at the upper limit of this range. Deliberate inclusion of perchlorate in a fixed concentration cannot be recommended as a way to enhance sensitivity, since its effects are variable and somewhat time-dependent. Complete removal is obviously desirable prior to assay if perchloric acid is used to extract tissues; precipitation as the potassium salt is less satisfactory in this respect than neutralization with a basic anion exchange resin.

Sensitization of the frog rectus abdominis by perchlorate is of some interest in itself. There is evidence that it is analogous to the potentiation of contraction by other foreign anions according to their position in the Hofmeister series[17]. Perchlorate has been shown to reduce chloride permeability and prolong the active state in frog sartorius muscle[18], and is roughly equivalent to thiocyanate in this respect. Other authors place perchlorate beyond thiocyanate in the lyotropic series[19].

In summary, perchloric acid is probably best avoided as an extracting agent prior to bioassay of acetylcholine because of the variable sensitization it produces particularly when other drugs may be present. When it is used for this purpose, its subsequent removal with an ion exchange resin appears desirable.

Zusammenfassung. Die Reaktion des mit Eserin vorbehandelten musculus rectus abdominis des Frosches auf Acetylcholin liess sich durch Oxotremorin ($2.0 \cdot 10^{-5} M$), Arecolin ($2.0 \cdot 10^{-5} M$), Aceclidin ($1.0 \cdot 10^{-4} M$) und Carbachol ($2.0 \cdot 10^{-7} M$) verstärken, während Atropin ($1.0 \cdot 10^{-5} M$) hemmend wirkte. Perchlorationen in Konzentrationen über $2 \cdot 10^{-4} M$ sensibilisierten den Froschmuskel erheblich und reduzierten die Grenzdosis, bei der eine Potenzierung durch Substanzen mit muskarinähnlicher Wirkung beobachtet wird. Alle beschriebenen Reaktionen liessen sich durch Gehirnextrakte verstärken, in denen zuvor Acetylcholin durch Kochen in alkalischer Lösung zerstört worden war.

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[17] P. Horowicz, Pharmac. Rev. *16*, 193 (1964).
[18] E. J. Harris, J. Physiol. *141*, 351 (1958).
[19] G. Boehm, Arch. exp. Path. Pharmak. *146*, 327 (1929).

Increased Level of 5-Hydroxyindoleacetic Acid in Cerebrospinal Fluid from Infantile Hydrocephalus

Carlsson, Falck and Hillarp[1] have suggested that 5-hydroxytryptamine (5-HT), as well as noradrenalin and dopamine, are transmitters in the central nervous system. Bogdanski, Weissbach and Udenfriend[2] reported that 5-HT appears in the cerebrospinal fluid (CSF) of rats and dogs following administration of 5-hydroxytryptophan, the precursor of 5-HT. However, it has not been possible to show the presence of 5-HT in CSF under normal conditions using sensitive spectrophotofluorometric methods. On the other hand, 5-hydroxyindoleacetic acid (5-HIAA) can be fairly easily determined in CSF. Ashcroft and Sharman[3] and Sharman[4] showed that the level of this acid is increased in CSF from hydrocephalic patients. Their materials were not used for diagnostic purposes.

The clinical diagnosis of hydrocephalus is fairly easy in advanced cases. However, with the new surgical method (ventriculo-venous-shunts) in which the results are uniformly better[5], the demand for early diagnosis, and hence earlier treatment, has increased. The early treatment produces better results.

The conventional diagnostic methods using pneumoencephalography and ventriculography carry a certain risk. They are not suitable for screening purposes. A simple laboratory test has so far not been evaluated, and our intention has been to produce a diagnostic method based upon determination of acidic monoamine metabolites in CSF. We have investigated hydrocephalic children and children suspected of hydrocephalus. Some preliminary results were presented at the Meeting of Scandinavian Neurologists in 1964[6].

Both groups have been subjected to a careful clinical investigation using other available methods, e.g. encephalography, ventriculography and echo-encephalography[7], in order to establish the correct diagnosis. Only children up to 1 year of age have been included in this report, i.e. cases of the so-called infantile hydrocephalus. 75 children were investigated. 39 of them had hydrocephalus and were operated upon. The remaining 36 were suspected of having the disease but further investigations could not verify the diagnosis.

The values of 5-HIAA in CSF of the 75 children are tabulated in the Table. Group II consists of non-hydrocephalic children undergoing investigations for various neurological conditions. It is seen that the level of 5-HIAA in the hydrocephalic group is in all cases above 0.09 µg/ml,

5-HIAA	Group I 'hydrocephalic'	Group II 'non-hydrocephalic'
$\leqq 0.08$	0	23
0.09–0.10	1	10
0.11–0.14	7	3
$\geqq 0.15$	31	0
Total	39	36

[1] A. Carlsson, B. Falck, and N.-Å. Hillarp, Acta physiol. scand. *56*, Suppl. 196 (1962).
[2] D. F. Bogdanski, H. Weissbach, and S. Udenfriend, J. Pharm. *122*, 182 (1958).
[3] G. W. Ashcroft and D. F. Sharman, Nature *186*, 1050 (1960).
[4] D. F. Sharman, Thesis, University of Edinburgh 1960.
[5] D. Yashon, J. Neurosurg. *20*, 105 (1963).
[6] H. Andersson and B.-E. Roos, Acta neurol. scand. *41*, Suppl. 13, 149 (1965).
[7] I. Sjögren, Acta neurol. scand. *41*, Suppl. 13, 13 (1965).

in contrast to the 'normal group' where the levels in nearly all cases are below 0.11 μg/ml.

