and nucleic acids[2,21,22], after the irradiation, solid sodium chloride was added to the solutions to give a 1 *M* concentration. DNA was then precipitated by adding 4 ml of absolute ethyl alcohol, centrifuged, washed with 2 ml of ethyl alcohol-water 80:20 (V/V) and redissolved in 2 ml of water. The solutions so obtained were utilized for the determination of the radioactivity, using a liquid scintillation counting system Beckman LS 150[23].

On the bases of these radioactivity measurements, the amounts of furocoumarins linked to DNA have been calculated, expressing them as percentages of the amount of furocoumarins initially present in the solutions. The results obtained are reported in the Figure. It appears clearly that the various furocoumarins have a very different photoreactivity with DNA.

In order to define in numbers the photoreactivity of each compound, we have calculated from the data obtained the time of irradiation which was necessary to give a linkage to DNA corresponding to 20% of the amount of furocoumarin initially present. On the bases of the numbers obtained, considering as equal to 100 the photoreactivity of psoralen, the relative photoreactivities of the compounds have been calculated.

The results are reported in the Table, together with the relative skin-photosensitizing activity of the various substances obtained in the test on guinea-pig skin[18].

As it appears, the 2 activities, in vivo and in vitro, are rather parallel. The higher skin-activity of a furocoumarin seems therefore to be due to a greater amount of substance linked to DNA, even if further studies have to be made to clarify whether the difference in the type of photoaddition which can take place between furocoumarins and DNA[3,4] may have an influence on the biological consequences of the photoreaction.

In conclusion, we may say that the results now obtained confirm that the photoreaction with DNA is connected with the photosensitizing effects exerted by furocoumarins on the skin[24].

Riassunto. È stata determinata la fotoreattività con DNA nativo di un gruppo di furocumarine per irradiazione a 365 nm. I valori ottenuti sono risultati in accordo con quelli di attività fotosensibilizzatrice cutanea posseduti dalle stesse sostanze.

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[21] G. RODIGHIERO, F. DALL'ACQUA and G. CHIESA, Rc. Accad. naz. Lincei *42*, 510 (1967).

[22] F. DALL'ACQUA, S. MARCIANI and G. RODIGHIERO, Z. Naturforsch., in press.

[23] The solution of scintillator had the following composition: 120 g naphthalene, 75 mg P.O.P.O.P. [2,2'-phenylene-*bis*(5-phenyl-oxazole)], 4 g P.O.P. [2,5-diphenyl-oxazole] dissolved in dioxane up to 1000 ml of solution.

[24] These studies were aided by Consiglio Nazionale delle Ricerche, Roma.

An Autoradiographic Study of the Mechanism of Neural Induction in the Chick Embryo

Induction of neural tissue formation in vertebrate embryos is a well-known phenomenon and is easily accomplished experimentally by interacting competent ectoderm with small pieces of the primary organizer such as the dorsal lip of blastopore of an early amphibian gastrula or the anterior third of a chick primitive-streak embryo. It has been shown several years ago that a molecular transfer occurs during such an inductive interaction; the molecules involved in the transfer presumably either directly or indirectly set off a biochemical mechanism that causes derepression of specific genes in the reacting system. Synthesis of specific RNA species has been demonstrated in a number of differentiating systems and it may be expected that such a synthesis would take place in the competent ectoderm during interactions leading to the formation of neural tissue. Equally relevant to the study of the mechanism is an understanding of the mode of participation of the inductor molecules in the mechanism. The experiments reported here confirm that specific RNAs are synthesized in the neural induction system and also suggest a possible mode of action of the inducing molecule.

