Die Angaben von WALDO und WIMSATT[2] deuten für die Kontrollgruppe der von ihnen verwendeten Versuchstiere in die gleiche Richtung. Arbeiten von NICHOLAS und SEIDEL haben die hohe Regulationsfähigkeit von Blastomeren des Ratten- bzw. Kaninchenkeimes ergeben, andererseits zeigen die Ergebnisse von DALCQ und seiner Schule schon am befruchteten Ei Bezirke bestimmter Differenzierung.

Herrn Prof. Dr. H. LETTRÉ danke ich für die Förderung der Arbeit.

H. WRBA

Institut für experimentelle Krebsforschung der Universität Heidelberg, den 18. Dezember 1955.

Summary

On the fourth day of development of the rat embryo all of the stages are found from the two-cell stage to the blastocele stage even among embryos from the same litter. On the seventh day, they all show egg-cylinder stages without exception. The time interval between cleavages from the second to the sixth day of development is not constant and, in fact, varies over a wide range, even among embryos from the same litter.

[2] C. M. WALDO und W. A. WIMSATT, Anat. Rec. *93*, 363 (1945).

DISPUTANDUM

The Mechanism of Reactions Catalyzed by Thiamine Pyrophosphate

The present article constitutes an attempt to rationalize, from the point of view of organic chemistry, the manifold enzymatic reactions catalyzed by the agency of thiamine pyrophosphate (DPT$^+$). The biochemical elucidation of DPT$^+$ catalyzed oxidative decarboxylation of pyruvate was summarized by GUNSALUS[1] who proposed a scheme shown in the equations (1)–(4) for the generation of acetyl Coenzyme A

$$(\mathrm{CoA{-}S{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}CH_3})$$

from pyruvate.

(1) $\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}O^-} + \mathrm{DPT^+} \rightleftharpoons \left[\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}:DPT}\right] + \mathrm{CO_2}$

(2) $\left[\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}:DPT}\right] + \mathrm{S(\ddot{S})R} \rightleftharpoons \left(\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}{:}S{-}R}\right)^- + \mathrm{DPT^+}$

(3) $\left(\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}{:}S{-}R}\right)^- + \mathrm{CoA{-}SH} \rightleftharpoons$

$$\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}S{-}CoA} + \left(\mathrm{HS{-}R}\right)^-$$

(4) $\left(\mathrm{HS{-}R}\right)^- + \mathrm{DPN^+} \rightleftharpoons \mathrm{S(\ddot{S})R} + \mathrm{DPNH}$

$$R = -(CH_2)_4\text{–}COOH$$

[1] I. C. GUNSALUS, *Mechanism of Enzyme Action* (John Hopkins Press, Baltimore 1954).

According to this scheme, reaction 1 is visualized as a heterolytic cleavage to yield CO_2 and a substance which is regarded by GUNSALUS (probably only formally) as a carbanion $\left(\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}(-)}\right)$ coordinated with DPT$^+$. This compound then reacts in the second step with lipoic acid to regenerate DPT$^+$ and to give the acetylated reduced form of lipoic acid. Acetate is then transferred to CoA–SH and lipoic acid regenerated by oxidation with DPN$^+$.

The intermediate complex of DPT$^+$ and $\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}(-)}$ is also capable of undergoing various other transformations among which the reaction with carbonyl compounds deserves attention:

$$\left[\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}:DPT}\right] + \mathrm{\overset{\overset{\mathrm{O}}{\|}}{C}{-}R} + \mathrm{H^+} \rightleftharpoons \mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}\overset{\overset{\mathrm{OH}}{|}}{CH}{-}R} + \mathrm{DPT^+}$$

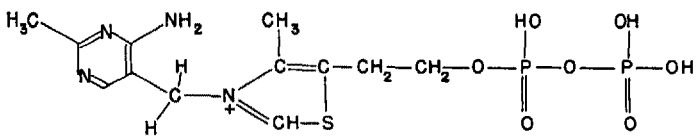
In this manner for instance acetoin may be formed and it has been emphasized by GUNSALUS that this reaction does not require lipoic acid.

