THE ROLE OF GANGLIONS IN THE MOTILITY OF BIVALVE SHELLS

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Summary

Experiments with fresh-water mussels *(Unio pictorum)* gave the following results:

Removal of the visceral ganglions is followed by a complete loss of the power of movement and by the opening of the shell. The animals become immobile and display no periodic activity.

Viscerally ganglionectomized animals respond to electrical and mechanical stimuli applied to the anterior margin of the mantle. It was demonstrated that the contraction of the shell elicited by mechanical stimulation originates solely from the anterior adductor, while both anterior and posterior adductors are responsible for a similar reaction to electrical stimulation.

This finding prompted the authors to make a morphological analysis which showed that the extirpation of the visceral ganglion does not involve a complete denervation of the posterior adductor.

The residual innervation may be traced to the region around the posterior adductor since the isolation of the latter provokes instantaneous stoppage of any further functioning. After such isolation no response can be obtained to any stimulus, however effective it may have proved in previous experiments.

Removal of the cerebral ganglions is much better tolerated by the test animals than of the visceral ones. Responses of cerebrally ganglionectomized mussels to mechanical and electrical stimuli are similar to those of animals deprived of the visceral ganglions.

Treatment of ganglionectomized animals with potassium chloride provokes instantaneous contraction, provided one of the adductors is normally innervated. This phenomenon led to the conclusion that K^+ acts via the peripheral nervous system.

Treatment with NaCl, $CaCl_2$ and $MgCl_2$ fails to elicit contraction. If KCl is applied after these salts, the usual reaction following treatment with K^+ will ensue, but contraction will be much less pronounced.

Introduction

The present authors have been engaged for some time in investigations concerning the nervous system of invertebrates, especially the elucidation of their neuromuscular relationships. Fresh-water mussels — *Unio pictorum* in the first place — were principally used in the pertinent experiments. The results of neuro-histological investigations were earlier described [1]. It was stated there that both thicker and thinner nerve fibres supply the innervation of the adductor muscles of fresh-water mussels, that these muscles themselves are devoid of nerve cells, and that, therefore, the particular behaviour of the adductors must be attributed to other factors. It was also stated that the earlier findings of BRÜCK [4], BOWDEN and LOWY [3] could not be correct. There are, on the one hand, no nerve endings in the adductor muscles of either the *Anodonta* or the Unionidae similar to the motor end plates of the higher orders, as postulated by Brück, nor is — on the other hand — the picture concerning innervation of the adductor, as presented by Bowden and Lowy, in accordance with real facts, since the figures shown in their picture are but artefacts.

Our continued investigations had the aim to elucidate the role played by particular ganglions in the innervation of the adductors of the *Unio pictorum,* and to study the effects of various experimental interventions. The present paper is intended to give an account of those experiments which were carried out with a view to elucidating the effect of a removal of certain ganglions upon the adductors, i. e. upon the mechanism which closes and opens the valves of the shell.

Extirpation of the visceral ganglions

Instantaneous closure of the shell follows the removal of the visceral ganglion ; it opens again after the lapse of 2 to 3 days and remains open, with a range of 5 to 7 mm. between the two valves of the shell. It is characteristic of these animals that they do not spontaneously close their shell, display no periodic activity, and are immobile ; it is only exceptionally that they extrude their leg and change their place. On an average, the ganglionectomized animals died 20 to 25 days after the operation. Having first examined the functioning of the adductors, we fixed the ganglionectomized animals at various intervals for the purposes of histological analyses.

Fixing the animals in an appropriate manner [2] we transferred the movements of the shell, i. e. the adductors, to a recording apparatus which enabled us to register the effect of the different stimuli in a mechanical way. The mechanical, electrical and chemical responses given by viscerally ganglionectomized mussels may be grouped as follows.

Mechanical stimuli applied to the posterior margin of the mantle have no effect whatever, nor is any response perceivable if the siphon is touched. A single touch of the anterior margin of the mantle induces contraction (Fig. 1*a*) : it is however partial and followed by a further contraction upon being touched again (Fig. 1*b*). Successively repeated mechanical stimulations result in a complete closure of the shell. This is performed by the anterior adductor, for mechanical stimuli applied to the anterior margin of the mantle fail to elicit any response after the anterior adductor has been cut through.

