Summary

With the aid of the tensiometer, it can be proved that histamine reacts in a specific manner with primary phosphoric acid esters. This proof is brought about by strongly increasing the capillary activity of cetyl phosphate by histamine on the interface chloroform water. The mechanism of this reaction is discussed. Simple secondary phosphoric acid esters and bi-secondary phosphoric acid esters are not influenced under the same conditions. A pronounced antagonistic effect of calcium ions antihistamine compounds may be proved with the same assays.

The point of attack of these compounds is the phosphate and not the histamine. The results of the tests confirm the assumption that the mode of action of the histamine and of the antihistamine compounds is connected with primary phosphoric acid esters.

Stability and Local Anaesthetic Effect in Some Derivatives of "Xylocaine"

Xylocaine (o,o'-dimethyl-N-diethylaminoacetylanilin), prepared by LÖFGREN in 1943, is one of the most successful recent local anaesthetics. In view of the favourable properties of this compound in clinical practice, a number of structural analogues has been prepared by various authors. From published work on the structure-activity relations in this series, particular interest attaches to the fact that the favourable properties of Xylocaine, and especially the duration of its action, are largely dependent on the presence of the two ortho-methyl substituents on the aromatic nucleus. This fact clearly emerges in the case of e.g. analogues derived from the isomeric xylidines and toluidines, and more strikingly still from a comparison with the demethylated analogues derived from o-toluidine and aniline. LÖFGREN[1], who was the first to note this effect, interpreted it in the light of the mechanism of action proposed by him, which was based on the inhibition of resonance between nitrogen atom and the aromatic nucleus. More recently HACH[2] has critized LÖFGREN's theory and submitted the view that lipophilic character is an important factor affecting the activity of Xylocaine and its isomeric and demethylated analogues.

Though the chemical stability of Xylocaine was noted already by LÖFGREN and later confirmed particularly by WOOD *et al.*[3], who studied the metabolism of this drug in dogs, this factor has, in our opinion, been given insufficient weight in theoretical considerations regarding the mechanism of action of this type of compound.

In consideration of the work of a number of earlier authors[4] concerning the stability of anilide bonds in simpler compounds, we came to the conclusion that the increase in activity observed with progressive substitution of the ortho-positions with methyl groups might be connected with the increased stability of the amide linkage. Compounds of the Xylocaine type are most likely to be initially attacked *in vivo* at the anilide bond (peptidases) with formation of the free aromatic amine and liberation of the acid moiety. This assumption is borne out by the work of THER[1], which has recently become available to us, on the Xylocaine analogue Hostacaine. The stability of the anilide linkage must needs affect the metabolism of these compounds in the organism which leads to the formation of inactive metabolites from the active compounds (cf. e.g. KINDLER[2]).

Table I.–Structures and Numbering of the Compounds Studied

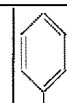
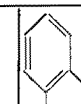
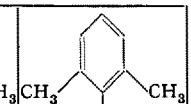
Structure R =	 NHCOR	 NHCOR	 NHCOR
$-O{\cdot}CH_2{\cdot}CH_2{\cdot}N(C_2H_5)_2$	"S 11"	"S 31"	"S 170"
$-CH_2{\cdot}N(C_2H_5)_2$ . . .	"S 200"	"S 201"	"S 202" (Xylocaine)

Table II.–Rate Constants of Acid Hydrolysis and Anaesthetic Activities

Compounds	Rate constant k (sec^{-1})	Activity	
		Surface anaesthesia (rabbit)	infiltration anaesthesia (guinea-pig)
"S 11"	$8{\cdot}22 \times 10^{-6}$	0·1	1·2
"S 31"	$3{\cdot}18 \times 10^{-6}$	0·54	1·0
"S 170"	$3{\cdot}32 \times 10^{-7}$	1·0	2·1
"S 200"	$8{\cdot}15 \times 10^{-5}$	(incomplete)*	0·12
"S 201"	$2{\cdot}64 \times 10^{-5}$	(4·5±2·0 min)*	0·24
"S 202"	$7{\cdot}27 \times 10^{-7}$	0·24 (52·5±2·0 min)*	1·4
Cocaine	–	1	–
Procaine	–	–	1

* Duration of complete anaesthesia after application of $M/3$ solution.

This hypothesis, which has been developed in more detail elsewhere[3], has now been subjected to experimental testing by measurement of the rates of acid hydrolysis of Xylocaine ("S 202") and its demethylated analogues ("S 200" and "S 201") as well as of the three structurally related carbamates ("S 11", "S 31", and "S 170") and comparison with the local anaesthetic activities of the these compounds. The structures of the substances studied are shown in Table I; the results are recorded in Table II.

The rates of hydrolysis were studied in 5 N-HCl at 99·5° C at an initial substrate concentration of 0·01 M and followed by an electrophotometric determination of the

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[2] K. KINDLER, Arch. Pharm. *266*, 19 (1928); *267*, 541 (1929); Forschungen u. Fortschr. *10*, 435 (1934).