We shall now assume that the above scheme is correct and discuss the mechanistic possibilities by which the postulated reactions could be rationalized as well as the nature of the "complex" reactive form of acetaldehyde.

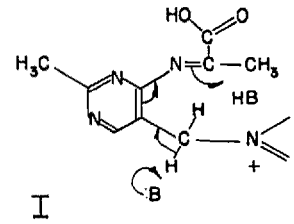
Inspection of reaction (1) reveals that pyruvate does not possess the proper setup for undergoing cleavage into CO_2 and $\mathrm{CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}(-)}$. Consequently, the function of DPT$^+$ must be such as to set the stage for an easy reaction in the manner desired. This cleavage is then catalyzed by the intervention of the respective specific protein.

The formula sequence I–VI shows the way in which this might be accomplished. DPT$^+$ may combine with pyruvic acid to give a Schiff base represented by the partial structure I. In compound I, the two hydrogens attached to the carbon, flanked on one side by the pyrimidine nucleus and on the other by the quaternary nitrogen, are highly activated and one of them may consequently be removed by base. The resulting *ylid* may then undergo an electron shift and a proton may attach itself to the former carbonyl carbon of the pyruvic acid moiety. If this is a concerted process, it may be visualized as represented by the arrows in formula I. An intramolecular process as shown in formula Ia could conceivably be considered as an alternative. The resulting compound II now meets requirements for an easy cleavage of the desired C–C bond in the manner indicated.

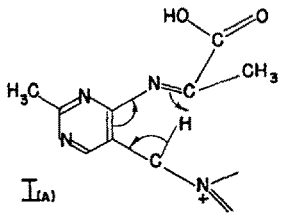
The product of this reaction (III) or rather the *ylid* derived from it by abstraction of one of the activated protons (IV) may now be identified with the complex $\mathrm{(CH_3{-}\overset{\overset{\mathrm{O}}{\|}}{C}:DPT)}$ postulated by GUNSALUS.



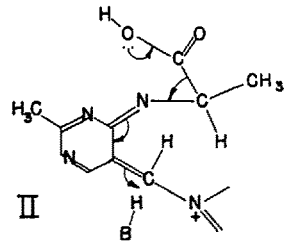
D.P.T. +



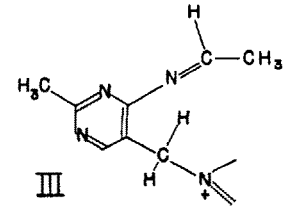
I



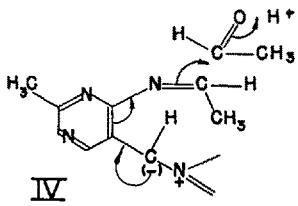
I(A)



