The Effect of Chloroacetophenone on the Inducing Capacity of Hensen's Node

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INTRODUCTION

IN a previous paper (Lakshmi, 1962) the effects of ω-chloroacetophenone (CAP), which is an irreversible —SH inhibitor, on the morphogenesis of chick embryos cultured *in vitro* were reported. Brachet (1950) suggested that the —SH-containing proteins might be active in induction. Rapkine & Brachet (1951) studied the effect of monoiodoacetate on the amphibian organizer and observed that the organizer region retained a high capacity for induction despite treatment with the inhibitor. The action of monoiodoacetate is reversible, hence it was felt desirable to investigate the action of CAP on the living organiser of chick, namely Hensen's node.

MATERIAL AND METHODS

Chick embryos at the primitive-streak stage were explanted *in vitro* by New's (1955) technique. These were treated with 0·0005 M CAP for 15 and 30 minutes, 0·001 M CAP for 15 minutes, and 0·0015 M CAP for 15 minutes. 0·1 ml. of the solution was added to the endodermal surface of the explanted embryos. Then they were incubated for 3 hours to re-initiate morphogenetic movements. The nodes were cut out, washed in Pannett–Compton solution three or four times to ensure removal of CAP, and were then grafted on to host chick embryos at the primitive-streak stage in order to test their induction capacity by Waddington's (1932) grafting method.

Control grafts of the node were made in the same way from donors which were treated with control solutions corresponding to the experimental solutions (Text-fig. 1 A, C, F).

The experimental CAP solution and the corresponding control solutions were made in the following manner: A 0·02 M CAP stock solution was prepared in 50 per cent. alcohol. Fifty per cent. alcohol served as control stock solution. To obtain the final CAP concentrations of 0·0005 M, 0·001 M, 0·0015 M and their corresponding control solutions the CAP stock and the control stock solutions were separately diluted with the required amount of fresh Pannett–Compton solution.

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Absolute controls were kept for the experiments with 0·0005 M CAP, in which nodes of embryos explanted in ordinary Pannett–Compton saline and further incubated for 3 hours, were grafted into chick embryos at the primitive-streak stage.

In all 128 experimental grafts (25 and 23 after 0·0005 M CAP for 15 and 30 minutes respectively, 68 after 0·001 M CAP treatment, and 12 grafts after 0·0015 M CAP treatment), 98 control grafts, and 52 absolute control grafts were made. All the experimental, control, and absolute control embryos were incubated for 18 to 20 hours after grafting, then fixed in acetic-alcohol (1 : 3), sectioned serially at 8 μ, stained in diluted Delafield's haematoxylin, and histologically examined. The important cases of induction are illustrated.

