2-dimensional electrophoresis, agar to starch-gel, method [7], (p. 129). All bands separated by agar-gel, except No. 9, appeared in starch-gel on an oblique straight line passing through the origin (Figure 3). This would indicate that component 9 is of different molecular weight and/or shape from the other ones. The fact that all fractions separated on agar-gel reappear when applied on starch-gel shows that none of the bands of this stage can be due to interactions with the electrophoretic medium. It also follows from this result that, apart from difference in number, bands on starch-gel and agar-gel cannot be compared directly.

At this stage of the investigation it is impossible to present any hypothesis which would incorporate these results within the framework of the general subunit theory of LDH-structure. Further extensive experiments with bidimensional electrophoresis, tests on differential inhibition and thermal stability of the isoenzymes and dissociation-recombination experiments[12] are envisaged, and these should eventually allow an interpretation of the multiplicity of Xenopus-LDH[13].

Zusammenfassung. Das Isoenzymmuster der Laktatdehydrogenase (LDH) wurde mittels Agar-Gel- und Stärke-Gel-Elektrophorese untersucht. Unbefruchtete Eier und Entwicklungsstadien vom 2-Zell-Stadium bis zum 15. Tag wurden als Totalextrakte analysiert; vom 15. Tag an wurden einzelne Organe geprüft und bis ins Adultstadium verfolgt. Die Entwicklung der LDH-Muster ist gekennzeichnet durch Zu- und vorübergehende Abnahme der Anzahl Isoenzyme und durch Verlagerungen der Aktivitäts-Intensitäten. Adultorgane weisen 7 Isoenzyme auf mit Stärke-Gel-Elektrophorese und deren 9 mit der Agar-Gel-Methode; dies steht im Gegensatz zu Untersuchungen an den übrigen Vertebraten-Gruppen, die in der Regel ein LDH-Muster von 5 Komponenten aufweisen.

YVETTE W. KUNZ and J. HEARN

Department of Zoology, University College, Dublin (Ireland), 30th March 1967.

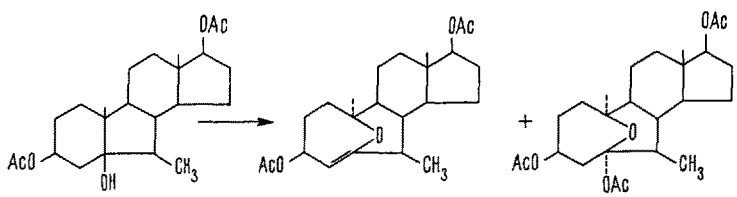
[12] A. BLANCO and W. H. ZINKHAM, Science *139*, 601 (1963); W. ZINKHAM, Science *142*, 1303 (1963).

[13] The authors are much indebted to Professor Dr. R. J. WIEME, Ghent, for demonstrating his agar-gel method and allowing them to work in his laboratory.

DISPUTANDUM

The Mechanism of the Lead Tetraacetate Oxidation of a B-Norsteroid

It has recently been shown[1] in our laboratories that the B-norsteroid I is oxidized with lead tetraacetate to form the bridged oxides II and III.



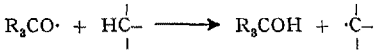
The direct formation of enol ethers or hemiketal acetates from tertiary monohydric alcohols by lead tetraacetate oxidation is unprecedented, and we should like here to present a brief review of the literature pertinent to this reaction, with some of our ideas regarding possible mechanisms for this transformation.

There is general agreement that the first stage in the oxidation of monohydric alcohols with lead tetraacetate is the formation of lead triacetate alkoxides[2], although such intermediates have rarely been isolated[3].

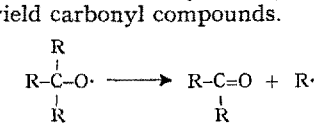
The alkoxide may decompose by either homolytic or heterolytic fission of the O-Pb bond. In the case of primary or secondary alcohols in polar solvents, heterolytic fission with concomitant elimination of a proton to form a carbonyl compound is the favored pathway[4]. On the other hand, in benzene solution, lead alkoxides of tertiary alcohols have been shown to undergo homolytic fission to form alkoxy and lead triacetate radicals[5,6].