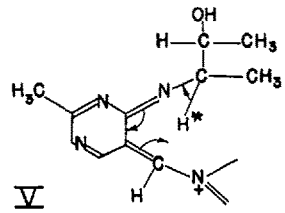
II



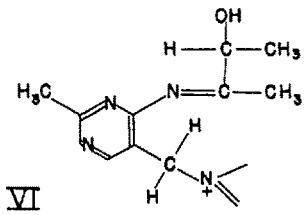
III



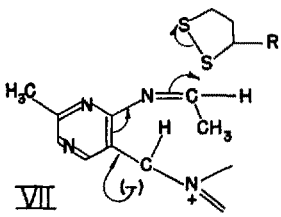
IV



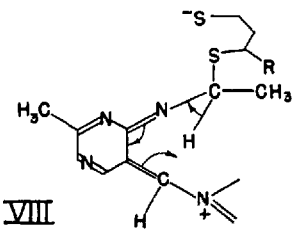
V



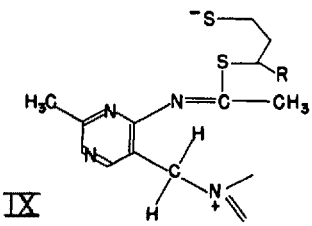
VI



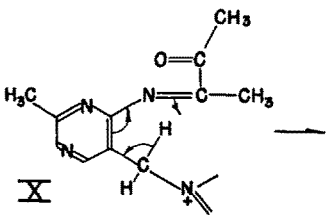
VII



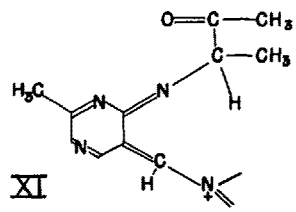
VIII



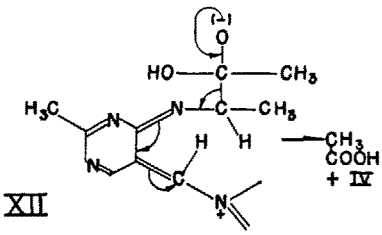
IX



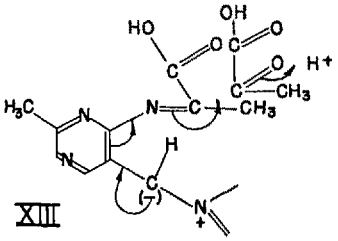
X



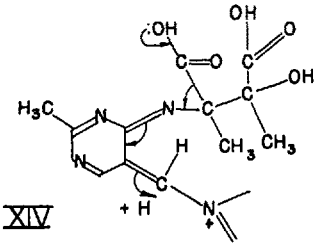
XI



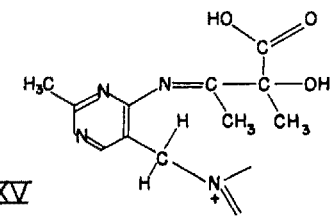
XII + IV



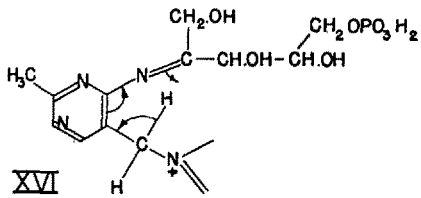
XIII



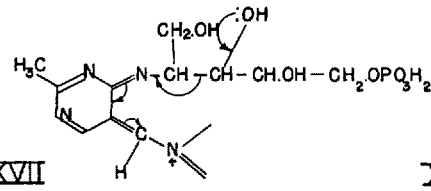
XIV



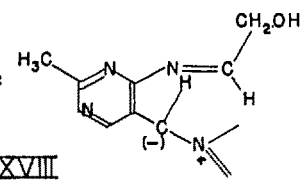
XV



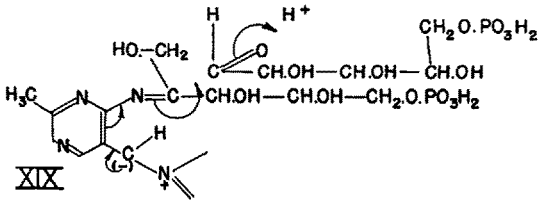
XVI



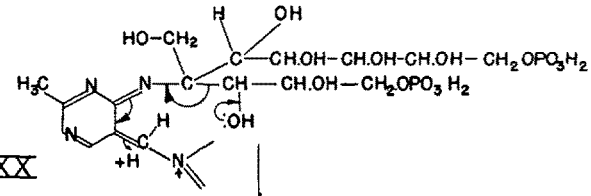
XVII



XVIII



XIX



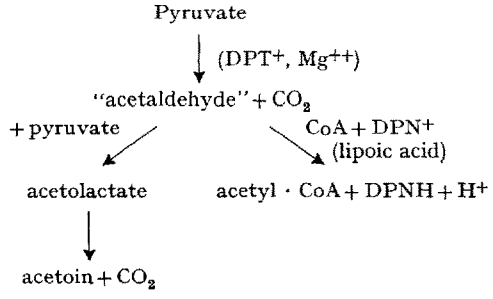
XX

GLYCERALDEHYDE PHOSPHATE + SEDOHEPTULOSE-7-PHOSPHATE + D.P.T. +

A reaction of IV with a carbonyl compound such as acetaldehyde may be easily envisaged and is shown by arrows in formula IV. This results in compound V in which the hydrogen marked by an asterisk is activated since its abstraction results in a mesomeric *ylid*. The compound may consequently undergo a shift of this hydrogen which may take place by the agency of an external base and acid or intramolecularly. Only the second possibility is illustrated by arrows in formula V. The product (VI) may now hydrolyse to DPT^+ and acetoin. The reaction of the (CH_3–CO:DPT) complex with lipoic acid, postulated by GUNSALUS, may now be easily rationalized as represented by the arrows in structure VII. The formation of acetyl lipoic acid *via* VIII and IX is based on considerations so similar to the ones discussed above that the formulae should be self-explanatory.

Another reaction to be considered is the cleavage of diacetyl with DPT^+ acting as acceptor for one half of the molecule while the other half is liberated as acetate[1]. Without going into a detailed explanation, it is obvious from the scheme X → XI → XII → IV that this may be achieved in a manner analogous to the reactions already discussed.