RESULTS

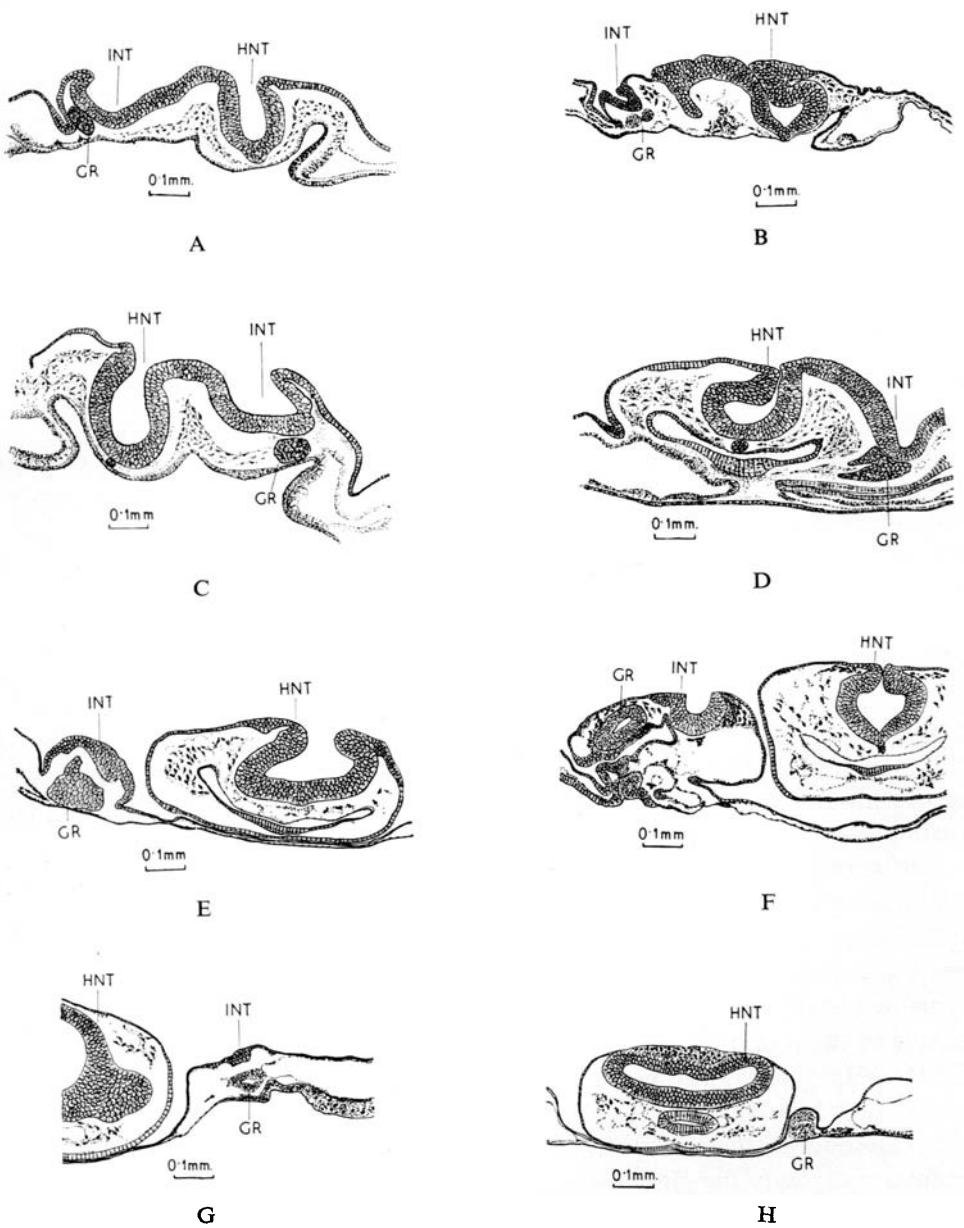
Out of the 128 experimental grafts 33 could not be taken for analysis for various reasons, such as grafts becoming covered with mesoderm or becoming lodged in between the splanchnic mesoderm and the endoderm, or near the heart or sometimes in the coelom. A few other cases were discarded because the grafts were degenerating though a strong induction was produced. In some cases the grafts could not be located though strong inductions were seen. Such cases were also not taken into consideration.

TABLE 1

Analysis of induction capacity of Hensen's node after CAP treatment

CAP concentration and duration	*Grafts made*	*Grafts considered*	*Strong inductions*		*Moderate inductions*		*Absence of induction*		*Control grafts*		*Absolute controls*	
			No.	*Percentage*	*No.*	*Percentage*	*No.*	*Percentage*	*No.*	*Percentage induction*	*No.*	*Percentage induction*
0·0005 M 15 mins.	25	20	11	55	3	15	6	30	15	80	52	84·6
0·0005 M 30 mins.	23	21	11	52·4	2	9·5	8	38·1	—	—	—	—
0·001 M 15 mins.	68	45	11	29·4	7	15·6	27	60	25	84	—	—
0·0015 M	12	9	—	—	1	11·1	8	88·9	6	83·3	—	—

The grafts treated with the different concentrations of CAP were studied from the point of view of differentiation. It was seen that 87·5 per cent. of those treated with 0·0005 M (15 minutes) and 81·2 per cent. of the ones treated with the same concentration for 30 minutes showed differentiation. After treatment with 0·001 M CAP for 15 minutes 50 per cent. of the grafts showed differentiation and the remaining grafts presented a loose disaggregated appearance. However, the cells of these grafts seemed healthy. The grafts treated with 0·0015 M CAP (15 minutes) did not show any differentiation at all and were represented by a mass of loose disaggregated cells but the cells seemed healthy (Text-fig. 1H). A control for this series is illustrated in Text-fig. 1F.



TEXT-FIG. 1 A, T.S. through a control induction for experiments of node grafts treated with 0·0005 M CAP for 15 minutes. B, T.S. through a strong induction produced by graft treated with 0·0005 M CAP for 15 minutes. C, T.S. through control induction for experiments of node graft treated with 0·001 M CAP. D, E, T.S. through strong and moderate inductions produced by nodes treated with 0·001 M CAP for 15 minutes. F, T.S. through a control for experiments of node treated with 0·0015 M CAP for 15 minutes. G, T.S. through a moderate induction produced by node treated with 0·0015 M CAP for 15 minutes. H, T.S. through a case showing absence of induction reaction to node graft treated with 0·0015 M CAP for 15 minutes. HNT, host neural tissue; INT, induced neural tissue; GR, node graft.

