nucleophiles towards the phosphoryl group, except when the leaving group is the anion of a fairly strong acid[5], as in *p*-nitrophenyl phosphate[6]. In the case of cyclic phosphates, it has been observed[7] that glycerol-1,2 cyclic phosphate is unaffected by aqueous ammonia at 100 °C for 1 h. Also, COVITZ and WESTHEIMER[8] have come to the conclusion that nitrogen bases act as general bases rather than nucleophilic catalysts in the hydrolysis of methyl ethylene phosphate. Finally it has been reported[9] that aniline attacks ethyl ethylene phosphate at carbon rather than phosphorus.

(2) The transesterification takes place by attack of the alkoxide anion. Many studies have been made on the nucleophilicity of such anions towards *p*-nitrophenyl-acetate[10], in particular by BRUICE et al.[11]. From these studies it would appear that in an equimolar mixture of two alcohols the more acidic one will give the highest percentage of transesterification because the decrease in nucleophilicity of the anion which accompanies the decrease in pK is more than compensated by the increase in the concentration of this anion. We do not have any precise data on the relative acidities of the hydroxyl groups of choline and ethanolamine. There is evidence that ethanolamine is somewhat more acidic than ethanol[12]. However, the presence of a positively charged nitrogen would also tend to increase the acidity and it seems reasonable to expect that choline is a stronger acid than either $H_3N^+–CH_2–CH_2OH$ or $H_2N–CH_2–CH_2OH$[13].

(3) The reactive species is the alcohol, not the alkoxide anion, and the amino group acts as a general base catalyst. Such a catalysis has been postulated, e.g. by JENCKS and CARRIUOLO[15], as contributing to the nucleophilicity of the serine hydroxyl in esterase. The amino group, by forming a hydrogen bond with the hydroxyl, would provide electrons to the oxygen, thus increasing its nucleophilicity. Such hydrogen bonding, whose existence in tetrachloroethylene solution is evidenced by IR-studies[16], would have to be mainly intramolecular if we want to explain why ethanolamine is more reactive than choline when present together at the same concentration. However, the reaction with choline could also be somewhat catalysed, intermolecularly, by the amino groups of ethanolamine. And the same could be true for hydrolysis.

In view of the possible relationship between the nucleophilicity of the alcohol group of amino alcohols and of serine in esterases, it may be relevant to note that some cyclic phosphates derived from *o*-hydroxy benzyl-alcohol have been reported[17] as powerful anticholinesterasic agents. Ethylene phosphate (calcium salt) has now been found[18] to have a weak anticholinesterasic action: At the concentration of $2.2 \cdot 10^{-2} M$ it produces 73% inhibition of horse serum (dilution 1:10) cholinesterase (substrate acetyl-choline) after 30 min incubation at 37 °C and pH 7.4.

Résumé. De nouvelles observations concernant les réactions de transesterification entre l'éthylène phosphate et les amino-alcools sont rapportées et des mécanismes possibles de cette réaction discutés.

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Folic Acid and Biotin on the Metabolism of One Carbon Unit: Utilization of β-Carbon of Serine for the Synthesis of Methionine

Several studies have been reported on relationships between biotin and folic acid. With regard to the nature of these relationships, two hypotheses were formulated. According to one, biotin may influence the synthesis of folic acid by intestinal microflora[1]. According to the other, biotin may be involved in the synthesis of coenzymatic forms of folic acid. In fact in the liver of biotin-deficient rats, the concentration of various folate coenzymes is decreased[2] and the capacity of these animals to convert folic acid to its activated forms[3] is reduced. This study has been undertaken in order to investigate the effect of biotin on the synthesis of methionine from serine and homocysteine.

Four groups of weanling male rats of the Wistar strain were fed ad libitum on the following diets respectively: purified basal diet, biotin-free diet, folic acid-free diet, biotin and folic acid-free diet[4]. After 60 days the animals

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were killed and the livers homogenized with 4 volumes of 0.08 *M* phosphate buffer pH 6.3. Aliquots of homogenate were incubated in a shaker at 37 °C for 2 h under nitrogen with 10 mg L-serine and 10 mg D,L-homocysteine; the final volume was 11 ml. The reaction was stopped by autoclaving, and methionine formed in the systems was assayed microbiologically with *Leuconostoc mesenteroides*, P. 60 ATCC 8042.