One interesting variation of the mechanisms presented is conceivable and results in greater simplicity of the electron shifts. STRECKER and OCHOA[2] recently studied oxidative decarboxylation and acetoin formation in certain bacteria which proceeded according to the following scheme:

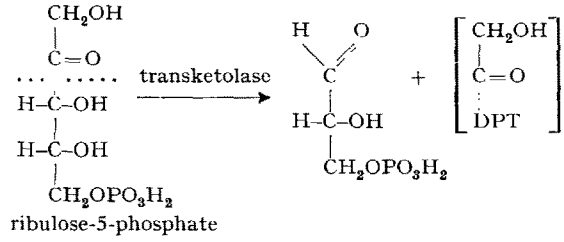


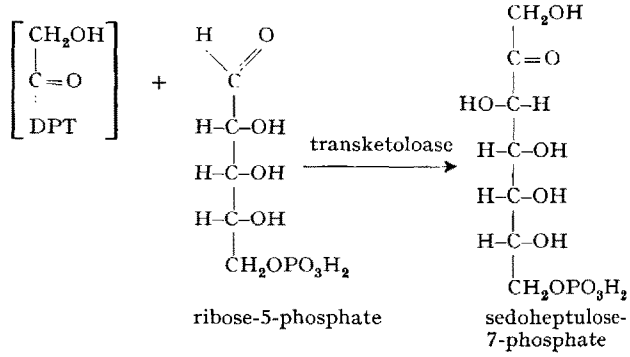
In spite of this scheme, they state later in the paper: "Separate experiments demonstrated that the pyruvate saturation level for acetyl phosphate formation was about 0·05 M while the acetoin-forming system was not yet saturated with 0·3 M pyruvate under the conditions employed ..... The above results do not seem to support the view represented in the scheme that 'acetaldehyde' is produced as free intermediate by a decarboxylating enzyme common to the acetoin and acetyl-CoA-forming systems. As it is difficult to imagine the existence of different products of pyruvate decarboxylation in view of identical cofactor requirements, the decarboxylation might be catalyzed by different proteins to give the same 'acetaldehyde' but this would remain bound to these proteins and undergo further reaction in this form."

While it is indeed difficult to visualize two different "active acetaldehydes" we can see readily (having identified the "acetaldehyde" with the *ylid* IV) that an *ylid* derived from the original Schiff base I and represented by XIII may attack carbonyl groups precisely in the same manner as IV. This would result in an alternate and mechanistically simpler possibility of formation of acetolactate. This is illustrated by the stages XIV and XV which are self-explanatory. Compound XV may then be hydrolyzed to acetolactic acid, which is decarboxylated as a β-keto acid by a separate enzyme.

