In order that this time be reduced to 2 o, it would be necessary for the enzyme and substrate to be concentrated within a small portion of the total ganglionic volume, such as at the nerve endings. Already evidence for a localization of this type has accumulated 4,5.

The calculation given above is merely an approximation, and serves only to show the enormous gap between the time for splitting under minimum and maximum conditions, and the necessity for localization of enzyme and substrate within the ganglion cell if the theory of chemical mediation is to hold.

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Choline Esterase in the Central Nervous System

The theory of transmission of nervous impulses to the voluntary muscles postulates the presence of a high concentration of choline esterase in nerve endings or their neighbourhood. We have previously demonstrated the accumulation of choline esterase at the end plates of muscle². We have now compared the rate of hydrolysis of acetylcholine in the grey and white matter of the spinal cord of the dog and found the concentration 10-20 times higher in the grey matter. 100 mgm. grey matter hydrolyse 7-9 mgm. acetylcholine in one hour, while 100 mgm. white matter split only 0.4-0.9 mgm.

In the central nervous system, as in muscle, choline esterase is found in high concentration in the tissue that contains nerve endings. This suggests that in the grey matter the enzyme may have a similar functional significance as at the end plates of muscle, that is, the rapid removal of acetylcholine, and that this substance may act as transmitter of nervous impulses in the central nervous system.

In the nervous system of crustaceans (lobster) there is also a higher concentration of choline esterase in the ganglion cells than in the nerve fibre.

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July 28.

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Specificity of the Salicylic Aldehyde Reaction of Csonka-Straub

Some time ago Straub (in Szent-Györgyi's laboratory) described a colorimetric method for the determination of pyruvic acid, similar to the method previously proposed by Csonka for the determination of acetone. In the presence of salycyclic aldehyde and concentrated potassium hydroxide, both substances develop a yellow colour, suitable for colorimetry. Having met with the necessity of determining pyruvic acid in the presence of several other ketoacids, I have made a study of the specificity range of this reaction and obtained the following results.

A positive salicylic aldehyde reaction is obtained with all compounds containing a CH₃CO group linked directly to a hydrogen or carbon atom; for example, acetaldehyde, acetone, ethyl acetoacetate, methylhexylketone, diacetyl, acetophenone, pyruvic acid, levulinic acid. The reaction is negative: (1) with carbonyl compounds that do not contain the above-mentioned group-formaldehyde, propionic aldehyde and higher homologues, ketobutyric acid and all higher ketoacids, fructose, dihydroxyacetone, acetonedicarboxylic acid; (2) with compounds containing a CH₃CO group linked to oxygen or nitrogen, that is O- and N-acetyl compounds, the CO group of which is not a genuine carbonyl group; for example, acetic acid, acetylglycine, acetylcholine, acetylsalicylic acid, triacetin.

The mechanism responsible for this very definite range of reaction specificity is obviously the following. In the first stage of the reaction salicylic aldehyde is condensed with the carbonyl compound to an oxybenzylidene derivative (Perkins' reaction):

$$\begin{array}{c}
OH \\
-CHO + R.CH_2.CO \dots \longrightarrow \\
OH \\
-CH = C.CO \dots \\
R
\end{array}$$

If a menthyl group be attached to the carbonyl group of the original compound, that is, in the case R=H, enolization will ensue in a strongly alkaline medium, leading to the formation of an enolate, the intense yellow colour of which is due to the system of cumulated double bonds:

$$OH \longrightarrow -CH = C - CO \dots + KOH \longrightarrow H$$

$$OH \longrightarrow -CH = C = C \dots + H_2O.$$

With R other than a hydrogen atom enolization and the formation of the cumulated double bond system is ruled out and no colour will appear, even though benzylidene condensation had taken place. With O- and N-acetyl compounds neither the condensation nor the enolization is obtained under the conditions of the colour test.

The salicylic aldehyde reaction is a sensitive test for the qualitative (and in many instances for the quantitative) determination of any methyl-carbonylderivative in the absence of other substances belonging to the same group. The reaction is very important as a valuable means for distinguishing these compounds from the homologous or substituted carbonyl derivatives. The specificity range outlined above should be especially kept in mind when making use of the reaction for quantitative studies on the metabolism of pyruvic acid, which may give rise to substances such as acetaldehyde, acetoin, acetoacetic and acetopyruvic acids and acetone, all falling within the specificity range of the Csonka-Straub reaction.

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