Our reaction was carried out using an excess of lead tetraacetate in anhydrous benzene solution at reflux in the presence of calcium carbonate. Under these conditions we may assume that the alcohol I was initially converted to an alkoxy radical[7]. It is the fate of this tertiary alkoxy radical which we wish to discuss.

Aliphatic tertiary alkoxy radicals are known to react by 2 different pathways. The first involves abstraction of a hydrogen atom to reform the tertiary alcohol and to give a new radical.

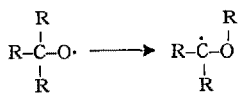


If this reaction occurs with the solvent, the starting alcohol is regenerated and can react anew to form a lead alkoxide. Where sterically feasible, intramolecular hydrogen atom transfer from carbon to oxygen is a favored pathway and the resulting radical alcohol may be transformed into a cyclic ether[8]. A second and more common route for reactions of tertiary alkoxy radicals is by β cleavage to yield carbonyl compounds.

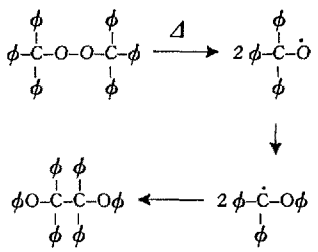


This process, which can occur concomitantly with intramolecular hydrogen abstraction, is generally favored with tertiary alcohols, and is less prevalent in secondary alcohols, although the factors which influence the ratio of abstraction to fragmentation are not completely understood (GREENE et al.[8]). In polycyclic systems, strain in both the starting alcohol and in the products can strongly influence this ratio (CAINELLI et al.[5]). The fragmentation process is reversible and can result in a change in the stereochemistry of both the α and the β carbon atoms of the alcohol[9].

A third possibility for reaction of tertiary alkoxy radicals is rearrangement by 1,2 migration. Although this



reaction has not been observed previously in aliphatic cases, corresponding aromatic examples are known. Triphenylmethoxy radical readily undergoes 1,2 aryl migration[10]. The driving force for this reaction is the stabiliza-



tion of the rearranged radical by resonance and possibly also the relief of strain. We wish to examine our reaction in the light of the above possibilities.

On homolytic cleavage of the lead alkoxide of alcohol I (Chart I), the alkoxy radical IV is generated. Models indicate that there are no hydrogen atoms near enough to the oxygen radical for reaction by intramolecular transfer. Reaction of IV with the solvent, benzene would regenerate the starting alcohol and form a phenyl radical, however, this reaction could not be detected[11].

We visualize 3 possible routes for the formation of the products II and III from radical IV. They will be discussed in turn.

[1] D. ROSENTHAL, C. F. LEFLER and M. E. WALL, Tetrahedron Lett. 3203 (1965); Tetrahedron *23*, in press (1967).

[2] R. CRIEGEE, in *Newer Methods of Preparative Organic Chemistry* (Academic Press, New York 1963), vol. II, p. 36.

[3] The isolation of lead hydroxy methoxy diacetate from methanol and lead tetraacetate has been reported by R. CRIEGEE, L. KRAFT and B. RANK, Justus Liebigs Annln Chem. *507*, 199 (1933).

[4] M. Lj. MIHAILOVIĆ, Z. MAKSIMOVIĆ, D. JEREMIĆ, Ž. ČEKOVIĆ, A. MILOVANOVIĆ and Lj. LORENC, Tetrahedron *21*, 1395 (1965).

[5] K. HEUSLER and J. KALVODA, Angew. Chem., Int. Edn *3*, 525 (1964); G. CAINELLI, B. KAMBER, J. KELLER, M. Lj. MIHAILOVIĆ, D. ARIGONI and O. JEGER, Helv. chim. Acta *44*, 518 (1961).

[6] It is possible that the lead atom plays a role in these mechanisms by complexing with the organic intermediates at certain stages. Our only attempt thus far to assess the importance of this factor was an unsuccessful effort to prepare the alkoxy radical corresponding to I from its hypochlorite. The reaction of I with chlorine monoxide or with hypochlorous acid resulted in the isolation either of the starting alcohol or polychlorinated products.

