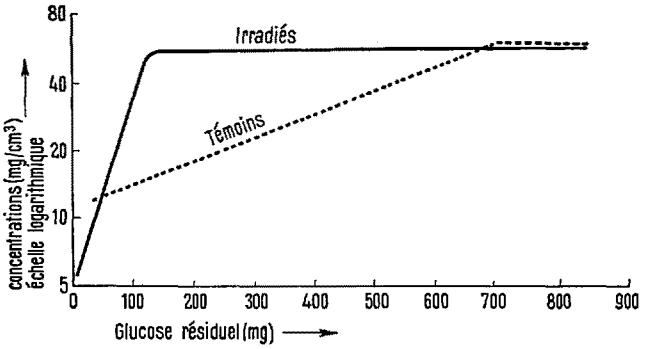

	Témoins	Irradiés
Glucose résiduel (mg)	1100 à 700	1100 à 100
Concentrations (mg/cm³)	60 ± 12	56 ± 10
Effectifs	14	16
	(t = 1,9)	
Glucose résiduel (mg)	700 à 0	100 à 0
Coefficient de corrélation	0,91	0,77
Probabilité	0,001	0,01
Pente de la droite de régression y en x	$1 \cdot 10^{-3}$	$8 \cdot 10^{-3}$
Effectifs	16	16

témoins et 32 cobayes irradiés totalement depuis 14 jours par une dose sublétale de rayons X (500 roentgens de 250 kV) et nous avons utilisé pour mesurer le volume de la solution gastrique et sa teneur en glucose les techniques décrites en [3].

Chez les témoins, le glucose ingéré, qui ne passe dans l'intestin que par petites fractions, reste dans l'estomac où il est dilué par les sécrétions de la muqueuse. Cette dilution se fait en deux temps: un premier temps, très court (moins de 15 min) abaisse la concentration à 60 ± 12 mg/cm³, puis il y a un arrêt; le glucose franchit le pylore sans que la concentration change jusqu'à ce qu'il ne reste plus que 700 mg de glucose dans l'estomac. A ce moment, la concentration baisse de nouveau en suivant une loi définie: entre le log des concentrations et le glucose résiduel il y a une corrélation hautement significative (coefficient de corrélation 0,91; $p = 0{,}001$; voir Figure et première partie du Tableau).

L'irradiation modifie la marche des phénomènes. La première dilution reste normale, aussi rapide, aussi importante. La concentration atteinte: 56 ± 10 mg/cm³ ne diffère pas statistiquement de celle des témoins (t de Fisher = 1,9), mais elle persiste jusqu'à ce que l'estomac ait évacué à peu près tout son contenu; c'est seulement lorsqu'il ne reste plus que 100 mg de glucose que la concentration s'abaisse de nouveau, mais elle le fait en suivant la loi normale. Le coefficient de corrélation 0,77 est significatif au seuil de probabilité 0,01, mais la droite représentant la fonction: log concentrations/glucose résiduel a une pente 8 fois plus forte que chez les témoins (Figure et deuxième partie du Tableau).

Pendant une longue période, les conditions d'absorption sont donc fortement perturbées chez les animaux irradiés, car c'est essentiellement la dilution que la solution ingérée subit dans l'estomac qui règle la vitesse de l'absorption intestinale[3,7]. Toutefois il n'est pas ici possible d'estimer l'importance, voire même le sens de cette perturbation dont les conséquences sont multiples, mais opposées. Alors que la persistance d'une concentration élevée accélère l'absorption[7], d'autres facteurs agissent en sens inverse; citons, l'absence d'eau, peut être aussi une moindre perméabilité à l'eau de la muqueuse intestinale analogue à celle que l'on a observée pour le péritoine[8], la diminution du volume gastrique, qui ralentit le transit pylorique[9].

L'intérêt des observations ci-dessus est de rattacher à un problème très général: perturbation radioinduite du métabolisme hydrique, un fait qui paraissait tout à fait étranger: la diminution de l'absorption intestinale des glucides.

Les droites de régression représentées dans la Figure ont été tracées par la méthode des moindres carrés. Le Tableau contient les statistiques relatives aux données expérimentales.

Summary. A sublethal dose of X-rays, delivered to guinea-pigs 14 days before a glucose absorption test, alters the pattern of water exchange between the blood and the gastric cavity. The normal dilution, which proves to be a main factor in the regulation of the intestinal absorption of sugar, is no longer achieved.

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Institut de Biologie, Paris (France), le 20 décembre 1960.

