt is increased in the lactating mammary gland[8]; while the opposite is found to hold true in the erythrocytes taken from patients with thyrotoxicosis[9].

The present communication reports results concerning the metabolism of glucose *via* the pentose phosphate pathway during (1) digestion, and (2) inflammation and repair. These particular physio-pathological processes have been selected for study because they are representative of the basic continuous functional responses by living tissues to maintain the integrity of the milieu interieur.

Experimental. The method of BLOOM and STETTEN[10] was used to indicate the relative utilization of the glycolytic and direct oxidative routes in glucose metabolism. The radioactive material (glucose-1-^{14}C and glucose-6-^{14}C, obtained from the Radiochemical Centre, Amersham) was diluted with inert substrate to give a specific activity of about 1.5 μc/mg. The glucose concentration in the Warburg flasks was 0.1%. Details of tissue preparation, incubation conditions and CO_2 determinations have been described in an earlier communication[1].

(a) *Study of glucose metabolism during digestion.* Adult, male Wistar rats, weighing 200–250 g, were fasted for 12 h. They were then allowed to feed for 15 min on a mixture of condensed milk, sugar and assorted grain, as well as being given water to drink. 2 h later they were decapitated, and slivers of the stomach wall (weighing about 100 mg) were taken and transferred to Warburg flasks. Sections were taken from the upper and lower half of the lesser curvature of the stomach, the latter tissue in the rat comprises an infinitely larger proportion of smooth muscle fibers.

(b) *Study of glucose metabolism during inflammation and repair.* Under light intravenous sodiumpentathol anesthesia, the femoral artery or vein was exposed in adult male mongrel dogs weighing about 10 kg. The vessel was then traumatized, by being crushed with artery forceps; by intramural injection of an irritant (0.2–0.5 cm^3 1% phenol or 0.5 cm^3 of 50% ethyl alcohol); or by being cut across and anastomozed. The animals were sacrificed 48–96 h after the intervention, at which time the affected part of the vessel was carefully dissected out and transferred to Warburg flasks.

Results and Comments. The results in the Table indicate that under conditions of digestion and during inflammation and repair glucose metabolism *via* the pentose phosphate pathway was considerably increased, although the quantitative increase cannot be precisely established because of the limitations of the technique employed[11,12].

Chemical reactions in biological systems are responses to physiological exigencies. Glucose, the major basic nutriment of animal tissue, is metabolized *via* at least two pathways – the glycolytic and the pentose phosphate. The former is the major and, in some tissues, the only measurable route of glucose metabolism under normal physiological conditions. The intermediate metabolites formed are utilized for the synthesis of the necessary cell constituents and, together with the Krebs cycle, this pathway provides the energy required for the many biochemical processes relating to cellular function and body 'work'.

Since the recognition of the pentose phosphate pathway in animal tissue, studies have revealed that the reaction sequence of glucose breakdown *via* this route provides the pentose sugars required for the synthesis of nucleic acids; it also contributes notably towards the formation

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