[7] This assumption is supported by the fact that when the alcohol I was irradiated at room temperature in benzene solution in the presence of lead tetraacetate, the enol ether II was formed. The reason that very little III was isolated can be explained by the fact that in the photolysis reaction the concentration of both reactants was very low. Since the reaction proceeds both thermally and photolytically, it is implied that alkoxy radicals rather than alkoxy cations are intermediates (J. KALVODA and K. HEUSLER, Chemy Ind. 1431, 1963).

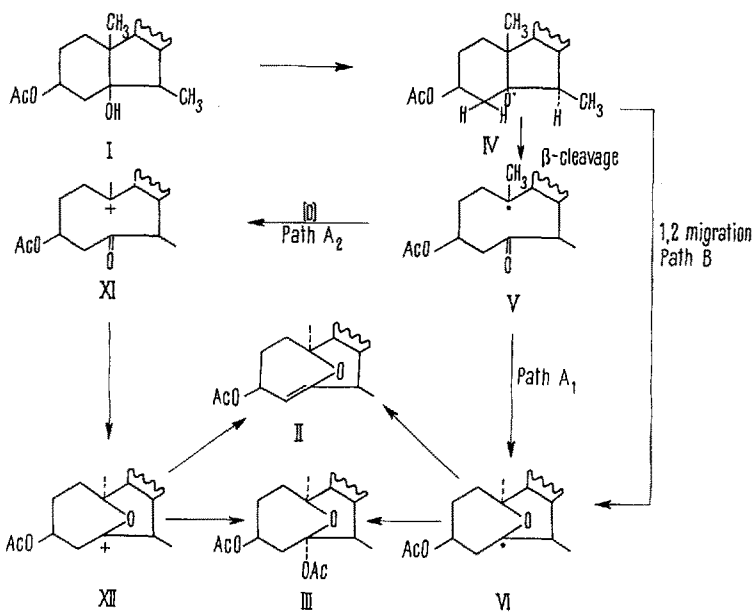
[8] G. CAINELLI, B. KAMBER, J. KELLER, M. Lj. MIHAILOVIĆ, D. ARIGONI and O. JEGER, Helv. chim. Acta *44*, 518 (1961); F. D. GREENE, M. L. SAVITZ, F. D. OSTERHOLTZ, H. H. LAU, W. N. SMITH and P. M. ZANET, J. org. Chem. *28*, 55 (1963).

[9] K. HEUSLER, J. KALVODA, G. ANNER and A. WETTSTEIN, Helv. chim. Acta *46*, 352 (1963).

[10] H. WIELAND, Ber. dt. chem. Ges. *44*, 2550 (1911); M. S. KHARASCH, A. C. POSHKUS, A. FONO and W. NUDENBERG, J. org. Chem. *16*, 1458 (1951).

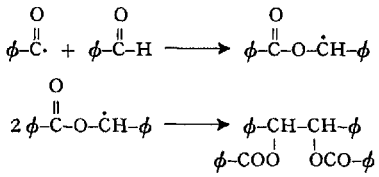
[11] It has been reported that the principal product of the reaction of phenyl radicals in benzene solutions is biphenyl[12], however, no trace of this compound could be detected in the non-polar fractions of the reaction mixture.

[12] B. M. LYNCH and K. H. PAUSACKER, Aust. J. Chem. *10*, 40 (1957); D. R. AUGOOD and G. H. WILLIAMS, Chem. Rev. *57*, 123 (1957).

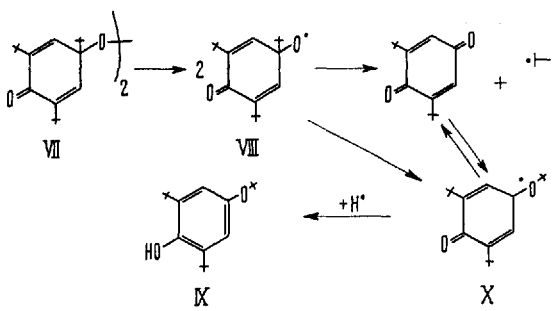


(1) *β-Cleavage – Radical Addition* (Path A_1). The tertiary alkoxy radical IV is bonded to a primary, a secondary, and a tertiary alkyl group. It has been shown that the relative ease of β-cleavage of alkyl groups in alkoxy radicals is in the order tertiary > secondary > primary[13]. Thus the expected cleavage product of IV would be radical V[14]. This radical, on recombination with the oxygen of the carbonyl group, could form the radical VI, which by loss of a hydrogen atom or gain of an acetoxy radical would provide the observed products II and III, respectively. If this mechanism were correct, it would exhibit a number of novel features.