Material and methods. White leghorn eggs were incubated to obtain primitive-streak stage embryos. The latter were explanted in watch glass[1] and used as recipients. Hensen's node (HN) pieces were prepared for grafting as follows: The nodes were excised; half the number were placed in Pannett-Compton (PC) saline for 3 h, and the other half in a solution containing F-1 histone (2.5 mg/ml) for 3 h. Following the treatment the normal and histone-treated node pieces were washed by repeated change of PC saline over a period of 60 min. In each recipient chick embryo 2 grafts (1 normal and 1 histone-treated) were made, 1 on either side of the primitive-streak nearer the margin of area pellucida at the level of the Hensen's node[2]. The cultures were incubated for 3 or 5 h. After the incubation a 30 min pulse of H^3-uridine (25 μc/0.4 ml per embryo) was given. At the end of the pulse the embryos were washed in PC saline at 0–4 °C, and fixed in Bouin's fluid.

The embryos were serially sectioned at 6 μ. The sections of each pair of normal and histone-treated grafts were arranged on 2 slides so that both contained sections of both grafts. One set of slides was treated with 5% TCA at 4 °C for 10 min to remove the precursor. Of the second set half the number of slides were incubated with 0.01 *M* phosphate buffer at pH 7.1 for 90 min; the other half were incubated with RNase 2.5 mg/ml in phosphate buffer at pH 7.1 for 90 min, and then extracted with cold TCA. On completion of the above procedure the slides were rinsed in double distilled water and dried off. They were

[1] D. A. T. NEW, J. Embryol. exp. Morph. *3*, 326 (1955).

[2] C. H. WADDINGTON, Phil. Trans. R. Soc. B, *221*, 179 (1932).

then coated with Ilford K-5 nuclear research emulsion and autoradiographed. H^3-butylmethacrylate sections were used as autoradiographic standards to enable proper comparison between the different batches of autoradiographs[3].

Nuclear grain counts were made in the ectoderm that was in contact with the normal and histone-treated grafts, and counts corresponding to each pair compared between themselves. If a statistically significant increase was found in any pair the corresponding slide which had been extracted with phosphate buffer was also counted in a similar manner in order to see if the difference had been maintained. All the slides that had been subjected to RNase treatment were also examined to make sure that no significant radioactivity remained in the nuclei after these treatments.

Results and discussion. It appears from these experiments that an inductive contact lasting 5 h is enough to trigger off the biochemical mechanism which produces a successful neural induction. BRAHMA[4] made a similar study and found that after 6 h of inductive contact, *Triturus* ectoderm showed greater nuclear labelling when given a 3 h pulse with H^3-uridine than the corresponding ectoderm which had not been placed in contact with primary organizer. He found, however, that the difference was abolished by buffer treatment before TCA extraction. The criterion we have used in the present experiments is the ability of the grafts to enhance synthesis of nuclear RNA (nRNA) in the ectoderm with which they are placed in contact. In the 3 h contact series only in one out of 15 cases the ectoderm in contact with HN graft showed an increase in nRNA synthesis as compared to the corresponding ectoderm that was in contact with histone-treated graft. This difference disappeared when the slide was treated with buffer before TCA treatment. When the duration of inductive contact was increased to 5 h, in 4 out of 7 cases the ectoderm overlying the HN graft showed greater nRNA synthesis than the ectoderm in contact with histone-treated HN graft. This difference was also found in the slides which had been treated with buffer before TCA extraction. The ectoderm cells overlying histone-treated HN graft showed no change in the level of synthesis with increase of duration of contact from 3–5 h. Normal ectoderm which was not in contact with either kind of graft synthesized nRNA in similar amounts. This might be due to the fact that histone-treated HN grafts did not possess neural inductive capacity[5]. But considerable changes occurred in the state of nRNA synthesis by ectoderm in contact with normal HN graft.