[7] M. Lourau, Exper. *15*, 192 (1959).
[8] P. Meyniel, Thèse de Doctorat en médecine (Bordeaux 1950), (Drouillard Ed.).
[9] J. N. Hunt et I. MacDonald, J. Physiol. *126*, 459 (1954).

PRO LABORATORIO

Utilization of the pH-Stat in the Study of Cellular Metabolism

The buffering properties of the systems employed in the study of cellular metabolism prevent appreciable changes of hydrogen ion concentration in the medium; therefore, in their presence, we cannot recognize directly the release of acid radicals and in brief the appearance of the protons produced by the cells as terminal step of their metabolic process.

In this note we report preliminary results on the study of cellular metabolism carried out on the basis of changes of hydrogen ion concentration in unbuffered solutions, as measured by an automatic titrator adapted as pH-stat[1].

We made use of a Titrigraph mod. TTT 1 A (Radiometer Copenhagen N.V. Denmark) with some modifications. The titration chamber was immersed in a thermostatically controlled bath; it was closed by a loose rubber stopper through which passed the two pH electrodes, the alkali delivery tip, the gas delivery tip and the glass rod of an electric stirrer. A low stirring speed of about 150 r.p.m. was choosen, to avoid damaging the cells. A magnetic stirrer could alternatively have been used. A hole in the stopper made possible the withdrawal of samples during the experiments.

These preliminary experiments were carried out with Yoshida's ascites cells and rat liver slices.

[1] C. F. Jacobsen and J. Léonis, C. R. Trav. lab. Carlsberg, Sér. chim. *27*, 333 (1951).

The ascitic fluid was suspended in 5 vol of isotonic saline solution and centrifuged twice at 4°C for 10 min at $350 \times g$, discarding the supernatant. The cells were then resuspended in the following medium: NaCl 0.154 Mol/100, KCl 0.154 Mol/4, $CaCl_2$ 0.11 Mol/3, $MgSO_4$ 0.154 Mol/1, KH_2PO_4 0.20 Mol/0.5, NaOH 0.1 *N* added until pH 7.4.

After centrifuging twice, the cells were resuspended in the same medium in the proportion of 1 vol packed cells to 5 vol of medium. The packed cell volumes were determined in a hematocrit after centrifuging for 20 min at $2500 \times g$. In some instances, dry weight determinations were also made. The liver slices were withdrawn and prepared in the usual way[2]. The titration vessel usually contained 12 ml of medium and 3 ml of cellular suspension (total number of cells about 150×10^6) or 15 ml of medium and about 60 mg of liver slices (dry weight). The solution was adjusted to the desired value of pH 7.4 with small quantities of NaOH N/50, and the experiments started as soon as temperature equilibrium was reached (2–3 min). The temperature of the bath was maintained at 37°. The concentration of the alkali used in the titration was of 0.02 normal, sufficiently low to avoid appreciable cell damages.

Before introduction of the cells, the liquid medium was saturated with gas and during the course a stream of a gas was blown over the surface of the liquid. Bubbling was avoided to prevent foaming.

Since the lysis of cells releases materials with buffering properties, we checked frequently the amount of lysed cells at the end of the experiments. The optical densities at 260 and 280 mμ showed that the percentage of cells lysed was close to that observed in the Warburg vessels (about 1.5%). The rate of cellular respiration, in absence of any substrate, is given by the amount of alkali required to neutralize the protons derived from the first dissociation of carbonate according to the reaction: $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$ and this is recorded by the pH-stat.

The Figure 1 shows a typical titration curve obtained with Yoshida's ascites cells in the presence of oxygen. When ascites cells are tested in presence of a substrate (glucose) under aerobic conditions, there are two sources of protons, carbonate and lactic acid. In absence of oxygen, there is only one source, namely lactic acid (Figure 2).

In the case of aerobic glycolysis, we made a correction of the amount of alkali required to neutralize the protons derived from carbonate, on the basis of the Crabtree effect (Figure 1 – dashed line). Since this correction was found to be very constant in different experiments, this value needs to be determined by the manometric measurements only once for the system under investigation.

The quantities of lactic acid released during the glycolysis, as estimated by colorimetric[3] and enzymatic[4] methods, are very close to those calculated from the equivalents of alkali delivered.