The attack of an alkyl radical on carbonyl oxygen is most unusual. We have found only 2 related examples in the literature. One is the addition of benzoyl radical to benzaldehyde[15]. The second is the thermal decomposition



of the peroxide VII[16] to form a mixture of 2,6-di-t-butylbenzoquinone and the phenolic ether IX. The



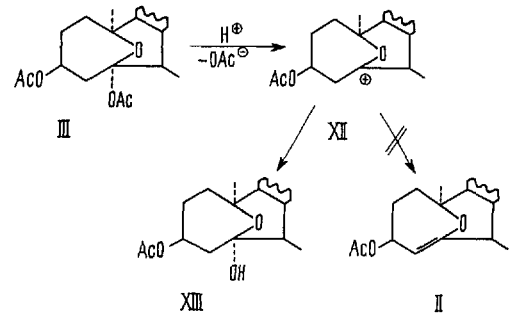
mechanism of this reaction is not known in detail, but the results can be explained by assuming that the alkoxy radical VIII formed by the cleavage of the peroxide breaks down to give the quinone and t-butyl radical which recombine to form the intermediate X[17]. Radical X may, however, also be formed directly from VIII by a 1,2 migration.

In any event both of these examples involve cases where the carbonyl group attacked is non-enolizable, and where the radical resulting from the addition is stabilized by being either benzylic or doubly allylic. Neither of these situations obtains in our case. The normal mode of reaction of alkyl radicals in the presence of an enolizable ketone is by hydrogen atom abstraction with the formation of an enolate radical[18]. It would therefore be expected that radical V would react by intramolecular hydrogen atom transfer from C-4 to C-10 or by loss of a β hydrogen atom to form olefins.

It has been shown[1] that the configuration at C-10 is retained during the oxidation of I. Although the product distribution of radical reactions may be influenced by steric factors[19], models of V indicate that the radical at C-10 can approach the carbonyl oxygen atom equally well from either face of the molecule. The alternative products with α oxide orientations are also sterically quite feasible. If the reaction does proceed by this mechanism, an unusually high degree of asymmetric induction would have to exist to account for the single configuration at C-10 in the products.

(2) *β-Cleavage-Ionic addition* (Path A_2). In a variant of the above mechanism the initial β-cleavage product V is oxidized by lead triacetate radical to the keto carbonium ion XI. This ion would be expected to react easily to form the bridged cation XII which would then either lose a proton or react with acetate ion to yield the products II and III, respectively.

This mechanism may be criticized for the following reason. In the course of our work[1] we showed that the ion XII is actually generated during the acid catalyzed solvolysis of III. This ion proceeds exclusively to the hemiketal XIII and shows no tendency to lose a proton to form the enol ether II[20].



It is of course true that the lead tetraacetate oxidation was carried out in an aprotic solvent and that the species XII generated under these conditions may react differently from a similar cation generated in aqueous medium. Still, this point would need to be clarified before acceptance of this mechanism, as would be the problem of the stereochemistry at C-10, mentioned before in connection with path A_1.

(3) *1,2 migration* (Path B). We wish to suggest that in the specific case under discussion, the reaction may proceed by a direct one-step migration of the 5,10 bond of the alkoxy radical from C-10 to oxygen, i.e. a direct rearrangement of IV to VI. In all aliphatic tertiary alkoxy radicals heretofore studied, 1,2 migration has not been observed. WALLING has stated[21], 'Extensive data on the

[13] J. D. BACHA and J. K. KOCHI, J. org. Chem. *30*, 3272 (1965); C. WALLING and A. PADWA, J. Am. chem. Soc. *85*, 1593 (1963).