Electrical stimulation provokes reactions of the same type as obtained by mechanical stimuli. Direct current of comparatively high voltage (20 to 90 V) was used to produce the required shock. Stimulation by means of inductors cannot be resorted to in myophysiology partly because of the low current-

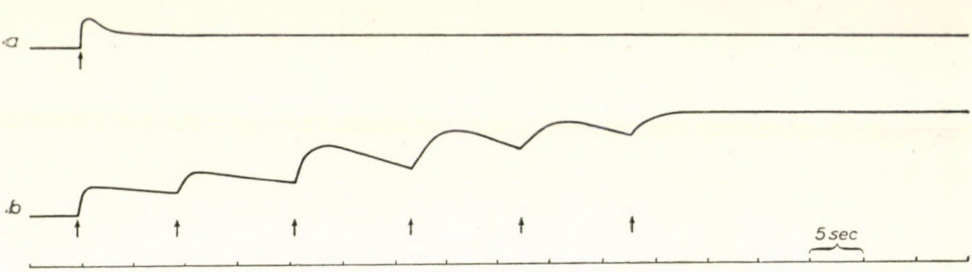

Fig. 1. Visceral ganglion removed. Mechanical stimulation of anterior margin of mantle : *a* — single mechanical stimulation, *b* — successively repeated mechanical stimulation

intensity and partly because of the fact that contact-reactions were caused by the poles of the inductor. Electric stimulation was therefore effected by dipping an electrode, each, on both sides of the fixed mussel in the water and then closing the circuit. This elicited contraction (Fig. 2*a*), the extent of which

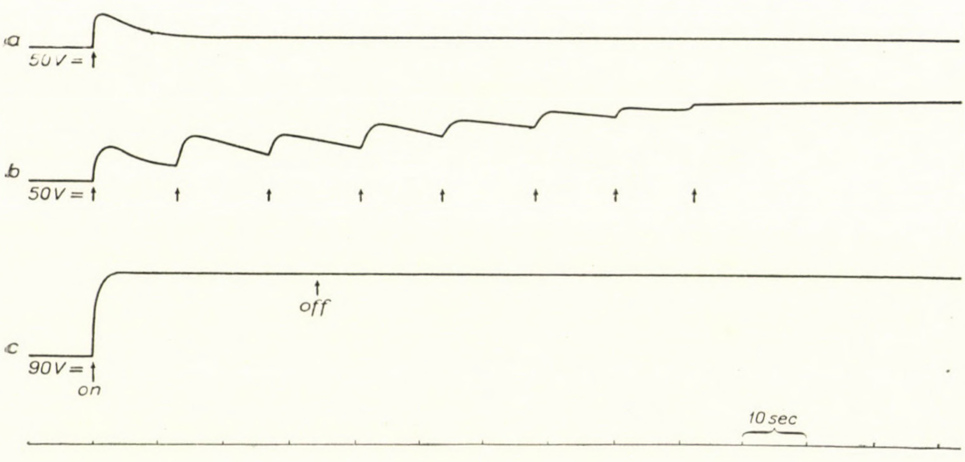

Fig. 2. Visceral ganglion removed. Electrical stimulation : *a* — single short electrical shock, *b* — several successive short shocks, *c* — single longer electrical stimulation

was proportional to the voltage and the duration of the current (Fig. 3). The number of successively applied electrical shocks released a corresponding number of adductor contractions and led, finally, to a complete closure of the shell (Fig. 2*b*). Contraction so obtained may last for some hours, whereas maximum contraction caused by mechanical means is followed by relaxation in a much shorter time. A single longer electrical shock produces maximum contraction (Fig. 2*c*), and relaxation ensues only long after the current has been switched off (this curve has no peak). It should be noted that it was only by closing, and never by opening, the circuit that reactions could be obtained.

