3 photoelectric cells. Further details of procedure can be found elsewhere[3].

Results. The only consistent effect observed in the treated animals was a decrease in fur shaking. This component shows some resemblance to the well-known dog behavior, but in mice the shaking movement is carried out much more rapidly, and there is one single shake at a time, an act in which either the entire animal or only its front part may be involved. The frequencies for treated and untreated animals, respectively, were: DBA, 8 and 19; C57BL, 35 and 75; F_1, 30 and 43; *se se*, 15 and 29; *se* +, 9 and 41. Evaluation by means of a non-parametric combining test[5] revealed that the decrease is highly significant ($p < 0.3\%$).

The other segments of the behavioral repertoire turned out to be practically unaffected. Moreover, there was no influence at all of treatment on locomotor activity for either strain.

Discussion. Most probably, fur shaking is elicited by irritations of the skin, e.g. by salt crystals and – in the field – by ectoparasites. The finding that chlorpromazine in the dosage used tends to block specifically the release of this act, whereas other motor functions are not interfered with, may be attributed to a lowering of sensitivity to itching in drugged animals. This is in keeping with a statement made in the literature[6] that chlorpromazine reduces the ability to perceive tactile stimuli. With higher doses, this primary effect will be masked by motor impairment[7,8].

Résumé. A en juger d'après les diminutions dans l'action de se secouer le pelage, une dose moderée de chlorpromasine, n'affectant pas les autres fonctions motrices, semble reduire, chez les souris, la sensitivité aux irritations de la peau.

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[8] The author wishes to thank Mrs. JANE HARRIS for her technical assistance.

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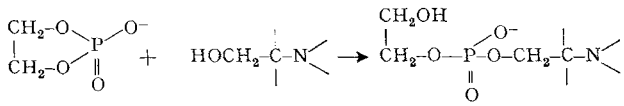
Further Observations on the Transesterification Reactions of Ethylene Phosphate with 2-Amino Alcohols

Some time ago it was reported[1] that ethylene phosphate reacts with 2-amino alcohols (ethanolamine, N,N-dimethylethanolamine, *tris*-(hydroxymethyl) amino-methane) in aqueous solution at weakly alkaline pH (8.3–9.4) to give transesterification products:



The reaction was favoured by an increase in pH and this suggested that the free NH_2 group (pKa = 9.45 in ethanolamine) played a role. This hypothesis has now been strengthened by the following experiment.

An aqueous solution (4 ml) of ethanolamine and choline chloride (3.4 *M* each, pH adjusted to 9.6 with hydrochloric acid) was made and ethylene phosphate dissolved in it (0.1 *M* calcium salt). The solution[2] was left at 37 °C for one week when it was treated in the usual way[1] with IRC-50, H^+ (6 ml) and Dowex 50, Na^+, 50–100 mesh (200 ml) resins, in order to remove the unreacted amino alcohols. In the solution obtained (recovery of phosphorus, 89%), a ratio N/P = 0.71 was found. The esterified ethanolamine and choline were determined after acid hydrolysis (HCl 1N, 1 h at 100 °C), the first by periodic acid oxidation, the second by the colorimetric procedure of HACK[3]. On a molar basis it was found that the amount of ethanolamine present corresponded to 59.5% of the total phosphorus, choline to 11.5%, the rest of the phosphorus being presumably present as glycol phosphate. The amphoteric character of the transesterification products was evidenced by passage over a column of Dowex 1 (Cl^-) at neutral pH, from which 70% of the phosphorus was recovered as expected, the retained part being again presumably glycol phosphate.

It appears therefore that the transesterification reaction is favoured by a free amino group, as partially present in ethanolamine but not in choline[4]. If this is the case, we may ask ourselves why. We have considered the following possibilities:

(1) The amino group provides nucleophilic catalysis by attacking the phosphoryl and forming a phosphoramidate intermediate. We think that this is unlikely for the following reasons: (a) We had no evidence[1] of the formation of such an intermediate in the reaction with ethanolamine. A single, amphoteric, ninhydrin positive product was obtained. (b) It appears that nitrogen bases are poor

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[2] A precipitate soon appeared. In the previously reported experiments[1] on the reaction with ethanolamine alone, a similar precipitation had also been observed when the concentration of the cyclic phosphate was 0.15 *M* but not when it was 0.05 *M*. However, this difference did not affect the yields, which were the same in both cases.

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[4] The precise interpretation of our result is complicated by the possibility that the transesterification products revert to the cyclic phosphate and at different rates. However, such reverse reactions, constantly tending to regenerate the cyclic phosphate should finally result in a complete hydrolysis to glycol phosphate. The relatively small amount of glycol phosphate found after one week may be taken as an indication that these reverse reactions do not take place to any great extent. A kinetic study should provide a more definite answer on this point.

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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