A possible source of error in this study might be that the cerebrospinal fluid in the hydrocephalic group was mostly obtained by ventricular puncture, in contrast to the non-hydrocephalic group where the liquor was withdrawn by lumbar puncture. Perhaps there is normally a difference between the level of 5-HIAA in ventricular fluid compared with lumbar spinal fluid. Further investigations were thus made in order to clarify this aspect. It is for many reasons impossible to obtain ventricular fluid from a normal child, so we had to rely upon 5 hydrocephalic children, where a double puncture could be made. The results of this separate study showed a slight decrease in the level of 5-HIAA when ventricular fluid was compared with lumbar spinal fluid. The lumbar values ranged between 0.15–0.22 μg/ml and the ventricular values between 0.13–0.28 μg/ml. In view of these data it does not seem probable that the different puncture technique has any influence upon the results. The control group is rather small but further investigations are to be carried out.

It is our impression that the values of 5-HIAA are higher the more centrally the obstruction is located. In some cases also homovanillic acid, the final metabolite of dopamine, was analysed in hydrocephalic CSF using the method of ANDÉN, ROOS, and WERDINIUS[8]. These values were also considerably increased. A more complete account will appear elsewhere.

Zusammenfassung. Die Konzentration der 5-Hydroxyindolessigsäure wurde in der Cerebrospinalflüssigkeit hydrocephalischer Kleinkinder (bis 1 Jahr) bestimmt. Die Werte waren deutlich erhöht. In einigen Fällen wurde die Konzentration an Homovanillinsäure, die ebenfalls erhöht war, bestimmt.

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[8] N.-E. ANDÉN, B.-E. ROOS, and B. WERDINIUS, Life Sci. *2*, 448 (1963).

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On the Mechanism of Potentiation of Kinins by Inhibitors of the Fibrinolytic System

In his review on bioactive peptides of plasmatic origin, LEWIS[1] first reported that cysteine potentiates the smooth muscle stimulating action of plasma kinin. Later, PICARELLI et al.[2] made a closer investigation of this effect and suggested that the potentiation of bradykinin possibly results from an inhibition of kininase present in the guinea-pig ileum. Later still, we could demonstrate that also inhibitors of the fibrinolytic system and peptones could potentiate the bradykinin action on the ileum[3,4]. When extending our study on further kinins (kallidin, eledoisin) and other bioactive substances (angiotensin, histamine, acetylcholine) we found that only the bradykinin and kallidin effects were potentiated. Fibrinolytic inhibitors and peptones in low concentration caused potentiation, while higher concentrations, for instance $> 0.1\,M$ ε-aminocaproic acid (EACA), diminished the bradykinin and kallidin effects in the same way as they inhibited the ileum's response to other bioactive substances.

It appeared not unlikely that intestinal tissues contain kininases which act specifically against bradykinin and kallidin, so that the potentiation would be the result of kininase inhibition. But also another explanation of the effect had to be considered; namely, that the different reaction patterns of bioactive substances with smooth muscle are responsible for the controversial effects when chemical compounds, otherwise known as inhibitors, interact with the respective reactive system. Such differences of the reaction pattern may be assumed the more, since bradykinin and kallidin, so-called slow-reacting substances, can be distinguished from other bioactive substances according to the time characteristics of their response.

In order to check the first mentioned of the two possible explanations of the potentiation phenomenon, we studied the degradation of bioactive substances in the presence of intestinal tissue. Homogenates from guinea-pig ileum were incubated with bradykinin, kallidin, eledoisin, and angiotensin respectively. The mixtures were kept at 30 °C and samples were taken at regularly spaced intervals. The activities of the samples were evaluated on the standard guinea-pig ileum preparation. Figure 1 shows the results of these experiments. While bradykinin and kallidin suffered a steep drop in activity, a longer incubation time was necessary for angiotensin to lose its activity; the activity of eledoisin, however, was only slightly diminished by contact with the homogenate.

In a further series of experiments we studied the effect of EACA, as a representative of the fibrinolytic inhibitors, on the degradation process. The same procedure as above was utilized, with the exception that EACA was present in the incubation mixture. It can be seen (Figure 2) that EACA from $0.1\,M$ concentration upwards inhibits the degradation depending on the concentration. We found no significant difference in the inhibitory exertion between bradykinin and kallidin on the one hand, kinins susceptible to potentiation by low concentrations of EACA, and on the other hand eledoisin and angiotensin, which are refractory to potentiation.

[1] G. P. LEWIS, Physiol. Rev. *40*, 647 (1960).

[2] Z. P. PICARELLI, O. P. HENRIQUES, and M. C. F. OLIVEIRA, Nature *18*, 77 (1962).

[3] W. DOLESCHEL, W. AUERSWALD, and H. SCHUBERT, Wien med. Wschr. *115*, 575 (1965).

[4] W. DOLESCHEL and W. AUERSWALD, Wien. med. Wschr. *116*, 130 (1966).