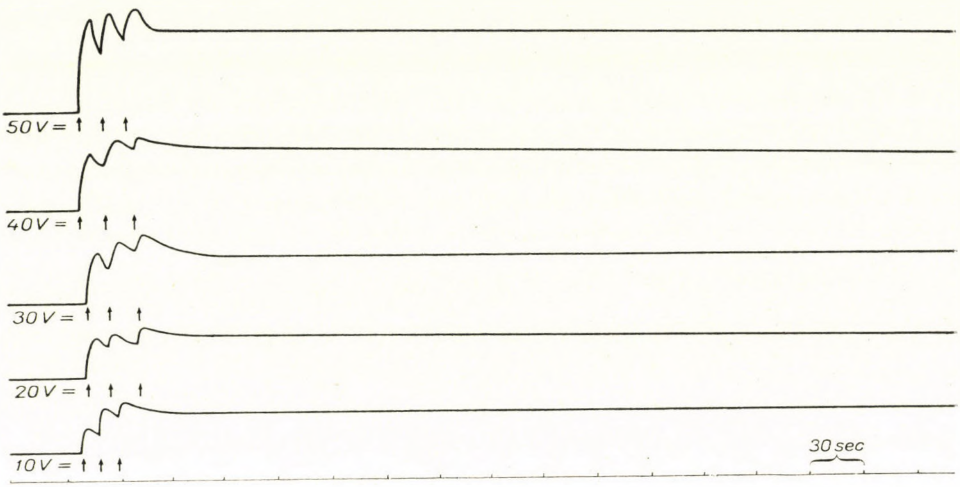

Fig. 3. Visceral ganglion removed. Three successive short electrical shocks. Increase of voltage increases contraction

It was surprising to observe that contractions produced by electrical shocks were effected by both adductors even on the 20th day following the removal of the visceral ganglion, and continued even after the normally innervated anterior adductor had been cut through (Fig. 4). It was this experiment which led us to suppose that — in contradiction to our earlier assumption —

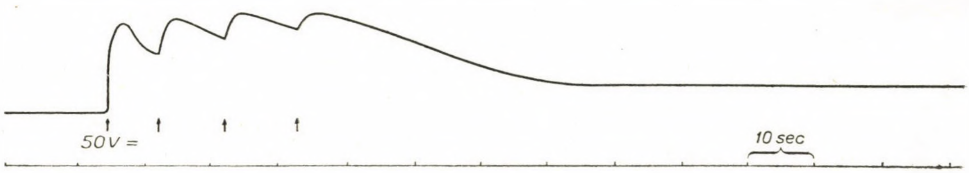

Fig. 4. Visceral ganglion removed, anterior adductor cut. Several successive short electrical shocks

the nerve fibres of the posterior adductor had not completely degenerated. This, again, made it probable that it is not solely the visceral ganglion which is responsible for the nerve supply of the posterior adductor.

This probability was further confirmed by the experiment in which a complete closure of the shell was obtained by applying a mechanical stimulus to — and only to — the posterior adductor 10 to 15 days after the removal of the visceral ganglion. Subsequent, very carefully performed histological analyses, too, pointed in this direction : we were able to demonstrate intact nerve fibres in the posterior adductor 14 days after the extirpation of the visceral ganglion.

It has already been stated that, in agreement with other authors [6, 8], we failed to discover nerve cells in the adductor itself in the course of our earlier experiments : this seemed to point to the possibility that there may be nerve cells in the connective tissue surrounding the adductor or in the commissures on the adductor, or, else, in the larger nerve trunks, and that these cells remain unimpaired even after the removal of the visceral ganglion. Our next step was to institute experiments for the elucidation of this phenomenon : having completely isolated the posterior adductors and stripped them of the surrounding tissues we saw the shell open immediately after the operation, and it was thereafter impossible to provoke a contraction of the posterior adductor by any stimulus that had always proved effective before. The inability to respond to stimuli was so complete that even electrical stimulus by means of electrodes applied directly to the posterior adductor failed to elicit any reaction.

After the histological examination of the mantles it could still be supposed that it is through the extremely large nerve trunks running in the mantles that impulses are transmitted from the cerebral ganglions to the posterior adductor by way of nerve fibres. In order to exclude this possibility we repeatedly observed the reactions of the anterior margin of the mantle of viscerally ganglionectomized animals. Since stimuli releasing such reactions invariably remained ineffective after the anterior adductor had been cut, we were led to the conclusion that, in animals whose visceral ganglions are extirpated, there remains no direct or indirect connection between the unimpaired cerebral ganglions and the posterior adductor. The experiments seemed thus to prove that the only possible connection existing in normal mussels between cerebral ganglions and posterior adductor goes through the said cerebrovisceral connectives.