It would be difficult to say what kinds of RNA are involved in this inductive process. Those synthesized in 3 and 5 h series have definite characteristics. The species synthesized in the 3 h series are extractable by plain buffer treatment while those of the 5 h series are not. Besides, the increase in nRNA synthesis in the 5 h series is 1.6 times greater than that seen in the 3 h series. This might indicate the difference in the rates of their synthesis. The pattern of synthesis of nRNA in a 3-hour pulse labelling is completely different[4]. It appears probable therefore that specific rapidly-labelled nRNAs are synthesized in the reacting system as a consequence of inductive interactions with the primary organizer. Inability of the histone-treated grafts to affect the state of nRNA synthesis might suggest that the inducer molecules normally complex with the histones on the DNA[6].

Synthesis of rapidly labelled nuclear RNA during embryonic induction

H^3-uridine in nuclear RNA
(grain counts/30 μ^2 nuclear area at 100 A.R.E.[a])

3 h		5 h	
HIST-HN-EPI[b]	HN-EPI[c]	HIST-HN-EPI	HN-EPI
57.38 ± 2.4	54.99 ± 1.9	75.6 ± 3.6	108.0 ± 7.2[d]
57.38 ± 2.0	58.82 ± 3.3	19.8 ± 2.0	54.9 ± 3.6[d]
49.50 ± 2.6	63.36 ± 4.3[d]	24.3 ± 1.3	27.9 ± 3.2
52.60 ± 3.1	58.59 ± 1.2	43.2 ± 2.3	52.2 ± 2.1[d]
34.44 ± 1.4	34.91 ± 1.9	85.5 ± 4.4[d]	63.0 ± 3.4
50.21 ± 2.3	42.33 ± 1.4	56.7 ± 4.4[d]	40.5 ± 3.2
41.60 ± 2.1	45.43 ± 1.0	24.3 ± 1.7	36.9 ± 1.6[d]
39.21 ± 1.9	32.75 ± 1.6		
43.04 ± 2.3	40.64 ± 2.3	Average: 47.0	54.8
51.65 ± 5.2	53.56 ± 6.0		
40.41 ± 1.4	41.60 ± 1.6	HN-EPI/HIST-HN-EPI: 1.17	
40.64 ± 1.3	41.36 ± 1.4		
38.23 ± 1.7	37.30 ± 1.8		
47.82 ± 1.9	46.62 ± 1.8		
39.84 ± 1.9	38.25 ± 2.1		
Average: 45.4	45.9		
HN-EPI/HIST-HN-EPI: 1.0			

[a] Nuclear grain counts were converted to grain counts at hypothetical 100 A.R.E. A.R.E. for the 3 h series was 17.0 and 11.3 for the 5 h series (A.R.E. is autoradiographic efficiency). [b] Ectoderm in contact with histone-treated Hensen's node (HN) graft. [c] Ectoderm in contact with normal HN graft. [d] Denotes statistically significant difference between the nRNA synthesized by the ectoderms.

Résumé. On a étudié de l'RNA nucléaire, synthétisé par des cellules ectodermes embryoniques qui avaient été mises en contact pendant 3 et 5 h avec des greffes organisatrices du nœud Hensen normales aussi bien que traitées par l'histone. On a suggéré un mécanisme probable par lequel des molécules inductrices pourraient produire une différenciation dans le tissu neural.

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Institute of Cancer Research, Royal Cancer Hospital,
London (England), 23 October 1968.

[3] The methacrylate sections were prepared by Mr. S. R. SCARFE who has devised his own procedures for their preparation. Autoradiographic efficiency, as calculated here, is the number of grain counts/100 disintegrations from a section of infinite thickness with reference to tritium.

[4] S. K. BRAHMA, J. Embryol. exp. Morph. *16*, 203 (1966).

[5] G. V. SHERBET and M. S. LAKSHMI, Nature *215*, 1089 (1967).

[6] Acknowledgments. The authors are grateful to Professor Sir ALEXANDER HADDOW for his interest in this work. This research project was supported by a grant from the Damon Runyon Memorial Fund for Cancer Research and also by grants from the British Empire Cancer Campaign for Research, the Medical Research Council, and the U.S. Public Health Service.

[7] Beit Research Fellow.