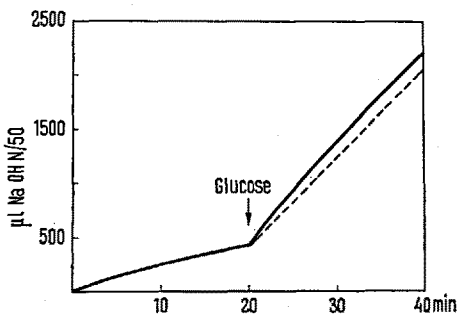

Fig. 1. Titration curve for Yoshida's ascites cells. Experiment No. 12. Gas: oxygen. $T = 37°C$. Cells 97.5 mg dry weight, glucose μMol 210. (The dashed line represents the correction of the glycolysis on the basis of the Crabtree effect (57%).)

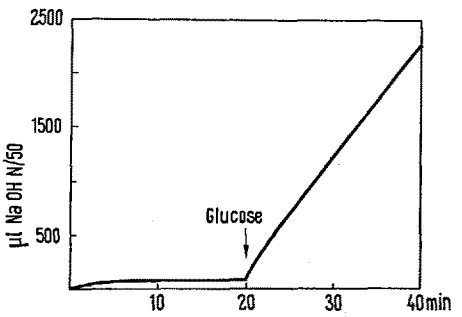

Fig. 2. Titration curve for Yoshida's ascites cells. Experiment No. 12. Gas: argon. $T = 37°C$. Cells 97.5 mg dry weight, glucose μMol 210.

The Table shows data on the respiration and glycolysis of Yoshida's ascites cells and rat liver slices, calculated both from titrimetric and manometric measurements. The values reported above can also be expressed in equivalents of proton neutralized. On this basis, the CO_2 released by Yoshida's ascites cells corresponds to 0.254×10^{-6} g protons/mg/h, the aerobic glycolysis to 1.00×10^{-6} and the anaerobic glycolysis to 1.4×10^{-6} g protons/mg/h from lactic acid.

On the basis of these experiments, it seems that the use of a pH-stat can improve the study of cellular metabolism by making it possible to modify such factors as temperature or the nature of the gas, to introduce different substrates, and to withdraw samples during the course of the experiments. It also greatly increases the speed of the measurements since only a few minutes are required to obtain adequate and reproducible results. The experiments can also be performed with large quantities of material and, since the high sensitivity of the instrument permits the detection of very small quantities of protons, it becomes possible to investigate the first stages of metabolic activity after the addition of a substrate.

	Titrimetric method			Manometric method		
	Q_{CO_2}[a]	μMol *L*(+)lactic acid[b]		Q_{CO_2}[a]	μMol *L*(+)lactic acid[b]	
		I[c]	II[d]		I[c]	II[d]
Yoshida's cells	5.7	1.0	1.4	6.6	1.2	1.4
Rat liver slices	4.06	–	–	4.25	–	–

[a] The amount of CO_2 is given in mm^3 at standard pressure and temperature per mg dry weight of tissue per h.
[b] This value means the μMol of *L*(+)lactic acid produced per mg dry weight of tissue per h.
[c] In the presence of oxygen.
[d] In the presence of argon.

[2] W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques* (Burgess Publishing Co. 1957).
[3] S. B. BARKER and W. H. SUMMERSON, J. biol. Chem. *138*, 535 (1941).
[4] G. PFLEIDERER and K. DOSE, Biochem. Z. *326*, 437 (1955).

Riassunto. È stata studiata la possibilità di seguire con un titolatore automatico impiegato come pH-stat l'andamento della respirazione e della glicolisi di cellule sopravviventi. I valori ottenuti sono in accordo con quelli descritti nella letteratura.

Rispetto al metodo manometrico sono segnalati alcuni vantaggi quali la rapidità delle determinazioni, la possibilità di variare più parametri sperimentali nel corso dell'esperienza nonchè la facilità di prelievo di materiale durante l'esperimento.

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Istituto Regina Elena per lo Studio e la Cura dei Tumori, Roma (Italy), March 6, 1961.

STUDIORUM PROGRESSUS

An Approach to Molecular Dynamics of Chemical Reaction

According to the theory of transition state[1], the reaction rate constant can be expressed as

$$k = \varkappa(kT/h)\ \{Q_{(t)}/Q_{(i)}\}\ \exp\ (-\Delta U_i/kT), \qquad (1)$$

where $\varkappa$ is the transmission coefficient, kT the statistical temperature which is the absolute temperature T multiplied by Boltzmann's constant k, h Planck's constant, $Q_{(t)}$ the partition function of the so-called transition state, $Q_{(i)}$ the partition function of the initial molecular system, and ΔU_i is the activation energy. The chemical reaction has been classified by HORIUTI into the two types, the one being an effusion-type and the other a diffusion-type, by the relations of the transmission coefficient $\varkappa \simeq 1$ and $\varkappa \ll 1$, respectively[2].