DISCUSSION

It will be seen in Table 1 that an increase in the concentration of CAP used for treatment has a telling effect on the induction capacity of the node. Even an increase of duration of treatment of 0·0005 M CAP for 15 more minutes has caused some decrease in the percentage of inductions produced. A study of the table will show that the percentage of induction has fallen from 70 to 11·1 with the increase of concentration of CAP from 0·0005 M to 0·0015 M. Apart from this fact it is also evident that a qualitative change in induction exists. The various induction cases were classified in two main categories; namely strong inductions and moderate inductions. In many cases the grafts induced either something approaching a secondary head structure or a neural tube of considerable dimensions. Such cases were described as strong inductions. For example, Text-fig. 1B illustrates a strong induction obtained from a graft treated with 0·0005 M CAP for 15 minutes. The cases where the grafts caused the formation of only a medullary plate or thick palisade were designated as moderate inductions. Text-fig. 1E, G are examples of moderate induction produced by node treated with 0·001 M and 0·0015 M CAP for 15 minutes respectively.

The experimental cases were studied from the point of view of differentiation of the grafts which seem to be affected by CAP. Increase of concentration of the chemical causes a decrease in the number of grafts differentiating. This suggests a direct action of CAP on the mesoderm of the graft. There is no reason to doubt this in view of the fact that Waddington (1932) observed that almost all the grafts of the anterior third of the primitive-streak differentiated into neural tissue. He further observed that all the grafts of the anterior third of the primitive streak which gave rise to inductions themselves differentiated into neural tissue. In the present work there have been cases where the grafts had differentiated into neural tissue and yet had not caused induction. Thus, in other cases where the graft had not differentiated and had not caused induction though the cells had remained healthy (Text-fig. 1H), this loss of inductive capacity may not be taken as due to the non-differentiation of the graft. The grafts treated with 0·0015 M CAP for 15 minutes also had not differentiated and had not formed any neural tissue by induction. Since the differentiation of the grafts themselves is due to their mesoderm, the non-differentiation of the treated grafts and the loss of inductive capacity they incur is a consequence of the CAP treatment.

It has already been mentioned that Rapkine & Brachet (1951) obtained inhibition of nervous system formation by removing the —SH groups through oxidation with oxidized glutathione and alloxane or with iodoacetamide. The experiments in which the same authors increased the concentration of —SH groups by treatment with cysteine can also be interpreted as indicating that the —SH groups play an important role in induction.

Brachet & Rapkine (1939) obtained formation of neural tissue in 37 per cent. of ventral explants treated with —SH-containing substances. They obtained

similar results again in later work (Rapkine & Brachet, 1951). In the latter paper they referred to their earlier experiments performed to study the inductive capacity of the dorsal lip of the blastopore of gastrulae treated with monoiodoacetate. About 80 per cent. of the grafts gave induction. They stated that the high percentage of induction was expected since the action of monoiodoacetate is reversible. But that of CAP is irreversible (Beatty, 1951) and therefore CAP causes a decrease in induction capacity with increasing concentration. It is to be noted that the reacting tissue in the present work is normal and only the graft which is implanted is treated with various concentrations of CAP.

The results of the present experiments indicate that —SH groups probably have an important role to play in the induction process. Hence the abnormalities of the nervous system produced by CAP in explanted chick embryos are probably due to interference with the normal induction process.

Brachet (1950), in discussing the distribution of —SH-containing proteins, considers the possibility that the reduction of certain dyes *in vivo* is caused by the —SH groups. However, he adds that 'such a conclusion is premature since the —SH groups were determined after fixation and there is nothing to prove that the living organizer is richer in —SH groups than the other regions of the egg'. But the action of CAP of blocking —SH groups in the living organizer leading to loss of induction capacity as observed in the present investigation, may perhaps be interpreted to mean the presence of —SH groups in the *living organizer*.

SUMMARY

1. The effect of chloroacetophenone at concentrations of 0·0005 M, 0·001 M, and 0·0015 M on the capacity of induction of Hensen's node has been studied.

2. A fall in the percentage of induction with increase of concentration of CAP is seen. A qualitative fall of induction is also observed as evidenced by a reduction in the number of strong inductions and increase in cases of absence of induction with increase of concentration from 0·0005 M to 0·0015 M. There were no strong inductions after 0·0015 M CAP treatment.

RÉSUMÉ

Effet de la chloroacétophénone sur le pouvoir inducteur du nœud de Hensen

1. L'effet de la chloroacétophénone sur le pouvoir inducteur du nœud de Hensen a été étudié aux concentrations de 0·0005 M, 0·001 M et 0·0015 M.

2. Une diminution du pourcentage d'induction obtenu s'est manifestée lorsque la concentration en CAP croît. Une diminution qualitative de l'induction s'observe également, se traduisant par la diminution du nombre d'inductions fortes, et l'augmentation du nombre des cas d'absence d'induction lorsque la concentration croît de 0·0005 M à 0·0015 M. Il n'y a pas de forte induction après un traitement à 0·0015 M de CAP.

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