Isolation of both adductors in perfectly healthy animals was followed by complete incapacity of movement : the adductors failed to contract even immediately after the intervention. Animals so treated did not survive longer than 36 hours during which time they responded to mechanical stimuli with a weak, to electrical ones with an intensive movement of the leg.

Extirpation of the cerebral ganglions

As in the case of visceral ganglionectomy, the removal of the cerebral ganglions is followed by convulsive contraction lasting 24 to 36 hours, whereafter the shell opens again, though to a lesser extent than after the extirpation of the visceral ganglions. Generally speaking, the animals seem to tolerate the removal of the cerebral ganglions better than that of the visceral ganglions. Analysis, in the case of cerebral ganglionectomy, is more difficult because the reactions of the normally innervated posterior adductor dominate the picture almost completely so that even weak stimuli provoke a durable and intensive contraction of the posterior adductor. As was to be expected, no effect was observed upon the anterior margin of the mantle having been touched after the removal of the cerebral ganglions, while the stimulus of the posterior margin of the mantle and the siphons elicited fairly strong, though sectional, contractions. Electrical stimulation provokes the same reactions as in viscerally ganglionectomized animals : the closing of the shell is likewise due to the action of both adductors, and the responses are quite analogous to those described in connection with the posterior adductor (Fig. 5).

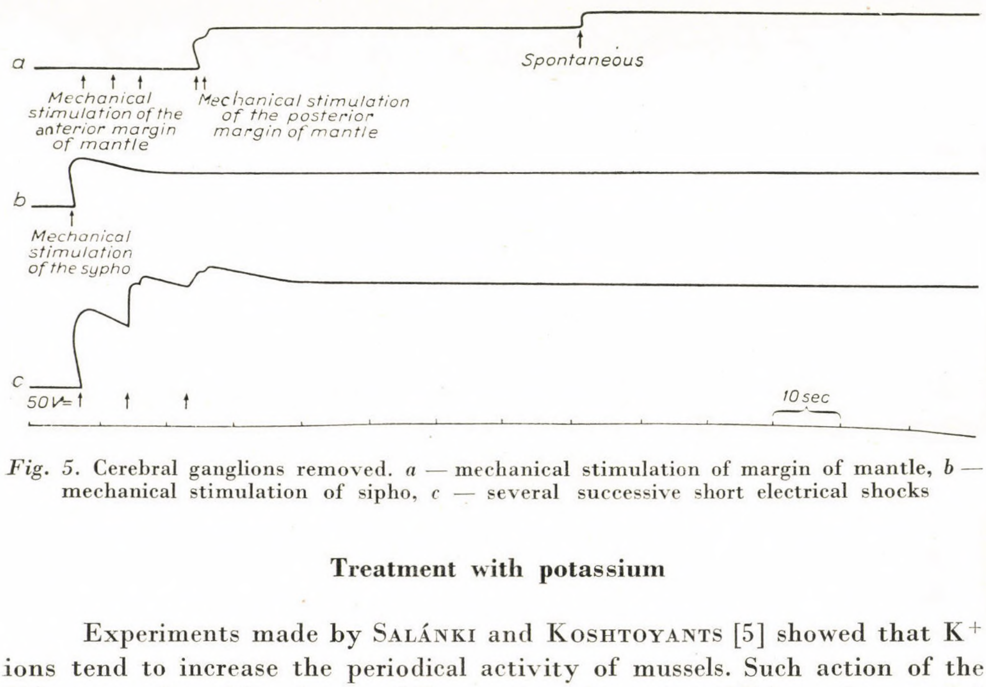

Fig. 5. Cerebral ganglions removed. *a* — mechanical stimulation of margin of mantle, *b* — mechanical stimulation of sipho, *c* — several successive short electrical shocks

Treatment with potassium

Experiments made by SALÁNKI and KOSHTOYANTS [5] showed that K^+ ions tend to increase the periodical activity of mussels. Such action of the K^+ was explained by them as follows : since the action of K^+ can be arrested by means of succinates, fumarate, malonate and malic acid it was assumed by the said authors that K^+ interferes with the Szent-Györgyi—Krebs cycle and accelerates the liberation of energy. On the strength of this theory they also concluded that periodical activity is governed by environmental and not some intrinsic factors.