We should like to point out that all reactions discussed here may be rationalized by such an alternate simpler mechanism in which C–C cleavage *comes in after* and not before the attack on the acceptor molecule. The first representation was chosen in order to stay at present within the limits of the proposals of GUNSALUS.

Another important reaction type catalyzed by DPT^+ is the transketolase reaction[3]. A typical example of this is the following reaction sequence:





A cleavage of ribulose-5-phosphate as indicated by the dotted line again requires cooperation of DPT^+ to give an "active form" of glycolic aldehyde which then reacts with ribose-5-phosphate. The formulation of these changes is analogous to all other DPT^+ catalyzed reactions that we have discussed and will be given only briefly.

The Schiff base of DPT^+ and ribulose-5-phosphate (XVI) may undergo a proton shift to XVII. This shift is arbitrarily formulated as an intramolecular one. Compound XVII is now set up for cleavage of the desired C–C bond as shown by arrows. The product of the electron shift as illustrated is the *ylid* XVIII and glyceraldehyde phosphate. XVIII may now be identified with the "active form" of glycolic aldehyde which will attack the carbonyl group of ribose-5-phosphate in complete conformity with the reactions of the "active aldehyde" IV and give sedoheptulose-7-phosphate.

The alternate mechanism which involves attack on the ribose-5-phosphate aldehyde group prior to cleavage of the C–C bond is illustrated in the structure XIX and XX.

While there does not appear to be enough evidence at present to make a decision, the alternate mechanism seems attractive by reason of its greater simplicity.

[2] H. J. STRECKER and S. OCHOA, J. biol. Chem. *209*, 313 (1954).

[3] For a summarizing reference see E. RACKER, *Advances in Enzymology*, Vol. 15 (Interscience, New York-London 1954), p. 141. – See also E. RACKER, G. DE LA HABA, and J. G. LEDER, J. Amer. chem. Soc. 15, 1010 (1953). – B. L. HORECKER and P. Z. SMYRNIOTIS, J. Amer. chem. Soc. *75*, 1009 (1953).

Note added in proof: A prepublication copy of this paper has been sent to Dr. K. BLOCH (Harvard). We wish to thank Dr. BLOCH and Dr. WESTHEIMER for informing us that an identical mechanism for the decarboxylation reaction of pyruvate has been proposed in a discussion by R. B. WOODWARD, W. S. JOHNSON, J. BADDILEY, and F. H. WESTHEIMER.

K. WIESNER and Z. VALENTA

Chemistry Laboratory, University of New Brunswick, Fredericton, Canada, December 1, 1955.

Zusammenfassung

Es wird ein Mechanismus für verschiedene enzymatische Reaktionen, bei denen Thiaminpyrophosphat als Coferment wirkt, vorgeschlagen.

PRO EXPERIMENTIS

Mesure de l'oxydation phosphorylante à l'aide de phosphore radioactif dans la cellule hépatique

KREBS et ses collaborateurs[1] ont mis au point, en 1953, une technique très élégante utilisant le phosphate inorganique marqué au phosphore radioactif (P^{32}) pour déterminer le quotient d'oxydation phosphorylante, c'est-à-dire le pouvoir de phosphorylation d'un homogénat de tissu animal. Cette méthode, basée sur l'équilibre dynamique existant entre le triphosphate d'adénosine (ATP) et le phosphate inorganique, utilise en fait l'action compétitive de l'oxydation phosphorylante, d'une part, et des phosphatases, d'autre part, selon le schéma suivant:

$$ADP + P_{inorg.} \underset{\text{« ATP'ase »}}{\overset{\text{Ox. ph.}}{\rightleftharpoons}} ATP$$