[14] Electronic factors are not the sole determinants of the mode of cleavage. In certain strained cyclobutanol systems, the normal direction of cleavage is reversed and primary groups are extruded in preference to secondary groups. M. AMOROSA, L. CAGLIOTI, G. CAINELLI, H. IMMER, J. KELLER, H. WEHRLI, M. Lj. MIHAILOVIĆ, K. SCHAFFNER, D. ARIGONI and O. JERGER, Helv. chim. Acta. *45*, 2674 (1962); J. FRIED and J. W. BROWN, Tetrahedron Lett. 1677 (1966).

[15] F. F. RUST, F. H. SEUBOLD and W. E. VAUGHAN, J. Am. chem. Soc. *70*, 3258 (1948).

[16] C. D. COOK, R. C. WOODWORTH and P. FIANU, J. Am. chem. Soc. *78*, 4159 (1956).

[17] W. H. STARNES, JR. and N. P. NEUREITER, J. org. Chem. *32*, 333 (1967).

[18] E. W. R. STEACIE, *Atomic and Free Radical Reactions* (Reinhold Publishing Corp., New York 1954), Chaps. IV and V.

[19] F. D. GREENE, C.-C. CHU, and J. WALIA, J. org. Chem. *29*, 1285 (1964); P. S. SKELL and P. D. READIO, J. Am. chem. Soc. *86*, 3334 (1964).

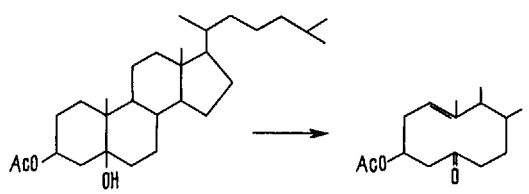
[20] Compound II was shown independently to be involved in an alternate ionization to form an allylic carbonium ion with very different solvolysis products. The distinction between the cations derived from β alkoxy allylic alcohols by the protonation of the α carbon atom and the ion obtained by protonation of the hydroxyl group has been made previously. E. WENKERT and D. P. STRIKE, J. Am. chem. Soc. *86*, 2044 (1964).

[21] C. WALLING, in *Molecular Rearrangements* (Ed. P. DE MAYO; Interscience, New York 1963), p. 418.

decomposition of tert-alkoxy radicals, where the substituents are alkyl, show a breakdown to ketone and an alkyl radical rather than alkyl migration.' Even so, we feel that in our system special circumstances exist which may favor a 1,2 migration mechanism.

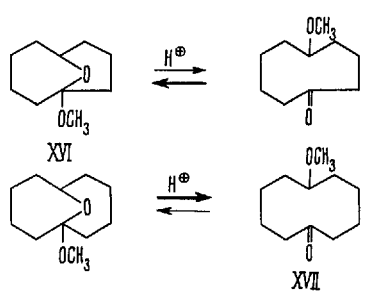
Simple molecular orbital theory predicts that 1,2 migrations in radicals would be less favored than in the corresponding cations because in the transition state the additional electron of the radical must be located in a non-bonding molecular orbital, with the result that the energy required to go to the transition state is greater in the radical than in the cation. We believe that in our case, because of strain, the ground state of the starting alcohol I and its derived alkoxy radical are of sufficient energy to allow radical migration to occur. This is mainly due to the five-membered B ring which is joined to 2 six-membered rings, one by a trans fusion. It is also possible that the presence of the 3 vicinal β substituents at C-10, C-5, and C-6, along with the 3β-acetoxy group adds additional strain to the molecule. The rearranged product, on the other hand, has oxygen containing six- and seven-membered rings, each in a stable chair conformation. The release of strain going from IV to VI may provide the needed driving force for this reaction.

In the case of a closely related, but less strained steroid alcohol, lead tetraacetate oxidation takes a different course. MIHAILOVIĆ and co-workers recently reported[22] that 5β-cholestane-3β, 5β-diol, 3-acetate (XIV) reacts with lead tetraacetate under conditions identical with ours to form a mixture of cis-trans keto olefins XV. Similar results were also observed using mercuric oxide[23].



XIV XV

The difference in behavior of the normal and B-nor steroid alcohols may be explained as follows: it is known that there is a distinct difference in the tendency of nine- and ten-membered rings to form bridged compounds. In nine-membered rings, bridging, where possible, is distinctly favored. For example, when 5-hydroxycyclononanone[24] and 6-hydroxycyclodecanone[25] are treated with methanol and acid, the former forms the bridged methyl ether (XVI) while the latter gives the open-ring keto ether (XVII).