It has already been stated that spontaneous movements of the shell come to a standstill after the removal of the visceral ganglions. Relying on the said theory we assumed that an introduction of K^+ ions would restore spontaneous shell movement at least to some extent, especially if the mechanism of the action of K^+ was really such as assumed by the authors. K^+ was applied by us in the form of KCl, and the required concentration of K^+ was produced in the same water in which the test animals were kept during the experiments. Our very first experiment, in which K^+ was introduced in the water, resulted in an instantaneous contraction of the slightly open shell of viscerally ganglionectomized animals. To obtain marked effects, we used highly concentrated (usually 0,2%) KCl. Neither NaCl, nor $CaCl_2$, nor $MgCl_2$ provoked a similar effect. If the preparation is adequately washed after a treatment with NaCl or $CaCl_2$, the K^+ reaction can be provoked anew, but the effect will be weaker because Na^+ and Ca^{++} diminish the action of K^+ (Fig. 6*a*, *b*). KCl induces furthermore a prompt and fairly frequent periodical activity (Fig. 6*c*).

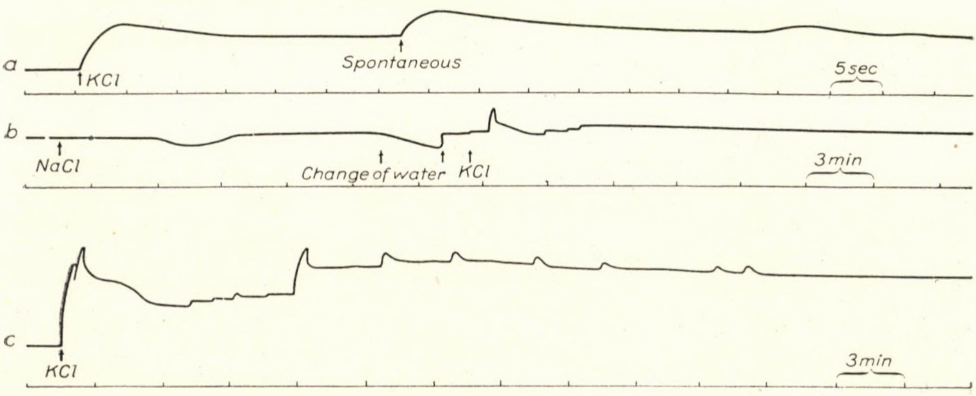

Fig. 6. Visceral ganglion removed. *a* — contraction caused by KCl, *b* — NaCl ineffective; after washing, renewed contraction effected by KCl, *c* — contraction and periodic activity under the effect of KCl

The fact that no contraction is provoked by KCl in animals the visceral ganglion of which has been removed and the anterior adductor cut through, leads to the inference that it is on the normally innervated adductors and through the peripheral nervous system that K^+ ions exert their influence. This, again, allows the conclusion that the theory of SALÁNKI and KOSHTOYANTS has to be somewhat modified. It is obvious that the periodic activity depends on environmental factors. The observed instantaneous reaction argues, however against the probability of a mechanism as postulated by the said authors: what actually happens seems to be that — by means of chemoreceptors and possibly other receptors that have remained intact — potassium is able to produce just that stimulation which elicits the contraction of the unimpaired adductor.

If the test animals are kept in KCl solution for a longer period the valves of the shell will be seen to reach almost maximum contraction which is promptly followed by relaxation after the preparation has been washed. We interpret this phenomenon by regarding the presence of highly concentrated K^+ as a continuous stimulation so that as long as it lasts all consequent reactions will last too. We think, therefore, that contraction following treatment with K^+ is a consequence of a clearly external stimulation and no spontaneous activity. Since the K^+ cannot be regarded as a natural stimulant, it is further assumed by us that reactions elicited there by are not adequate but probably connected with ionic processes occuring in the course of neural conduction in which the K^+ plays a prominent role.

The results here described apply to a part of our investigations only, and there are still problems which await solution. It is, for instance, not yet clear how a muscle can tolerate the action of a comparatively high concentration of KCl without suffering any considerable damage. Our investigations are to be continued as soon as further experimental material becomes available.