Nous avons modifié les techniques de dosage établies par KREBS[1], en particulier sa détermination des phosphates marqués au P^{32}, séparés par chromatographie sur papier. Nous nous sommes mis, par contre, entièrement dans ses conditions d'expérience biochimiques. Nous avons préféré mesurer la radioactivité directement sur les chromatogrammes, alors que KREBS élue les différentes fractions phosphates de la bande de papier, et qu'il procède aux examens chimiques et physiques dans les éluats. Nous avons fait passer les chromatogrammes sous un compteur de Geiger, relié à l'intégrateur «Precision Ratemater» de Tracerlab et à un milliampèremètre enregistreur de Trüb & Täuber. Le chromatogramme est entraîné automatiquement par friction à l'aide d'un tambour se trouvant sur la prolongation de l'axe entraîneur du milliampèremètre enregistreur. Le diamètre du tambour entraînant le papier étant le même que celui faisant avancer le papier d'enregistrement, nous avons la même vitesse linéaire pour les deux bandes, ce qui facilite la détermination des r_f des substances séparées; en introduisant des repères, nous pouvons superposer les chromatogrammes et les courbes enregistrées.

Les Figures 1 et 2 nous montrent respectivement l'aspect général du dispositif adopté et le détail de l'entraînement du chromatogramme. Nous pouvons encore voir sur la Figure 1 l'enregistrement d'une courbe, les grands pics représentent l'activité du phosphate inorganique, les petits celle de l'ATP. La planimétrie des surfaces obtenues nous donne la répartition du P^{32} dans les différentes fractions phosphates.

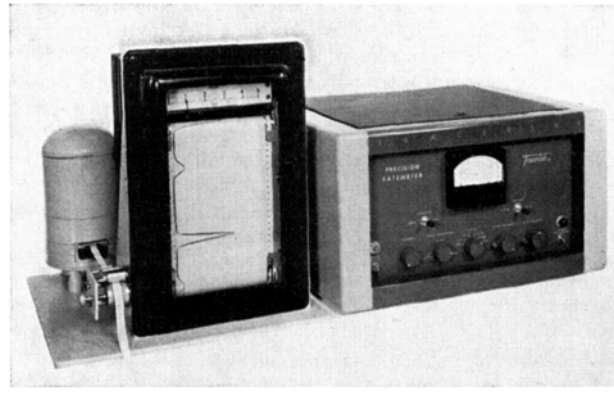


Fig. 1.

Nous avons pu bénéficier pour la mise au point de cet appareillage de l'expérience de l'un d'entre nous[2] qui avait déjà construit précédemment un système semblable pour la mesure de la radioactivité de substances séparées par chromatographie ou par électrophorèse sur

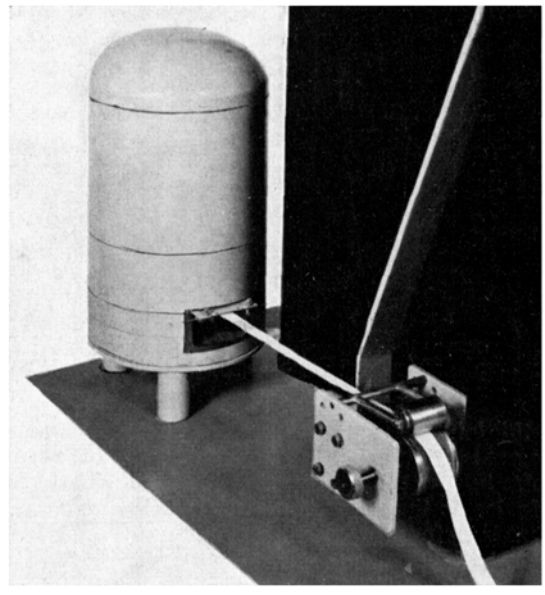


Fig. 2.

papier. L'avantage de l'appareil que nous venons de décrire brièvement, et que nous pouvons faire passer un plus grand nombre de chromatogrammes dans l'appareil (par exemple pendant une nuit et même plus) sans avoir à le recharger, avec un minimum d'encombrement.

[1] H. A. KREBS, A. RUFFO, L. V. EGGLESTON et R. HEMS, Biochem. J. *51*, 107 (1953).

[2] P. LERCH et S. NEUKOMM, Schweiz. med. Wschr. *81*, 515 (1954).