The data of the Table show that in the systems with the liver homogenate of biotin-deficient rats, and in those of folic acid-deficient rats, the quantity of methionine formed is significantly lower than in control rats ($P < 0.05$ and $P < 0.01$ respectively). In the systems with the liver homogenate of biotin and folic acid-deficient rats, the quantity of methionine formed is lower, not only when compared with control rats ($P < 0.001$) but also with folic acid-deficient rats ($P < 0.05$).

It appears from these results that methionine is synthesized in the liver of biotin-deficient, and biotin and folic acid-deficient rats less effectively from homocysteine and serine than in control rats and folic acid-deficient rats respectively.

This finding is in agreement with the observations of MARCHETTI and TESTONI[5], who reported that the methylation processes in biotin deficiency are markedly impaired. In fact *N*-methylnicotinamide excretion in urine is lower in biotin-deficient rats than in the animals fed on vitamin.

Besides, the methionine synthesis from homocysteine and betaine in the liver homogenates of biotin-deficient rats is lower than that in rats receiving biotin. Therefore, from the results it may be deduced that biotin is able to influence, through its action on the storage of folate coenzymes, the synthesis of methionine and probably the process of methylation generally.

Effect of biotin and folic acid on the synthesis of methionine by rat liver homogenate. Values give the mean result ± S.E. of mean of 6 determinations

Group	Animals in experiment	μg Methionine formed/g fresh tissue
1	Control	198 ± 11.64
2	Biotin deficient	162 ± 12.09
3	Folic acid deficient	139 ± 7.39
4	Biotin and folic acid deficient	116 ± 5.00

Riassunto. La sintesi di metionina da serina e omocisteina risulta notevolmente diminuita nel fegato di ratti carenti di acido folico e biotina, non solo rispetto ad animali controllo, ma anche a quelli carenti solo di acido folico. La biotina pertanto sembra influenzare l'utilizzazione del β-carbonio della serina per la sintesi del metile della metionina probabilmente attraverso il suo effetto sulla disponibilità dei coenzimi dell'acido folico nel fegato.

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Die Zahl der Mitochondrien im Aortenendothel bei experimenteller Stenose

Bei vergleichenden Untersuchungen des Endothels der grossen Blutgefässe vom Kalb und anderen Tierspezies konnten HACKENSELLNER et al. statistisch signifikante Unterschiede im Mitochondrienbesatz des Endothels der verschiedenen Standorte des Kreislaufes feststellen[1-4]. Sie führten diese auf die Stoffwechselbedingungen des Endothels zurück, wobei Beziehungen zwischen dem Sauerstoffgehalt des Blutes und dem Druck in den einzelnen Kreislaufabschnitten wahrscheinlich gemacht werden konnten.

Wir stellten uns die Aufgabe, die Zahl der Mitochondrien des Endothels der Aorta von Kaninchen bei veränderten Kreislaufbedingungen zu untersuchen. Wir bedienten uns dazu eines Modells der Aortenstenose, das durch Einengung des Durchmessers der Aorta abdominalis um etwa die Hälfte erzielt wurde (nähere Einzelheiten bei GÖTZE und MEYER[5]). Wir erreichten dabei deutliche Druckdifferenzen in den Gefässabschnitten ober- und unterhalb der Drosselungsstelle; ausserdem Zeichen einer Linksherzüberlastung. Von Gruppen zu je fünf operierten Kaninchen wurden nach 1, 3, 7, 14 und 21 Tagen die Mitochondrien des Endothels der Aorta ober- und unterhalb der Stenose ausgewertet; weiterhin gelangte eine gleich starke Kontrollgruppe zur Untersuchung.

Die Darstellung der Mitochondrien wurde nach einer von MEYER und HACKENSELLNER angegebenen Methode durchgeführt[6]. Die Auszählung erfolgte als Blindversuch am Projektionsbild der Schwarz-Weiss-Negative. Dem Untersucher waren weder die Herkunft der abgebildeten Mitochondrien noch die Versuchsdauer bekannt. Die gewonnenen Werte stellen ein relatives Äquivalent dar; es entspricht etwa einem optischen Flachschnitt durch die Endothelzelle. Die Ergebnisse unserer Versuche sind aus der Tabelle zu entnehmen.

Es ist daraus folgendes ersichtlich: (1) Die Kontrolltiere weisen in jedem Falle unterhalb der angenommenen Stenose mehr Mitochondrien pro Zelle auf als oberhalb. (2) Unter dem Einfluss der Einengung kommt es oberhalb zu einer Erhöhung der Werte und unterhalb zu einer Erniedrigung. Diese Zu- bzw. Abnahme ist schon einen Tag

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