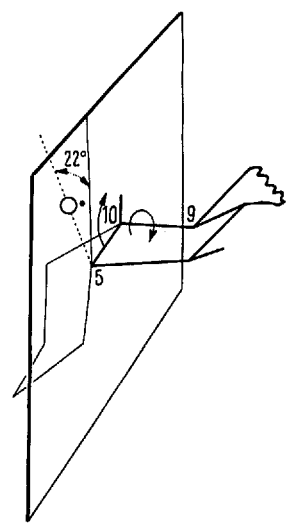


XVII

The particular facility of nine-membered rings to bridge in preference to forming open-ring tautomers may be further seen in compound XIII which does not give a semicarbazone and which exists exclusively (by IR) as a hemiketal.

It may therefore be argued that in the B-nor series, rearrangement of IV to VI by 1,2 migration completely avoids the high energy intermediate V, while in normal steroids the ketone form corresponding to V is more favored and the observed products reflect this difference.

By assuming a direct migration mechanism another possible explanation for the differences observed between the five- and six-membered B ring cases suggests itself. Models show that in the radical IV, movement of the 5,10 bond is restricted to the vicinity of a plane[26] which is swept out by the rotation of C-10 about the 9-10 bond. In intermediate IV the oxygen radical lies only 22° from this plane. On the other hand, when the B ring is six-membered, this angle is 60°. Thus, 1,2-migration in IV would require far less distortion of normal bond angles than would be necessary in normal steroids, resulting in a transition state of lower energy.



In contrast to the mechanism involving the ketone intermediates V or XI, a direct migration mechanism would predict retention of configuration at C-10, which explains the observed R configuration at C-10 in the products II and III.

After 1,2 migration, the product, radical VI, would then be expected to react irreversibly[27] by the loss of a hydrogen atom at C-4 or by capture of an acetoxy radical to form the products II and III respectively.

In conclusion, we must emphasize that at this time we are able only to outline the alternative mechanisms. While it is tempting to suggest that this reaction is the first bona fide example of a 1,2 alkyl migration in an alkoxy

[22] M. Lj. MIHAILOVIĆ, Lj. LORENC, M. GAŠIĆ, M. ROGIĆ, A. MELERA and M. STEFANOVIĆ, Tetrahedron *22*, 2345 (1966).
[23] M. AKHTAR and S. MARCH, J. chem. Soc. c, 937 (1966).
[24] R. CRIEGEE and H. ZOGEL, Ber. dt. chem. Ges. *84*, 215 (1951).
[25] R. CRIEGEE and W. SCHNORRENBERG, Justus Liebigs Annln Chem. *560*, 141 (1948).
[26] This surface is actually a cone, however, for small displacements it approximates a plane.
[27] The possibility that the 2 products II and III were interconvertible was excluded by showing that each product was stable to the reaction conditions.

radical, a clear-cut choice between the aforementioned possibilities must await further work[28].

[28] We wish to acknowledge the support of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, USA under Contract No. SA-43-ph-4351. We should also like to thank Dr. J. KALVODA for stimulating discussions.

Zusammenfassung. Für die Bildung neuartiger Oxydationsprodukte, die bei der Reaktion eines B-Norsteroidalkohols mit Blei-tetraacetat entstehen, werden zwei Mechanismen vorgeschlagen: Die bekannte Spaltungs-Additions-Reaktion eines Alkoxy Radikals und die bisher unbekannte Umlagerung eines Alkyl-tertiäralkoxy Radikals.

DAVID ROSENTHAL

Research Triangle Institute, Research Triangle Park (North Carolina 27709, USA), 28th March 1967.

PRO EXPERIMENTIS

L'absorption intestinale de l'eau chez le lapin

Les recherches sur l'absorption gastrointestinale de l'eau[1,2] ont démontré, entre autres, que l'intestin grêle absorbe beaucoup plus rapidement que l'estomac[3,4] et que 99% de l'eau absorbée emprunte la voie porte[1,5]. Malgré la rapidité avec laquelle l'eau est absorbée, il reste que la lumière du tractus gastro-intestinal contient toujours une certaine quantité d'eau qui, chez le lapin, représente environ 12% de l'eau corporelle totale[5]. Ce % demeure essentiellement inchangé après un jeûne complet de 24 h[6]. Utilisant l'oxyde de deutérium, GOTCH et al.[7] ont trouvé que 2% de l'eau corporelle totale se trouve dans l'intestin grêle. On a aussi démontré que l'épithélium intestinal laisse passer l'eau dans les deux sens[1,2].