However, $\varkappa$ is a correction factor brought somewhat formally into the expression of rate constant. For the reaction of effusion-type ($\varkappa \simeq 1$), it can be considered that the transition state method of Eyring's is a good approach. But it must be supposed that the Eyring's theory is inapplicable to the reaction of diffusion-type, because the method is based on the equilibrium model of chemical reaction whose original concept can be found in the classical treatment of ARRHENIUS'[3]. Therefore, it will be desirable to deal with the problem from the more general point of view.

This paper describes an approach to the non-equilibrium model of chemical reaction from the molecular dynamical theory. One of our purposes in this work is to find the mutual relationships among the several theoretical treatments of reaction rate from a unified and general situation.

In the approach based on the non-equilibrium model, the problem is reduced to the diffusion problem of particles over a potential barrier from a potential-hollow corresponding to the initial configurations of reaction system to another hollow belonging to the final configurations of product system on the adiabatic potential energy surface[4]. If the degree of freedom of the reacting molecular system is given by f, the transition (hyper-) surface of the dimension $(f-1)$ can be defined in the vicinity of the 'watershed' of a potential barrier. A profile of the potential energy surface in the two dimensional case is shown diagrammatically in Figure 1. Our interest is focused upon the diffusion flux of the representative points of reaction system across the transition surface in phase space as a consequence of Brownian motion. It may be safely said that Brownian motion in the molecular level is responsible for diffusion process in the macroscopic level, in a broad sense. As shown in Figure 2, the behaviour of the flow of the representative points can be likened to the motion of Brownian particles as a whole. Such a Brownian particle-like motion results from the irregularly fluctuating forces due to the interaction between the reaction system and the surroundings. The reacting system is presumed to accept or lose energy through the fluctuating forces from the surroundings. Then the passage of the reacting system in the vicinity of the top of potential barrier to the final state taking the molecular configurations of product can be achieved by means of a large number of fluctuations.

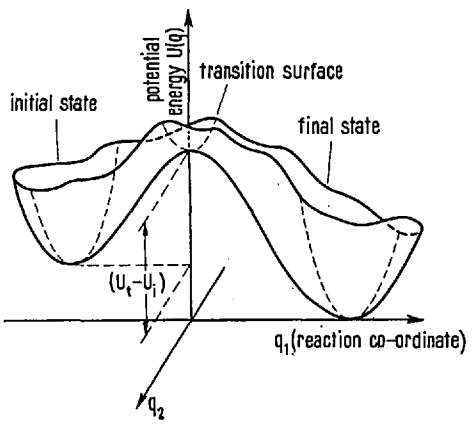

Fig. 1. A profile of two-dimensional potential energy surface.

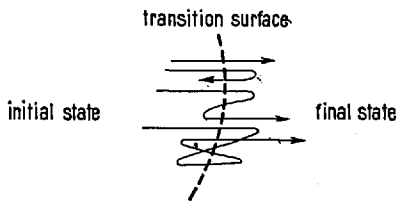

Fig. 2. Brownian particle-like motion of the representative points in the neighbourhood of the transition surface.

In the f-dimensional phase space, the co-ordinates of position are called $(q_1, q_2, \ldots, q_f)$, the conjugate momenta are $(p_1, p_2, \ldots, p_f)$, and the reduced masses with regard to those degrees of freedom are $(M_1, M_2, \ldots, M_f)$, respectively. The co-ordinates are chosen so that these normalize each other in the neighbourhood of the transition surface, and q_1 is selected as the reaction co-ordinate.

The Hamiltonian H_r of the reaction system is given by

$$H_r = \sum_{\alpha=1}^{f} p_\alpha^2/2\,M_\alpha + U(q_1, q_2, \ldots, q_f), \qquad (2)$$

where $U(q_1, q_2, \ldots, q_f)$ is the potential energy of the system.

The potential energy of the system is developed in the power series of the displacements around the saddle-point in the transition surface, whose molecular configuration is specified by suffix t, as the following:

[1] S. GLASSTONE, K. J. LAIDLER, and H. EYRING, *Theory of Rate Processes* (McGraw-Hill, New York 1941).

[2] J. HORIUTI, Pap. Inst. Phys. Chem. Res. Tokyo *34*, 1174 (1938); Bull. Chem. Soc. Japan *13*, 210 (1939).

[3] S. ARRHENIUS, Z. phys. Chem. *4*, 226 (1889).

[4] H. A. KRAMERS, Physica *7*, 284 (1940).