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DIE ROLLE DER GANGLIEN IM BEWEGUNGSMECHANISMUS DER MUSCHELSCHALE

Die Untersuchungen von Malermuscheln *(Unio pictorum)* ergaben folgende Resultate:

Nach Entfernung der viszeralen Ganglien werden die Tiere bewegungsunfähig, die Schale öffnet sich. Die Tiere sind bewegungslos, eine periodische Aktivität ist nicht vorhanden.

Tiere mit entfernten viszeralen Ganglien reagieren auf eine mechanische Reizung des Mantelrandes an der vorderen Körperhälfte, sowie auf elektrische Reizungen. Es wurde nachgewiesen, daß das Schließen der Schale infolge mechanischer Reizung im Wege des vorderen Schließmuskels geschieht, während in der Reaktion auf elektrische Reizungen beide Schließmuskeln teilnehmen.

Auf Grund der letzteren Beobachtung wurde auch der morphologische Nachweis erbracht, daß die Entfernung des viszeralen Ganglions hinsichtlich des hinteren Schließmuskels keine vollkommene Denervation bedeutet.

Ferner wurde festgestellt, daß die restliche Innervation vom Gebiet um den hinteren Schließmuskel ausgehen dürfte, da das Isolieren des letzteren die sofortige vollkommene Funktionsunfähigkeit zur Folge hat. In solchen Fällen erweisen sich alle, in früheren Untersuchungen effektive Reizungen als wirkungslos.

Die Entfernung der Gehirnganglien vertragen die Versuchstiere bedeutend besser als die Extirpation der viszeralen Ganglien. Die Reaktionen der Tiere mit entfernten Gehirnganglien auf mechanische und elektrische Reizungen sind analog der Reaktion derjenigen Tiere, bei denen die viszeralen Ganglien entfernt wurden.

KCl vermag bei Tieren mit entfernten Ganglien momentan eintretende Kontraktionen hervorzurufen. Vorbedingung dieser Kontraktionen ist das Vorhandensein eines Schließ-

muskels mit intakter Innervation. Auf diese Weise gelang es festzustellen, daß die Wirkung von K^+ im Wege des peripherischen Nervensystems erfolgt.

NaCl, $CaCl_2$, und $MgCl_2$ vermögen keine Kontraktion hervorzurufen. Wurde KCl nach den erwähnten Stoffen angewendet, so erfolgte zwar die nach K^+ übliche Reaktion, doch war die Kontraktion bedeutend kleiner.

РОЛЬ НЕРВНЫХ УЗЛОВ В МЕХАНИЗМЕ ДВИЖЕНИЯ РАКОВИНЫ ДВУХСТВОРЧАТЫХ МОЛЛЮСКОВ

Исследования перловиц *(Unio pictorum)* привели к следующим результатам:

После удаления висцеральных ганглий животные проявляли неспособность двигаться, раковина открылась. Животные были неподвижными и не показывали периодичной активности.

Животные с удаленными висцеральными ганглиями реагируют на механическое раздражение края мантии передней части тела, равно как и на электрические раздражения. Авторам удалось доказать, что закрытие раковины осуществляется после механического раздражения действием переднего сфинктера, в то время как в реакции на электрическое раздражение участвуют оба сфинктера.

На основании последнего наблюдения также и морфологически было выявлено, что удаление висцеральных ганглий не имеет последствием полной денервации заднего сфинктера.

Далее было установлено, что остаточная иннервация повидимому происходит из области около заднего сфинктера, ибо изолирование последнего моментально вызывает полную функциональную неспособность. В таких случаях все раздражения, которые в прежних экспериментах были эффективными, остались безрезультатными.

Удаление мозговых нервных узлов животные переносят гораздо лучше, чем экстирпацию висцеральных ганглий. Реакции животных с удаленными мозговыми нервными узлами на механические и электрические раздражения тожественны с реакциями животных, у которых провелась экстирпация висцеральных ганглий.

Хлористый калий вызывает у животных с удаленными ганглиями моментальную контракцию. Предусловием этой контракции является присутствие сфинктера с неповрежденной иннервацией. Таким образом удалось выявить, что действие K^+ происходит посредством нервной системы.

Хлористый натрий, хлористый кальций и хлористый магний не в состоянии вызывать контракции. Если хлористый калий применялся после вышеуказанных веществ, то имела место появляющаяся после K^+ обычная реакция, однако, контракция была значительно меньше.

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