Malgré les nombreuses connaissances déjà acquises sur ce sujet, il nous a paru opportun d'étudier l'absorption intestinale de l'eau à l'aide d'une technique simple, rapide et très efficace. Cette technique nous permet de suivre l'absorption in vivo et d'établir une équation à partir de laquelle il est possible de calculer la quantité moyenne d'eau transférée en fonction du temps.

Matériel et méthodes. Nous utilisons des lapins albinos mâles de la souche Nouvelle-Zélande, pesant entre 2270 et 3160 g. Nous les mettons à la diète hydrique 24 h avant l'expérience et à diète absolue 12 h plus tard. Nous anesthésions l'animal à l'aide de Nembutal sodique injecté dans la veine marginale de l'oreille (30–50 mg/kg). Ensuite les 2 veines fémorale et porte sont cathétérisées suivant la technique déjà décrite[8,9]. Après nous être assurés du bon écoulement du sang dans les cathéters, nous injectons dans le duodénum 2 ml de sérum physiologique contenant 30 μCi d'eau tritiée. Nous prélevons simultanément les échantillons sanguins des 2 veines à l'aide de seringues tuberculines aboutées aux cathéters. Avant chaque prise, nous prenons soin d'enlever de chaque cathéter le sang qui s'y trouve déjà (0.17 ml), prélevons immédiatement 0.35 ml avec une nouvelle seringue et retournons ensuite dans la circulation le sang préalablement retiré du cathéter. Nous centrifugeons l'échantillon sanguin et mesurons, suivant la méthode déjà décrite[10], l'activité de 100 μl du sérum surnageant.

Résultats et discussion. Nous considérons que l'activité mesurée dans le sérum fémoral traduit l'activité, dite résiduelle, au moment de la prise, alors que l'activité mesurée dans le sérum porte est la somme de cette activité résiduelle et de l'activité apportée par le transfert intestinal[9]. Nous pouvons donc suivre les variations de ces activités fémorale et porte en fonction du temps, comme le font voir les deux courbes de la Figure 1, obtenues chez un animal. A part quelques modifications des valeurs des activités, nous retrouvons des courbes de

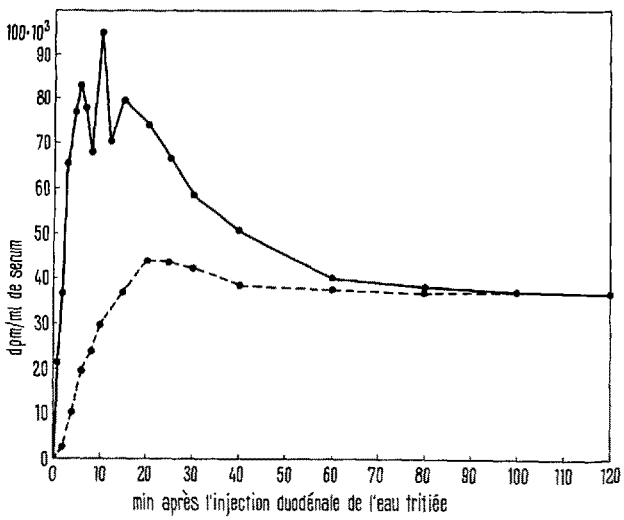


Fig. 1. Courbe-type représentant les variations temporelles de l'activité dans le sérum du sang porte (●——●) et dans le sérum du sang fémoral (●----●) chez un animal.

[1] T. H. WILSON, *Intestinal Absorption* (Saunders Company, Philadelphia 1962).
[2] G. WISEMAN, *Absorption from the Intestine* (Academic Press, New York 1964).
[3] J. F. SCHOLER et C. F. CODE, Gastroenterology *27*, 565 (1954).
[4] P. R. LEE, C. F. CODE et J. F. SCHOLER, Gastroenterology *29*, 1008 (1955).
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