[3] A. SEKERA, *New Trends in Local Anaesthetic Research* lecture presented at the Scientific Conference, held at Brno University, February 19th–20th, 1954.

liberated aromatic amine. The anaesthetic activities in Table 2 are expressed as the ratios of the molar concentrations of the compound studied (as the hydrochloride) and the standard giving the same anaestehtic effect. Each compound was tested for both superficial anaesthetic activity (rabbit cornea; cocaine as standard in conc. $M/100$) and for infiltration anaesthesia (intradermal application to guinea-pigs; procaine as standard in conc. $M/50$) so that its effectiveness might be judged on a broader basis. In the case of compounds "S 200" and "S 201", the first of these methods could not be used because of their low activity; the duration of complete anaesthesia of the cornea after applications of $M/3$ solutions of these substances and of Xylocaine for comparison were therefore determined.

The results obtained so far show that both in compounds of the Xylocaine series and their carbamate analogues there is a distinct parallelism between the local anaesthetic effect and the resistance to hydrolysis with increasing o-methyl substitution. The only exceptions are the results with compounds "S 11" and "S 31" in infiltration anaesthesia; the difference between the two values (1·2 and 1·0), however, lies within the limit of error for the biological method of testing used.

Final conclusions as to the possible bearing of these results on the mechanism of action of the compounds studied must be postponed until the measurements of hydrolysis in alkaline solution and the determination of the activation energies and frequency factors now in progress have been completed. On the basis of these results it should then be possible to decide whether the effect noted is of steric or electronic origin.

We should like cordially to thank Dr. J. RUDINGER for the translation of this paper, which he has kindly made for us.

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Zusammenfassung

Die Reaktionsgeschwindigkeit der sauren Hydrolyse von Xylokain, Diethylaminoacet-o-toluidid und Diethylaminoacetanilid und seiner Carbamat-Analoga wurde studiert: Die Stabilität dieser Verbindungen steigt mit fortschreitender Methyl-o-Substitution und verläuft mit ihren lokalanästhetischen Wirkungen parallel.

Spontaneous Peristaltic Activity in Arteries and Veins of Adult Goldfinches and Albino Mice Cultivated *in vitro*

In previous studies, the occurrence of a spontaneous peristaltic activity was shown in embryonic arteries of chick (ATTARDI, GANDINI, and MARCON[1]; TONI[2]) and rabbit (ATTARDI[3]) and in veins of chick embryos and newly hatched chickens explanted *in vitro* (ATTARDI and ATTARDI GANDINI[4]).

In this paper, evidence is presented that a similar contractile activity also occurs in arteries and veins of adult animals. For such investigations, animals of a small size, namely goldfinches (*Carduelis carduelis*) and albino mice (*Mus musculus*), were used, under the assumption that their vessels, owing to the small calibre and the thinness of the walls, should be in a suitable nutritive and mechanical condition for displaying a contractile activity in the plasmatic medium, into which they are explanted, as is the case with the embryonic vessels.

The isolated vessels were washed in physiological salt solution containing penicillin (50 units per cm^3). Usually segments of vessel of 2 to 3 mm in length were explanted. The technique used was the same as described in the earlier reports; the culture medium was composed of chick plasma and ten day old chick embryo extract in equal parts.

The results of the experiments are shown in the Table.

In the adult goldfinch, all arteries tested, both central and peripheral, showed a contractile activity *in vitro*. The fact should be noted that this activity occurred in a very high percentage of explants (about 100%), in contrast to what was found in the case of embryonic arteries. Of the veins examined, a contractile activity was exhibited by those of the portal system and by the umbilical vein, a fact which had already been described in chick embryos and newly hatched chickens; moreover by some veins of the posterior portion of the body (coccygomesenteric vein, hypogastric vein, renal portal vein, femoral vein, external iliac vein, common iliac vein (anastomosis)[1]: in the latter veins a spontaneous contractility of a peristaltic type had also been observed in chick embryos in the last period of incubation and in newly hatched chickens (personal data not yet published).

The valve shaped as a perforated diaphragm which is situated where the common iliac vein empties into the efferent renal vein (SPANNER[2]) shows *in vitro* a rhythmical contractile activity, which is at present the object of a more detailed study.

In the arteries, the contractile activity is in most cases noticeably powerful and appears as typically coordinated. The contraction manifests itself as a contractile wave which almost always starts from one extremity of the explanted segment and propagates along the latter at a varying speed in the different explants, and is followed by a relaxation which likewise takes place successively in the following portions of the vessel. It is therefore justifiable to describe these contractions as of the peristaltic type, bearing in mind, of course, that their more or less typical character may be influenced by the particular conditions under which the explanted vessels are placed, on account of their isolation from the organism and of the lack of any internal pressure to keep their walls extended. The contractile activity starts immediately after explantation, or at least within the first hour, and generally becomes exhausted within 6–12 h; in several cases, however, persisting for longer periods, up to 2–3 days, although much weakened. The frequency of the contractions is generally higher in the more peripheral arteries. In the veins the contractile activity, which is likewise of a peristaltic type and fairly powerful, generally begins within the first hour and lasts from a few hours to 2 or 3 days.

[1] G. ATTARDI, E. GANDINI, and L. MARCON, Boll. Soc. ital. Biol. sper. *24*, 1333 (1948).
[2] G. TONI, Boll. Soc. ital. Biol. sper. *29*, 6 (1953).
[3] G. ATTARDI, Boll. Soc. ital. Biol. sper. *25*, 1057 (1949).
[4] G. ATTARDI and D. ATTARDI GANDINI, Exper. *11*, 37 (1955).

[1] For the above indicated veins the terminology proposed by R. SPANNER, Morph. Jb. *54*, 560 (1925), has been used.
[2] R. SPANNER, Morph. Jb. *54*, 560 (1925).